AN INVESTIGATION OF COMBINED EFFECTS OF AQUEOUS INFUSIONS OF BLACK TEA (*CAMELLIA SINENSIS*), *OCIMUM* SPP. AND *STEVIA REBAUDIANA* IN MODULATING ANTIOXIDANT, ANTIGENOTOXIC AND CANCER PREVENTIVE POTENTIAL

Thesis Submitted for the Award of the Degree of

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in

Zoology

By

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2023

DECLARATION

I, hereby declared that the presented work in the thesis entitled "An investigation of combined effects of aqueous infusions of black tea (*Camellia sinensis*), *Ocimum* spp. and *Stevia rebaudiana* in modulating antioxidant, antigenotoxic and cancer preventive potential" in fulfillment of the degree of Doctor of Philosophy (Ph. D.) is the outcome of research work carried out by me under the supervision Dr. A. Najitha Banu, working as Assistant Professor, in the Department of Zoology/School of biotechnology and biosciences of Lovely Professional University, Punjab, India. In keeping with the general practice of reporting scientific observations, due acknowledgments have been made whenever the work described here has been based on the findings of another investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "An investigation of combined effects of aqueous infusions of black tea (*Camellia sinensis*), *Ocimum* spp. and *Stevia rebaudiana* in modulating antioxidant, antigenotoxic and cancer preventive potential" submitted in fulfillment of the requirement for the reward of the degree of **Doctor of Philosophy (Ph.D.)** in the Department of Zoology/School of biotechnology and biosciences, is a research work carried out by Khushboo Guleria, 11616697, is a bonafide record of his/her original work carried out under my supervision and that no part of the thesis has been submitted for any other degree, diploma or equivalent course.

(Signature of Supervisor)

Name of supervisor: Dr. A. Najitha Banu

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ABSTRACT

Black tea is the most famous and popular consumed beverage due to its numerous health- promoting qualities around the world. According to the meta-analysis reports, drinking black tea (BT) may have mild to moderate impacts on the four biggest causes of fatalities globally- cancer, Type II diabetes, stroke, and coronary artery disease linked to atherosclerosis. Combining black tea with other medicinal plants used to make herbal teas or tisanes will boost its capacity to promote health. The goal of the current study was to identify the antioxidant, antigenotoxic, and lung cancer prevention benefits of black tea alone and in combination with other herbal teas [*Ocimum gratissimum* (OG), *Ocimum sanctum* (OS), *Ocimum canum* Sims (OC) & *Stevia rebaudiana* (St as an additive)]. DPPH, ABTS, NO (cell-free assays) as well as LPO and hemolysis (*ex vivo*) assays were used to assess the antioxidant properties of aqueous infusions. Utilizing combination index values (CI), isobolograms, and polygonograms, researchers examined the antioxidant interactions between black tea and herbal tea. Folin-Ciocalteau reagent and an aluminum chloride colorimetry assay were used to measure the total amount of phenolic and flavonoid content. A chemical substance or agent that can harm/cause damage to chromosomes or DNA known as "genotoxin" was determined by H_2O_2 - DNA damage (genotoxicity) assay. By assessing the cytotoxicity against the A549 lung cancer cell line and the *in vivo* determination of benzopyrene [B(a)P] induced pulmonary lesions in mice. The findings showed that in comparison to other infusions, the BT in combination with OG had the highest quenching capabilities between BT and herbal teas, and the antioxidant interaction found was nearly additive. Whereas, Black tea combination with *O. sanctum* (BTOS) exhibited moderate antagonism to antagonism, and Black tea combination with *O. canum* Sims (BTOCS) showed antagonism respectively. To further evaluate the antioxidant capacity of the binary mixture of BT and OG at five different ratios (3:1, 2:1, 1:1, 1:2 & 1:3) antioxidant parameters were undertaken. The BT and OG combination (3:1) demonstrated maximum radical scavenging activity, TPC & TFC. BTOG (3:1) was further combined with *Stevia rebaudiana* (whole leaves). BTOGSt was found to be most effective free radical scavenging effect then other combinations. Moreover, in antigenotoxicity BTOGSt were found to be most effective in inhibiting H_2O_2 – induced DNA damage. The TPC was found

maximum in OG followed by BT & St whereas, TFC was observed to be high in BT as compared to OG & St. While, in combination there was no significant difference was noted in all mixtures (BTOG, BTSt, OGSt & BTOGSt). The chemo preventive potential of lung cancer in BT, OG, BTOG (3:1), and stevia (as an additive) was further investigated. The aqueous infusions of BT, OG, St, BTOG & BTOGSt were more cytotoxic against the A549 lung cancer cell line as compared to OG. The histopathological examination revealed that BT, OG, St, BTOG & BTOGSt demonstrated a similar protective effect against B(a)P-induced carcinogenesis (dysplastic, hyperplastic & emphysema, papillary progression, mononuclear cell infiltration, early stage of carcinoma). Thus, from the perspective of their antioxidant potential or interactions & anticancer potential, this work offers a scientific foundation for creating and designing beverages containing BT mixtures, which may improve BT's ability to reduce oxidative stress-related disease.

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KHUSHBOO GULERIA

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ABREVIATIONS

APPENDIX 1

1.	**ICMR**	**Indian Council of Medical Research Survey**
2.	**GATS**	Global Adult Tobacco Survey
3.	**PAH**	Polycyclic Aromatic Hydrocarbons
4.	**WHO**	World Health Organization
5.	**IARC**	International Agency for Research on Cancer
6.	**BMI**	Body Mass Index
7.	**WCRF**	World Cancer Research Fund
8.	**EMRO**	East Mediterranean Region
9.	**WPRO**	Western Pacific Region
10.	**PAHO**	Americas region
11.	**AFRO**	Africa region
12.	**EURO**	Europe region
13.	**SEARO**	South-East Asia region
14.	**NCRP**	National Cancer Registry Programme
15.	**PBCR**	Population-Based Cancer Registry
16.	**$DALY_{AMI}$**	Disability Adjusted Life Years$_{\text{Adjusted Mortality to Incidence ratio}}$
17.	**$YLLs_{AMI}$**	Years of Life Lost$_{\text{Adjusted Mortality to Incidence ratio}}$
18.	**$YLDs_{AMI}$**	Years Lived with Disability$_{\text{Adjusted Mortality to Incidence ratio}}$
19.	**NSCLC**	Non-small cell lung cancer
20.	**SCLC**	Small-cell lung cancer
21.	**ALK**	Anaplastic Lymphoma Kinase
22.	**EGFR**	Epidermal Growth Factor Receptor
23.	**MET**	Mesenchymal-Epithelial Transition
24.	**FGFR**	Fibroblast Growth Factor
25.	**PIK3CA**	Phosphotidylinositol-4,5 biphosphate 3 Kinase Catalytic subunit Alpha
26.	**BRAF**	Proto - Oncogene B - Raf and v – Raf murine sarcoma viral oncogene homolog B
27.	**ROS 1**	ROS proto-oncogene 1, receptor tyrosine kinase
28.	**RET**	Rearranged during transfection- Proto Oncogene

29.	**HER 2**	Human Epidermal Growth Factor Receptor 2
30.	**NTRK**	Neurotrophic Tyrosine Receptor Kinase
31.	**MEK**	Mitogen- activated protein kinase
32.	**KRAS**	Ki-ras 2 Kirstein rat sarcoma viral oncogene homolog
33.	**4 DCT**	Foyr-Dimensional Computed Tomography
34.	**CCRT**	Concurrent Chemoradiotherapy
35.	**ADME**	Adsorption, Distribution, Metabolism & Excretion
36.	**NNK**	Nicotine-derived nitrosamine ketone
37.	**NNN**	N-Nitrosonornicotine
38.	**B(a)P**	Benzo(alpha)pyrene
39.	**BPDE**	Diol Epoxide metabolites
40.	**AChR**	Agonist-acetylcholine receptor
41.	**CYP1A1**	Cytochrome A1
42.	**AhR**	Aryl Hydrogen Receptor
43.	**ROS**	Reactive Oxygen Species
44.	**CYP**	Cytochrome
45.	**BPdG**	r7, r8, r9 – trihydroxy - (- 10 - (N2-) deoxyguanosyl) - 7, 8, 9, 10 tetrahydrobenzo(a)pyrene
46.	**NDEA**	-N-Nitrosodiethylamine
47.	**BT**	Black Tea
48.	**GT**	Green Tea
49.	**BrDU**	Bromodeoxyuridine
50.	**TFs**	Theaflavins
51.	**EGCG**	Epigallocatechin Gallate
52.	**OG**	*Ocimum gratissimum*
53.	**DEN**	Diethylnitrosamine
54.	**BTPs**	Black Tea Polyphenols
55.	**PBP**	Polymeric Black Tea Polyphenols
56.	**DBTE**	Decaffinated Black Tea
57.	**GTA**	Glyceryltrioctanoate
58.	**CYP1A2**	Cytochrome A2

59.	**MNNG**	N-methyl-N'-nitro-N-nitroguanidine
60.	**Res**	Resveratrol
61.	**TPA**	Tetradecanoylphorbal
62.	**DMBA**	7,12-dimethylbenz(a)anthracene
63.	**AFB**	Alfatoxin B
64.	**TS**	Thesinensins
65.	**EC**	Epicatechins
66.	**EGC**	Epigallocatechin
67.	**ECG**	Epicatechin gallate
68.	**STAT**	Signal Transducers and Activators of Transcription
69.	**JNK**	c-JUN N-terminal kinase
70.	**COX-2**	Cyclooxygenase - 2
71.	**Nf-kB**	Nuclear Factor Kappa-Light chain enhancer of activated B-cells
72.	**FOX**	Forhead box O
73.	**iNOS**	Inducible nitric oxide synthase deficiency
74.	**BW**	Body Weight
75.	**H_2O_2**	Hydrogen Peroxide
76.	**.OH**	Hydroxyl Radical
77.	**OS**	*Ocimum sanctum*
78.	**OCS**	*Ocimum canum* Sims
79.	**St**	*Stevia rebaudiana*
80.	**BTOG**	Black Tea + *Ocimum gratissimum*
81.	**BTSt**	Black Tea + *Stevia rebaudiana*
82.	**OGSt**	*Ocimum gratissimum + Stevia rebaudiana*
83.	**BTOGSt**	Black Tea + *Ocimum gratissimum + Stevia rebaudiana*
84.	**ERK**	Extracellular signal – regulated Kinase
85.	**DBT**	Dehydrated Black Tea solids
86.	**DGT**	Decaffinated Green Tea
87.	**PPE**	Polyphenon E
88.	**B16M-F10**	Mother B16 line (Lung colony formation)
89.	**DPPH**	2,2-diphenyl-1-picryl-hydrazyl

90.	**ABTS**	2,2'-azino-bis (2- ethyl- benzothiazoline – 6 – sulfonic acid)
91.	**NO**	Nitric Oxide
92.	**TPC**	Total Phenol Content
93.	**TFC**	Total Flavonoid Content
94.	**LPO**	Lipid Peroxidation
95.	**TCA**	Trichloro acetic acid
96.	**NEDA**	-N-(1- Naphthyl) Ethylenediamine
97.	**$FeSO_4$**	Ferrous Sulphate
98.	**HCl**	Hydrogen Chloride
99.	**TBA**	Thiobarbituric acid
100.	**BHT**	Butylated Hydroxy Toulene
101.	**$AlCl_3$**	Aluminium Chloride
102.	**CSIR-IIIM**	Council of Scientific & Industrial Research – Indian Institute of Integrative Medicine
103.	**OD**	Optical Density
104.	**PBS**	Phosphate-buffered saline
105.	**GAE**	Gallic Acid Equivalent
106.	**QE**	Quercetin Equivalent
107.	**A_c**	Absorbance of Control
108.	**A_t**	Absorbance of Test samples
109.	**Na_2EDTA**	Ethylene nitrile tetra acetic acid disodium salt dihydrate
110.	**NaOH**	Sodium Hydroxide
111.	**A_0**	Absorbance of Control
112.	**A**	Absorbance
113.	**SRB**	Sulforhobodiamine
114.	**RPMI 1640**	10% fetal bovine serum with 2mM - glutamine
115.	**A549**	Type II pneumocyte lung tumor
116.	**CO_2**	Carbon Dioxide
117.	**T_z**	Time Zero
118.	**C**	Control growth
119.	**T_i**	Test growth

120.	**S.D**	Standard deviation
121.	**SPSS**	Statistical Package for Social Sciences
122.	**ANNOVA**	Analysis of Variance
123.	**EC_{50}**	Half – maximal effective concentration
124.	**µg/ml**	Microgram per mililitre
125.	**A**	Alveolus
126.	**B**	Bronchiolus
127.	**BV**	Blood Vessels
128.	**MNC**	Mononuclear Cell infilteration
129.	**PUFA**	Polyunsaturated Fatty Acids
130.	**AAPH**	2,2'-Azobis (2- amidino propane) dihydrochloride
131.	**AIDS**	Acquired Immunodeficiency Syndrome
132.	**SOD**	Superoxide Dimutase
133.	**MTT**	(3 – (4, 5 – Dimethylthiazol – 2 –y) – 2, 5 – Diphenyl tetrazolium Bromide
134.	**HCT-116**	Human Colorectal Carcinoma Cell Line (Adult Male)
135.	**HT-460**	Non- small cell lung cancer stem – like holocones
136.	**HT-29**	Human Colorectal Adenocarcinoma Cell Line
137.	**MCF-7**	Breast Adenocarcinoma 1
138.	**NH-313**	Mouse NIH/Swiss embryo
139.	**NCI-H187**	Cellosaurus cell line
140.	**COPD**	Chronic Obstructive Pulmonary Disease
141.	**MCF-57**	Human Breast Cancer Cell Line
142.	**THP-1**	Immortalized Monocyte – like cell line
143.	**BTCs**	Black Tea Combinations
144.	**CI**	Combination Index
145.	**RR**	Relative Risk
146.	**NAP**	Napthalene
147.	**5MC**	5 - methylchrysene
148.	**IcdP**	Indeno[1, 2, 3 – cd] pyrene
149.	**DBaiP**	Dibenzo[a, i] pyrene

150.	**DBahA**	Diben[a,h] anthracene
151.	**BkF**	Benzo[k] fluoranthene
152.	**BjF**	Benzo[j] fluoranthene
153.	**BbF**	Benzo[b] fluoranthene
154.	**BaA**	Benz[a] anthracene
155.	**NSAR**	N - nitrososarcosine
156.	**NPYR**	N - nitrosopyrrolidine
157.	**NPIP**	N - nitrosopiperidine
158.	**NMOR**	N - nitrosomorpholine
159.	**NDMA**	N - nitrosodimethylamine
160.	**NDELA**	N - nitrosodiethanolamine

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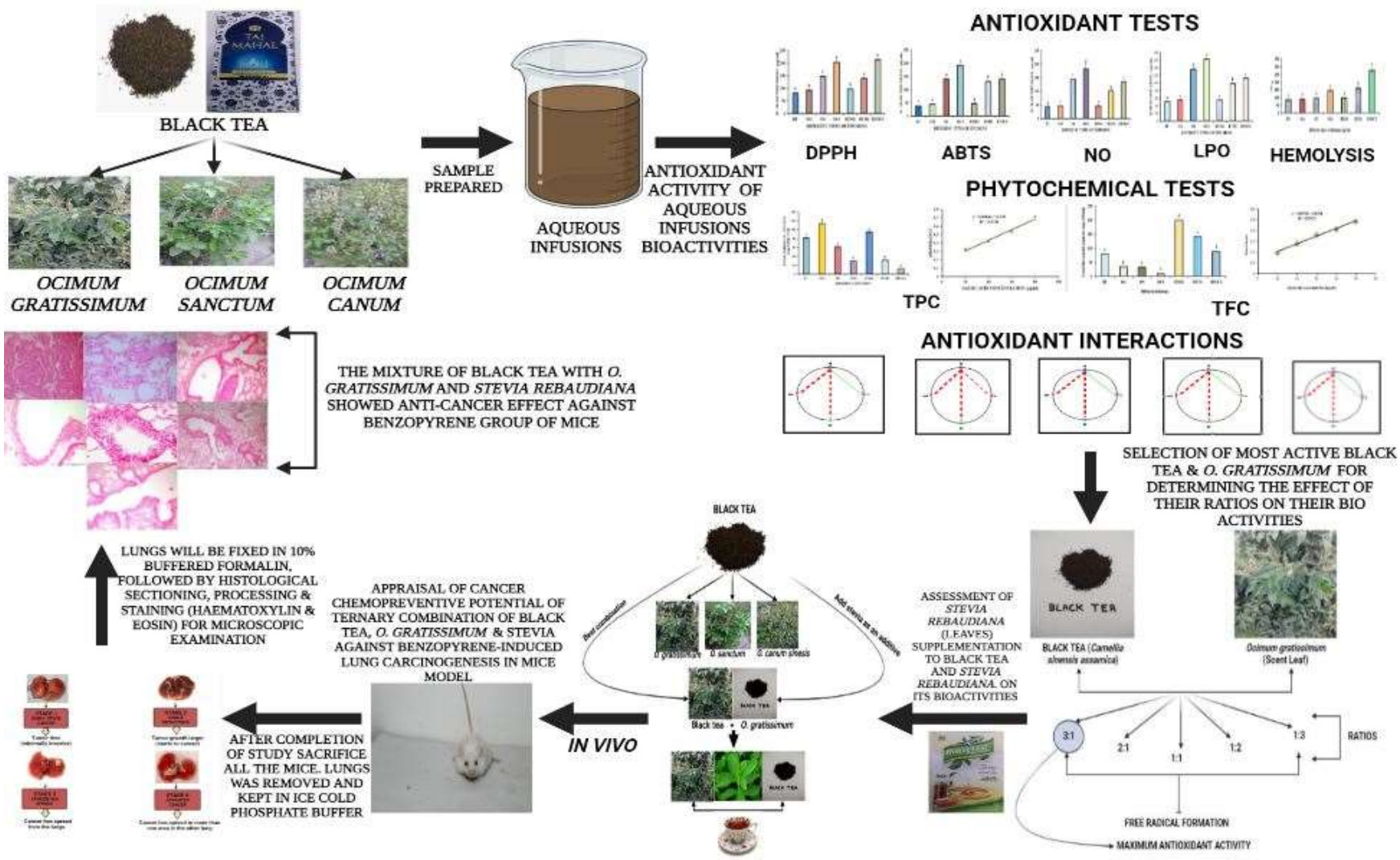


Caption for graphical abstract: Stevia supplementation as an additive in Black Tea with *O. gratissimum* (ternary) mixture for making a refreshing beverage is used to cure illnesses caused by oxidative stress.

CHAPTER 1

INTRODUCTION

1 Introduction

A complex combination of environmental and genetic factors that coordinate carcinogenesis is the biological disease of cancer (Go *et al*., 2015; Motofei, 2022). One of the main reasons for death worldwide. In India, 26.7-29.8 million cases will increase from 2021 to 2025 (Kulothungan *et al*., 2022). The northeastern region of India had the highest cancer burden compared to the country's southern and central parts (Kulothungan *et al*., 2022). Men had a higher rate of it. According to the Indian Council for Medical Research's 2016 report on the "Burden of cancers in India," seven malignancies accounted for more than 40% of the total disease burden. Cancers of the lungs, breast, esophagus, mouth, stomach, liver, and cervix are among the top 10 cancers (Kulothungan *et al*., 2022) as shown in **Fig.1**.

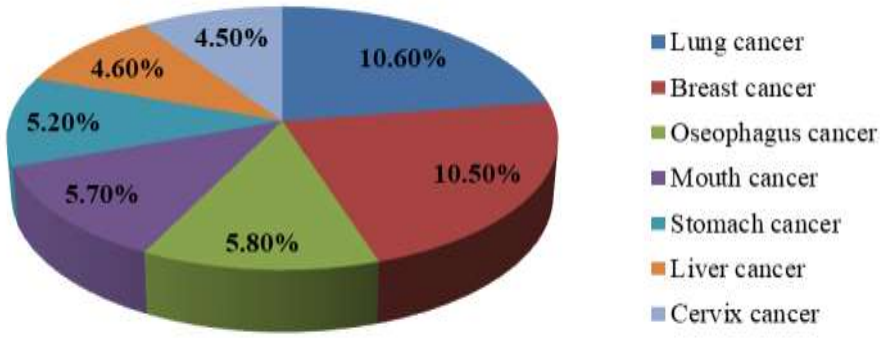


Fig.1: Pie chart showing ICMR report of different types of cancers along with their incidence percentage; 2016 (India)

According to estimates, there will be an increase in male cancer cases in India from 706,740 in 2015 to 942,363 in 2025. Similarly, female patients will increase from 666,144 in 2015 to 853,612 in 2025. The prostate, lung, tongue, and mouth will be the male contributors with the most impact by 2025, while the gallbladder and breast will be the female contributers with the biggest effect on society (Prasad and Dhar, 2018).

According to reports, there will be 2.2 million new lung cancer cases worldwide in 2020, and 1.8 million people will die. In 2020, smoking contributed to

11.4% of new instances of cancer and 18% of cancer-related deaths (Nath *et al*., 2022). India is the world's second-largest tobacco consumer and third-biggest producer. In India, there are 267 million smokers of cigarettes or around 28.6% of the population (42.4% of men and 14.2% of women) [Global Adult Tobacco Survey – 2 [GATS] – 2 2016-2017: India fact sheet; http://gatsatlas.org]. Cigarette smoke has a considerable detrimental impact on health, particularly cancer in India. If current smoking trend and population growth continues, the number of current smokers worldwide will reach 2 billion by 2030 (Asthana *et al*., 2016). Cessation of cigarette smoking, which is recognized to be the primary factor causing lung cancer, is the most excellent strategy to prevent lung cancer (Burzic *et al*., 2022). About 7500 chemical substances, including polycyclic hydrocarbons (PAHs) and nitrosamines, which are either free in the gas phase or coupled with aerosol particles are present in cigarette smoke and are responsible for most of the toxicity caused by smoking (Hecht and Hatsukami, 2022; Zhan *et al.*, 2023). When smoke is inhaled, chemicals like carbon monoxide, tar, aromatic amines, ethylene oxide, benzene, butadiene, volatile hydrocarbons and metals, arsenic, cannabis, and tobacco-specific nitrosamines are deposited in the airways (alveoli) and air sacs, which cause the lungs to become congested. Immediately following this, tissues change and break the thin layer of alveoli (Smith et al., 1997; Smith et al., 2000; Hecht, 2003 Hecht, 2003; Talhout *et al*., 2011; Li and Hecht, 2022) as illustrated in **Fig. 2**.

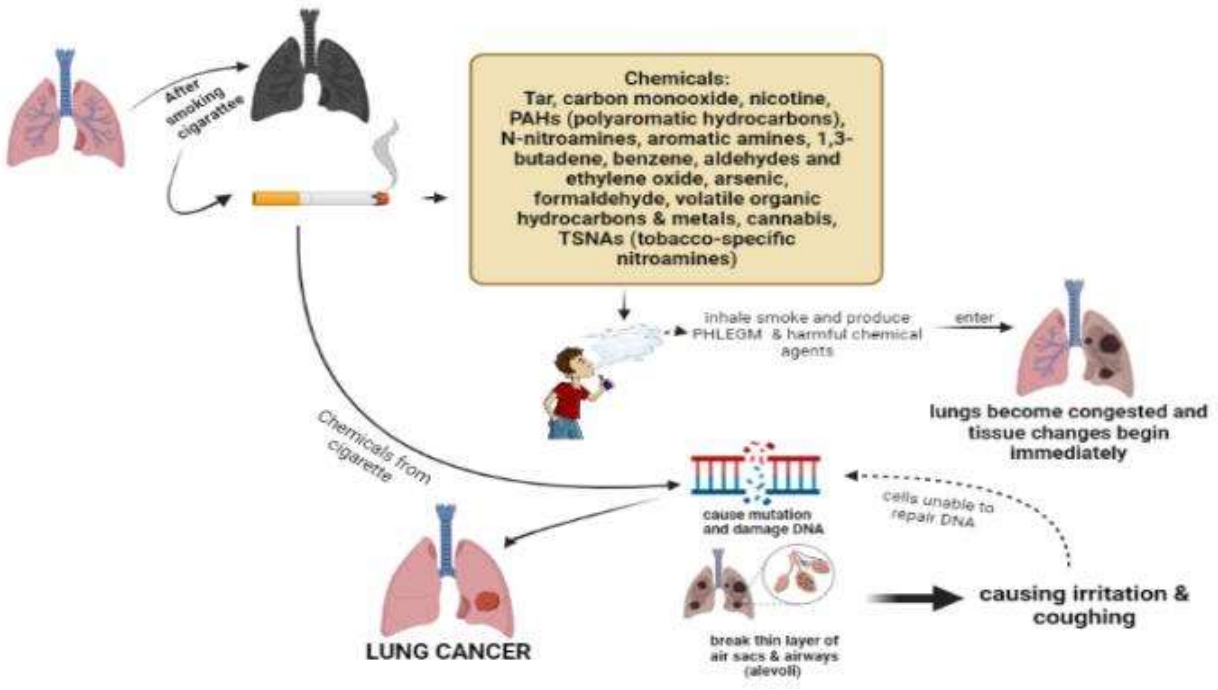


Fig. 2: Diagrammatic representation of different steps showing impact of smoking on lungs (created in www.Biorender.com)

Incomplete combustion of organic matter occurs during human and industrial activities such natural gas, engines, refuse vehicle traffic, home heating (cooking), natural processes like carbonization, and tobacco smoking. This results in the formation of PAHs, a class of chemicals and one of the most common environmental contaminants (Singh *et al*., 2022; Zhu *et al*., 2022). Several hundred PAHs are known to act as mutagenic and carcinogenic to humans. B(a)P was detected as the first carcinogen in cigarette smoke as well as one of the most well-studied potent PAHs that contains many heterocyclic compounds which can be generated by incomplete combustion or ubiquitous in our environment and is used as a toxicological surrogate or prototype for all carcinogenic PAHs (Das and Ravi, 2022; Singh *et al*., 2022).

Many techniques or therapies for cancer prevention, like radiation therapy, chemotherapy, surgery, etc., are used (Altun and Sonkaya, 2018; Abadi *et al*., 2022). They may harm patient's quality of life and therapy development. Tiredness, decreased appetite, vomiting and diarrhea, hair loss, dry mouth, constipation, and change in taste are the most common side effects of chemotherapy's most common side effects (Milliron *et al*., 2022; Gutte and Deshmukh, 2023).

Using botanicals, dietary phytochemicals, and other agents of natural origin having natural having potential to reverse, delay, inhibit, or reduce carcinogenesis, is a cancer prevention technique that is gaining impetus (Chen *et al*., 2018) as depicted in **Fig. 3.** Recent research suggested that a combination of phytochemicals or plant extracts may be more efficient in reducing the risk of acquiring cancer than a single phytochemical or plant extract. The quest for a viable cancer-preventative agent remains ongoing (Kapinova *et al*., 2007; Wang *et al*., 2015; Stockert and Hill, 2018).

Tea consumption has various health advantages, including cancer prevention, weight loss, and heart or liver disease control (Bagherniya *et al*., 2018; Bag *et al*., 2022). Tea drinking has become a phenomenal global culture in recent years, which is primarily contributed to its natural capabilities compared to traditional tea consumption, which is determined mainly by its flavor and sympathomimetic effect (nasal decongestants) (Chen and Lin, 2015; Bag *et al*., 2022). After water, tea is the second most popular drink, made from *Camellia sinensis* and extensively consumed as a beverage, which is far better than other types of drinks (Sinija and Mishra, 2008; Samanta, 2022; Wang *et al*., 2022; Sun *et al*., 2022). It is manufactured from *Camellia sinensis* leaves, which also yielding white, green, and oolong teas. The significant

difference in these tea types is based on processing (oxidation/fermentation) (Konarikova *et al*., 2015; Chen *et al*., 2022; Wong *et al*., 2022). Black tea is one of the highly oxidized form of tea in which the phenolic compounds are converted into thearubigins and theaflavins, responsible for antimutagenic, antipyretic, anticarcinogenic, and antioxidant activities towards peroxy radical damage (Botting et al., 1999; Khan and Mukhtar, 2013; Chen *et al*., 2022; Wong *et al*., 2022).

Black tea's chemopreventive properties and its gradients, especially theaflavins, are responsible for the inhibition of tumor development that is due to various mechanism like modulation of carcinogen-induction of apoptosis, suppression of transcription factors, metabolizing enzymes (Katiyar and Mukhtar, 1996; Kohlmier *et al*., 1997; Khan and Mukhtar, 2015; Phan *et al*., 2022).

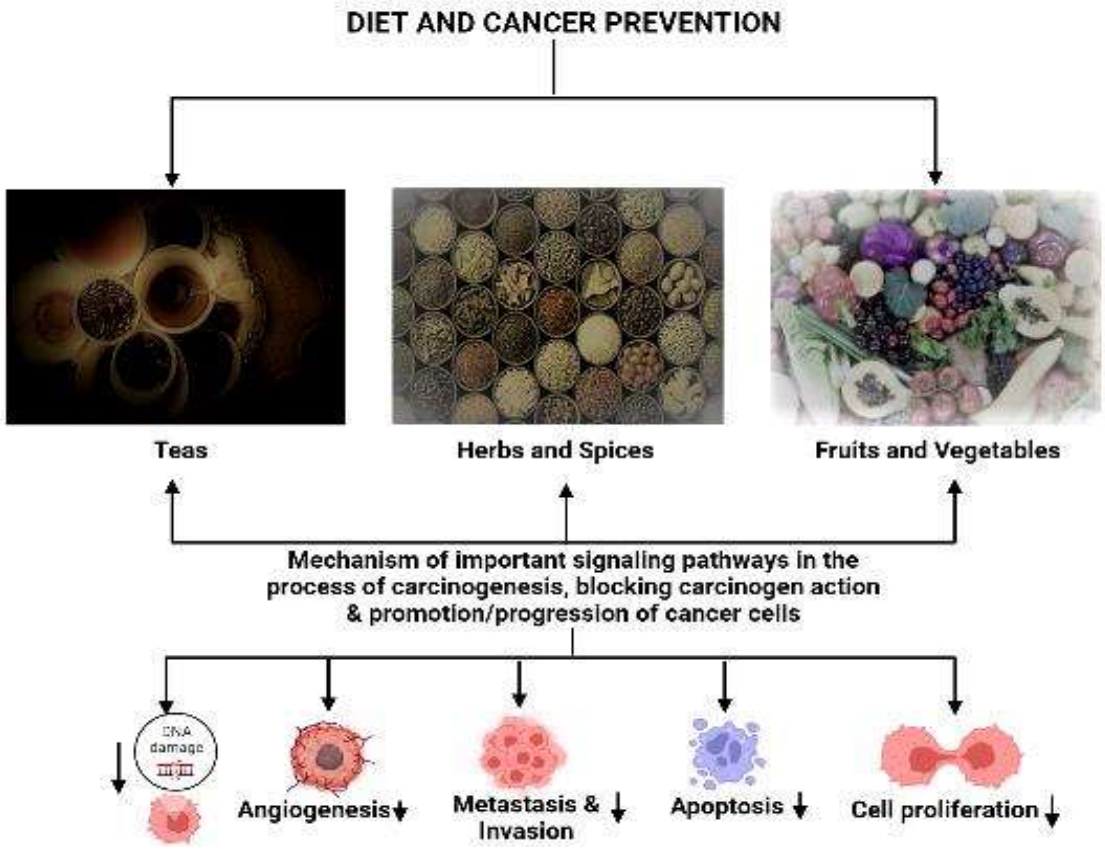


Fig. 3: Represents the dietary/natural agent's role in cancer chemoprevention (created in www.Biorender.com)

Black tea is known to shows antioxidant and anticancer properties in the laboratory, but clinical research has produced contradictory findings on black tea (Korir *et al*., 2014; Zhang *et al*., 2015; Carloni *et al*., 2016). Some studies have proved that it has a minor impact on cancer (Gardner *et al.*, 2007; Ganmaa *et al*., 2008; Tang *et al*., 2009; Nie *et al*., 2014). At the same time, some epidemiological studies have

revealed that black tea may act as cancer preventative (Baker *et al*., 2007; Bai *et al*., 2014; Cassidy *et al*., 2014; Wang *et al*., 2014).

In India (*Ocimum* spp.) is generally added to black tea for flavor and medicinal properties. It is one of the common herbs in the family Lamiaceae. Since ancient times, multiple countries have used this plant as a traditional herb because of its potent perfume and flavor. There are different species of tulsi; routinely used ones are *Ocimum sanctum* Linn, *Ocimum canum* Sims, and *Ocimum gratissimum* L. commonly known as Shyama or Krishna or purple leaf tulsi, Dulaltulsi, and Rama or green leaf tulsi, respectively (Tewari *et al*., 2012). Numerous medical, biological, and pharmacological characteristics, including antifungal, antiviral, antibacterial, antimalarial, antiallergic, antihypertensive, anticancer, and antioxidant activities, were present in different plants parts of tulsi (Kulkarni and Adavirao, 2018). Several phytoconstituents, including the sesquiterpene hydrocarbons caryophyllene, carvacrol, eugenol, and methyl eugenol, are present in tulsi essential oil (Pandey *et al*., 2016). The leaves and stem of tulsi contain phenolic compounds such as cirsilineol, circimaritin, apigenin, cirsilineol, eugenol, rosameric acid, ursolic acid, luteolin-7-O glucuromide, apigenin-7-O-glucuronide, molludistin, orientin, sesquiterpenes, monoterpenes viz. β-sitosterol, cholesterol, camphene, campesterol, stigmasterol, nerol, bornyl acetate, β-elemene, vicenin and orientin (flavonoids) (Bhattacharyya *et al*., 2013).

Several studies showed that when different plant extracts are mixed, they can act synergistic, additive, or in antagonistic manner. Synergism means “enhancement” when two plant extracts/phytochemicals/drugs are combined, and it gives more effect than a single plant species. Antagonism implies the impact of both herbs is less than the sum of the effect of two plant herbs whereas, Additivity means when two herbs' effect is equal to the sum of the effect of two herbs taken separately (Chou, 2010; Malongane *et al*., 2017). Black tea and resveratrol, as well as black tea and curcumin, were found to interact synergistically (George *et al*., 2011; Eldeen *et al*., 2015). Although tulsi is commonly used as an additive in black tea, the interaction of black tea with different *Ocimum* species is still unmapped.

The rise of obesity cases and the diabetes epidemic led to the development and commercialization of tea-based beverages supplemented with low-calorie aspartame (artificial sweetner) obtained from leaves of *Stevia*, sorbitol and sucrolose. *Stevia*

rebaudiana, also called “Sweet Leaf” and belongs to the family Asteraceae. It contains steviol glycosides, which are present in leaves and have a sweet flavor (Brahmachari *et al*., 2011). Among these, interest is generated for Stevia whole leaf extract as it not only acts as a sweetener but also contains phytochemicals that have antioxidant and anticancer properties that may further enhance the health-promoting properties of black tea (Jayaraman *et al*., 2008; & Shukla *et al*., 2009). In this background this study is undertaken to formulate and evaluate the combination of black tea with three different *Ocimum* spp. (*Ocimum gratissimum*, *Ocimum sanctum* and *Ocimum canum*) for antioxidant potential, the binary combination exhibiting maximum radical scavenging will be supplemented with *S. rebaudiana*. Furthermore, the effect of black tea, *Ocimum* spp., and *S. rebaudiana* as single infusion and combination are scrutinized for antigenotoxic and lung cancer preventing potential.

CHAPTER 2

REVIEW OF LITERATURE

2 Review of Literature

2.1 Cancer

"Cancer" refers to any illness that can affecting any part of the body. The term "metastasis" describes the quick development of cancerous cells that spread outside their usual bounds, can infect surrounding biological structures, and spread to other organs (Liu *et al*., 2022). Cancer development proceeds through various stages. Biological, chemical and physical stimuli interact with a person's genetic components to produce these modifications (Smolarz *et al*., 2022; Bergerot *et al*., 2022) as shown in **Fig. 4**.

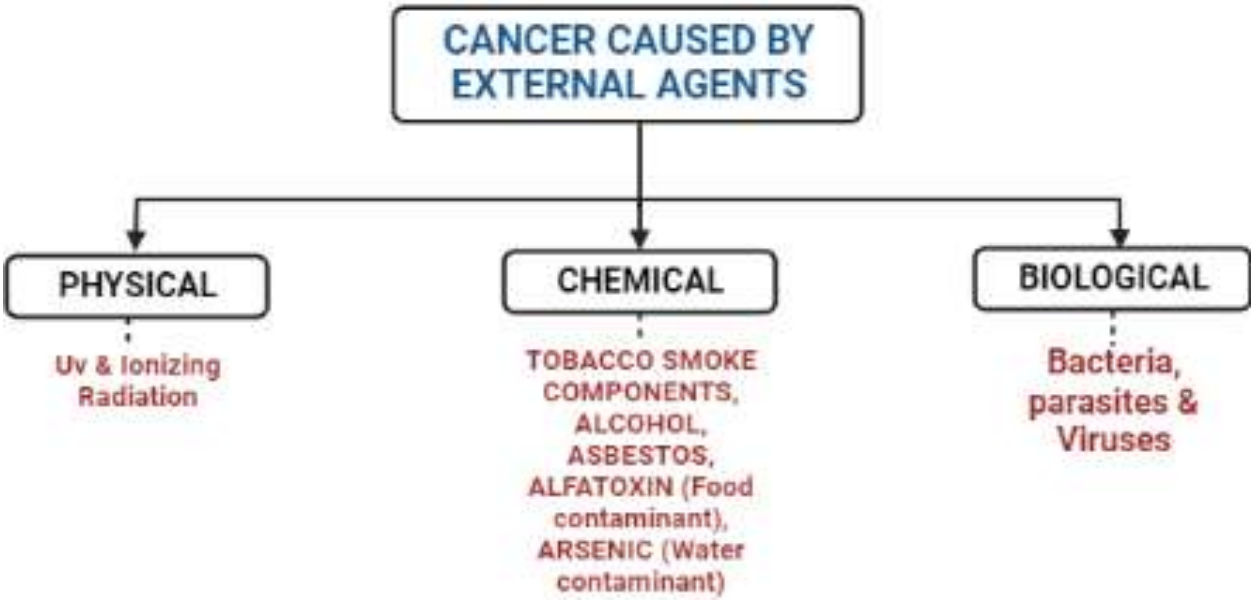


Fig. 4: Represents the various types of external agents causing cancer (created in www.Biorender.com)

WHO and IARC (International Agency for Research on Cancer) maintains a classification of cancer-causing substances (**Fig. 4**). Age-related increase in cancer incidence are most likely caused by an accumulation of factors responsible for particular tumors that shows increase with the advancement of age. In addition to the high overall risk, aging may decrease cellular repair processes effectiveness (Carroll *et al*., 2022).

Normal body cells have a predictable cycle of growth, division, and death. Until adulthood, a person's normal cells divide more quickly in the early years of life. Following that, most body cells only divide to replace damaged or dying cells and heal injuries (Granger *et al*., 2002; Giovannucci, 2022).

Cancer cells arise mainly due to DNA damage over time. Every cell has genetic material within the nuclear membrane, which controls every action the cell takes (Reinhardt *et al*., 2007; Palii et al., 2008; Farhan *et al*., 2022). Cancer cells do not repair damaged DNA (Quellette *et al*., 2022; Groelly *et al*., 2023). Inherited malignancies exist because people can inherit DNA that has been damaged (Groelly *et al*., 2023). However, environmental factors such as tobacco smoke, chemicals, viruses, or excessive sunlight can all damage/mutate a person's DNA.

Cancer's stage represents the size of the tumor and how far it has expanded from its origin. The grade describes how cancerous cells appear, as shown in **Fig. 5**.

Invasion: (Genetically altered cell)

A tumor develops when a healthy population of cells (beige) has a genetic alteration that increases their propensity to divide when resting usually (Liu *et al*., 2022).

Hyperplasia: (Localized stage)

Whenever a tissue or organ has more cells than is typical due to uncontrolled cellular proliferation caused by these additional cells' rapid division, the risk of cancers rises without physiological cues (Yan *et al*., 2022)

Dysplasia: (Regional speed/pre-cancer)

In addition to proliferating excessively, the offspring of this cell appears abnormal within a tissue or organ. It is not cancer, but it may sometimes become cancer when a rare mutation that alters cell behavior occurs. It can be moderate, mild, or severe, depending on how much of the tissue or organ is affected (Anastasiou *et al*., 2022).

STAGES OF CANCER:

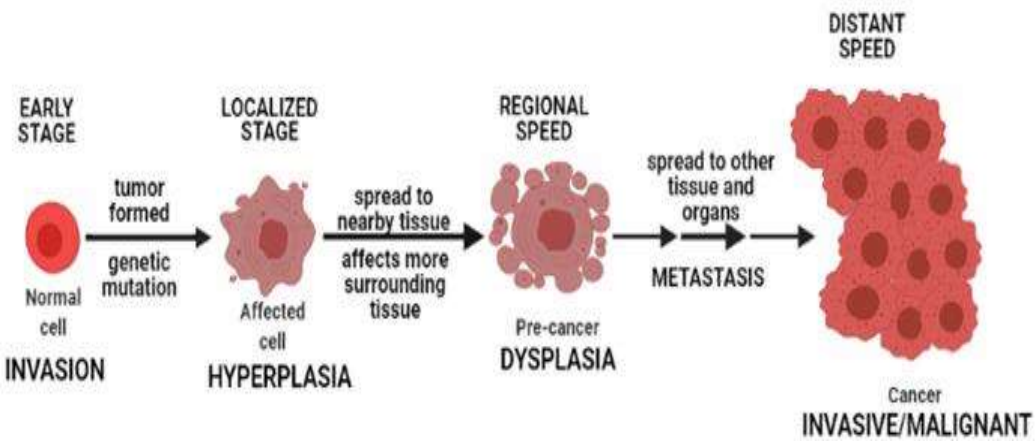


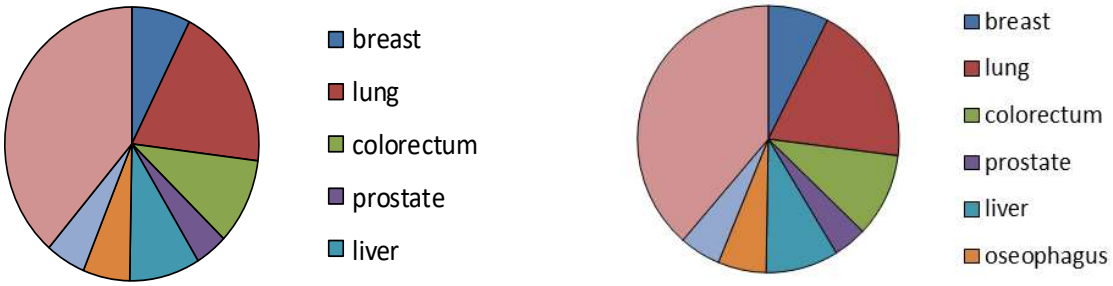
Fig. 5: Diagram showing different stages of cancer (created in www.Biorender.com)

Invasive/in situ cancer: (Malignant)

If genetic changes of tumor cells begin to invade beyond the tissue and cells into blood or lymph where cancer first developed and establish new tumors (metastases) throughout the body. This is referred to as invasive or malignant cancer (Ola, 2022).

Most common cancers in the world

The Globe Health Organisation has identified the four most prevalent illnesses globally: breast, lung, colon, rectal and prostate cancer. About one-third of cancer fatalities are brought on by tobacco smoking, having a high BMI and drinking alcohol (Bray *et al*., 2018) as shown in **Fig. 6**.



Number of new cases of cancer rises (both sexes, all ages), 2020

Number of cancer cases of deaths (both sexes, all ages), 2020)

(GLOBOCON, 2021)

Fig. 6: Pie chart shows increases in the number of cancer cases and fatalities in both males and females

According to one of the studies, most cancer cases were predicted to occur in the breast and lungs, followed by the colorectum, prostate, stomach, liver, cervix uteri, esophagus and other malignancies as illustrated in **Fig's. 7(a), (b) & Table 1**. However, the most common mortality death by a single type of cancer are lung cancer, as compared to the breast, colorectum, prostate, stomach, liver, esophagus, pancreas, and other cancers in both males and females of all ages, respectively, for 2020. The total cancer incidence cases (19292789) and mortality rate (9958133) were reported worldwide (Sung *et al*., 2021). GLOBOCAN 2021 stated the number of incidences, mortality and 5-year prevalence of both sexes in Europe, Asia, Caribbean and Africa, North America and Latin America.

It demonstrated that Asia (the largest continent) contained a higher number of cancer incidences (49.3%), the death rate (58.3%), and 5 years of prevalence (40.8%) followed by Europe (22.8%, 19.6%, and 26.7%), North America (13.3%, 7%, and 18.7%), Latin America and Caribbean (7.6%, 7.2%, and 7.6%), and Africa (5.7%, 7.1%, and 4.3%), respectively as depicted in **Fig. 9 and Table 2.**

Table 1: The estimated age – Standardized Incidence and Mortality Rates (World) in 2020, both sexes, All Ages (males and females) (Excl. NMSC – non-melanoma skin cancer) as per ASR 10,000.

Type of cancer	Incidence rate (%)	Number of cases (Incidence rate %)	Mortality rate (%)	Number of cases (Mortality rate %)
Breast	11.7	2261419	6.9	684996
Lung	11.4	2206771	18	1796144
Colorectum	10	1931590	9.4	935173
Prostate	7.3	1414259	3.8	375304
Stomach	5.6	1089103	7.7	768793
Liver	4.7	905677	8.3	830180
Cervix uteri	3.1	604127	-	-
Esophagus	3.1	604100	5.5	544076
Pancreas	-	-	4.7	466003
Other cancers	42.9	8275743	35.7	3557476

Source: GLOBOCAN, 2021

Incidence: the number of new cases that occur in a given time and geographical area, expressed as either the total number of cases per year or the average per 100,000 people per year.

Mortality is the number of deaths that occur in a given region and period, while the death rate is the number of deaths per 100,000 people per year.

Prevalence = number of cases/ population size

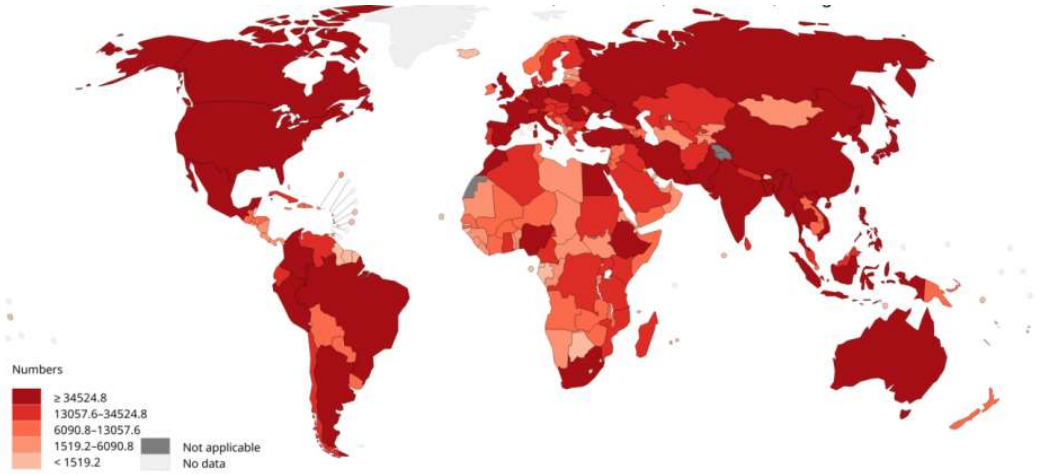


Fig. 7: (a) Cancer incidence and mortality statistics worldwide and by region (WHO, 2023)

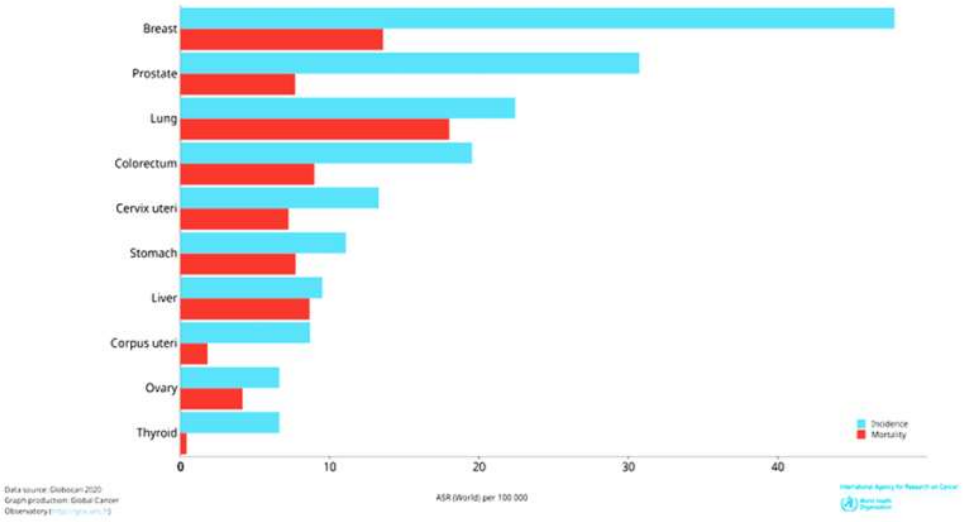


Fig. 7: (b) The World's Standardised Incidence and Mortality Rates for 2020, Including All Age Groups and Both Sexes (Excl. NMSC - non-melanoma skin cancer) (WHO, 2023)

Table 2: Estimated number of cancer occurrences and mortality in both sexes in the world (2020).

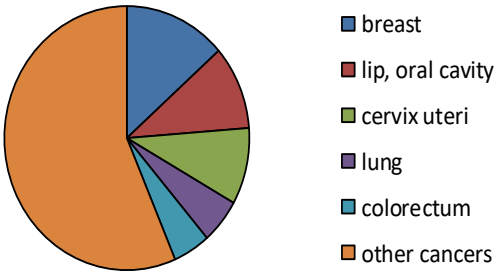
Countries	Incidence rate (%)	Number of cases (Incidence rate %)	Mortality rate (%)	Number of cases (Mortality rate %)
Eastern Africa	13.56	331233	9.91	222189
Northern Africa	14.61	307507	9.21	191081
Western Africa	11.69	247611	8.44	164930
South America	20.24	1095348	9.45	521389
North America	33.95	2556862	9.22	699274
Eastern Asia	21.91	6008355	13.10	3617104
South-Eastern Asia	15.62	1100037	10.00	689093
South-Central Asia	10.93	1951843	7.41	1258683
Central &Eastern Europe	25.50	1314193	13.36	695828
Western Europe	31.24	1424394	10.85	559671
Australia & New Zealand	40.43	235955	8.55	58744
Total World	20.44	19292789	10.65	9958133

Source: GLOBOCAN, 2021

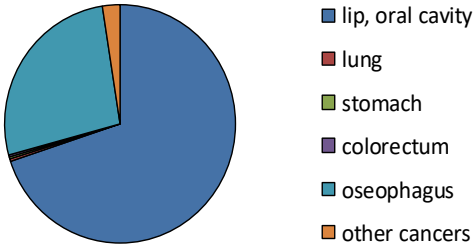
Incidence: the number of new cases that occur in a given time and geographical area, expressed as either the total number of cases per year or the average per 100,000 people per year.

Mortality is the number of deaths that occur in a given region and period, while the death rate is the number of deaths per 100,000 people per year.

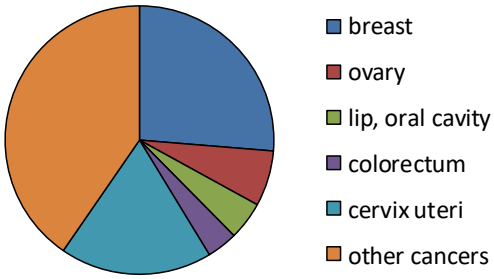
Prevalence = number of cases/ population size



New cases of cancer rise in sexes, 2020



New cases of cancer rise (all males), 2020



New cases of cancer rise (all females), 2020

Fig. 8: Cancer fatality rates and new cases are expected to increase in 2020 (GLOBOCAN, 2021)

In the case of India, the highest ratio of cancer was identified in the breast (females) and lip/ oral cavity (males) in both sexes as compared to other cancers. At the same time, the incidence and mortality rate were higher in the breast (females) than cervix uteri, ovary, and colorectum and lip/oral cavity. Whereas in males, maximum new cases of cancer were observed in the lung, lip/oral cavity followed by colorectum, stomach, and esophagus, respectively in 2020, as illustrated in **Fig. 8 and Table 3(a), (b) and (c)**. However, the total cancer incidence cases (1324413) and mortality rate (851678) were reported in Indian regions like Ahmedabad, Aurangabad, Banglore, Barshi, Bhopal, Cachar, Chennai, Delhi, Dibrugarh, Dindigul, Kamrup, Kollam, Karwar, Imphal/Manipur, Mansa, Meghalaya, Mizoram, Mumbai, Nagpur, Nagaland, Poona, Ratnagiri, Sangrur, Sikkim, Trivandrum, Tripura, etc. as shown in **Fig. 9**. It explained that the incidence and mortality cases of the 5-year prevalence of both sexes in India region are (197.1%) 2720251 (Globocan, 2021).

Table 3: (a) The estimated age – Standardized Incidence and Mortality Rates (India) in 2020, both sexes, All Ages (males and females) (Excl. NMSC) as per ASR 100,000.

Type of cancer	Incidence rate (%)	Number of cases (Incidence rate %)	Mortality rate (%)	Number of cases (Mortality rate %)
Stomach	6.3	40686	6.3	53253
Lung	8	51675	7.8	66279
Colorectum	6.3	40408	4.2	35385
Lip, oral cavity	16.2	104661	8.8	75290
Esophagus	6.2	40183	6.9	58342
Other cancers	57	368417	-	-

Source: GLOBOCAN, 2021

Incidence: the number of new cases that occur in a given time and geographical area, expressed as either the total number of cases per year or the average per 100,000 people per year.

Mortality is the number of deaths that occur in a given region and period, while the death rate is the number of deaths per 100,000 people per year.

Prevalence = number of cases/ population size

Table 3: (b) The estimated age – Standardized Incidence (India) in 2020, (males) (Excl. NMSC) as per ASR 100,000.

Type of cancer	Incidence rate (%)	Number of cases (Incidence rate %)	Mortality rate (%)	Number of cases (Mortality rate %)
Breast	13.5	178361	10.6	90408
Lung	5.5	72510	7.8	66279
Colorectum	4.9	65358	4.2	35385
Lip, oral cavity	10.3	135929	16.2	104661
Cervix uteri	9.4	123907	9.1	77348
Other cancers	-	1 324 413	-	851 678

Source: GLOBOCAN, 2021

Incidence: the number of new cases that occur in a given time and geographical area, expressed as either the total number of cases per year or the average per 100,000 people per year.

Mortality is the number of deaths that occur in a given region and period, while the death rate is the number of deaths per 100,000 people per year.

Table 3: (c) The estimated age – Standardized Incidence (India) in 2020, (females) (Excl. NMSC) as per ASR 100,000.

Type of cancer	Incidence rate (%)	Number of cases (Incidence rate %)	Mortality rate (%)	Number of cases (Mortality rate %)
Breast	26.3	178361	10.6	90408
Cervix uteri	18.3	123907	9.1	77348
Ovary	6.7	45701	3.8	32077
Lip, oral cavity	4.6	31268	8.8	75290
Colorectum	3.7	24950	6.9	58342
Other cancers	40.4	274196	-	-

Source: GLOBOCAN, 2021

Incidence: the number of new cases that occur in a given time and geographical area, expressed as either the total number of cases per year or the average per 100,000 people per year.

Mortality is the number of deaths that occur in a given region and period, while the death rate is the number of deaths per 100,000 people per year.

Fig. 9: Spread of cancer across states of India (WHO, 2023)

2.2 Lung Cancer

A growing problem related to health of humans in the world is cancer. Lung, breast, and colorectal are main types; diagnosed most frequently worldwide (WCRF, 2019). Most common cause of death in the world right now is lung cancer, which is prevelantly found in men (Ferlay *et al*., 2015). Lung, breast, colorectal, prostate, and skin cancer (non-melanoma) were the top five most frequent malignancies worldwide, according to stats released by global cancer in 2018 (Bray *et al*., 2018). Lung, colorectal, stomach, liver, and breast were the five main cancer-related causes of death. As a result, lung cancer remains most prevalent cancer and the main reason for cancer deaths worldwide (Adjei, 2019). In 2012, lung cancer claimed the deaths of over 1.6 million individuals. Various ecological and clinical investigations have demonstrated a link between cigarette use and the development of lung cancer. This "rarest form of disease" has, during the past century, been the primary reason for death via cancer in (Eastern Asia, Australia, Northern Europe, North America, and New Zealand) (Bray *et al*., 2018).

Lung cancer prevalence has risen over the last half-century. The usage of cigarettes was soon connected to the disease (Wynder and Graham, 1950; Didkowska *et al*., 2016). The theoretical justification and statistical literature both suggest that lower cigarette smoking prevalence can have real benefits. However, tobacco consumption, is on the rise globally, especially in developing nations like China and India (WHO, Global Adult Tobacco Survey China, 2016; Global Adult Tobacco Survey India, 2016). In India, lung cancer stands in fourth position in terms of several deaths it caused in 2018 (Bray *et al*., 2018). The tobacco crisis continues to propagate across the rest of the planet, which is causing a swift enhancement in lung cancer cases and related fatalities. By 2035, 3 million lung cancer fatalities are anticipated worldwide. From 1.1-2.1 million cases in 2012 to 2035, the figures for women and men will each double, with the gender disparity continuing at 0.5 million in 2012 and 0.9 million in 2035 (WHO, 2016) in **Fig. 10**. Mortality due to lung cancer is showing increment nationally as well as worldwide due to increasing tobacco consumption especially in developing nations (Doll and Hill, 1950; Lopez *et al*., 1994). Several ecological and medical researches have demonstrated a link between cigarette use and the development of lung cancer (Wynder and Graham, 1950; Wakefield *et al*., 2014; Claire *et al*., 2020).

The East Mediterranean region (EMRO) and the African area are predicted to experience the fastest growth. The estimated global mortality rate from lung cancer, broken down by area from 2004- 2035 is the Western Pacific region (WPRO) – 1,475,602 (50%), Americas region (PAHO) – 501,860 (17%), East – Mediterranean region (EMRO) – 64,477 (2%), Africa region (AFRO) – 33,534 (2%), Europe region (EURO) – 530,257 (18%) and South-East Asia region (SEARO) – 284,518 (10%), respectively as depicted in **Fig. 10(a).**

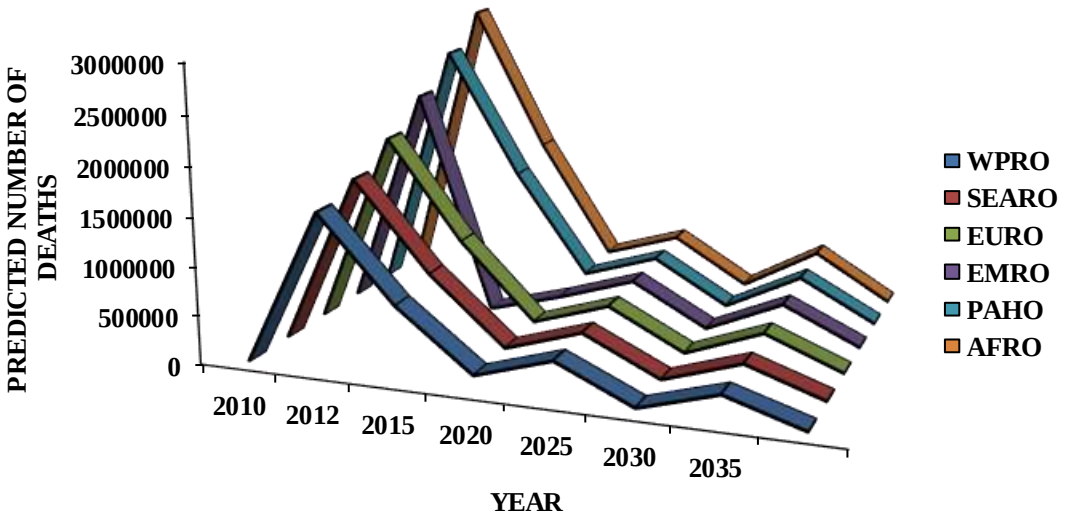


Fig. 10: (a) The fastest growth of mortality rate from lung cancer in male & female from 2004 – 2035 in East Mediteeanean region (EMRO), South – East Asia region (SEARO), Europe region (EURO), Western Pacific region (WPRO), Americas region (PAHO), & Africa region (AFRO) (WHO, 2016)

Lung cancer mortality rates range from 16–17% (45–64 age group) in the East Mediterranean region (EMRO), South-East Asia region (SEARO), and Africa region (AFRO), and are rising by 6 to 10 percent (pp) in the other locations. Similarly, middle-aged individuals recorded the highest rate of deaths in EMRO, AFRO, & SEARO (approximately 30%, 54-64 years) as seen in **Fig. 10(b).** In comparison, the lower percentage (10 pp) was noted in the Americas and Europe regions.

As a result, 30% of lung cancer deaths in all regions occurred in people 65 to 74 years old. In WPRO, EURO, and PAHO countries, lung cancer deaths after the age of 75 account for more than one-third of all fatalities, whereas they are no higher than 20% in the other regions (Bilano *et al*., 2015; Globocan, 2016). Population aging in more industrialized countries is mainly blamed for the rising absolute number of lung

cancer deaths, whereas the tobacco epidemic is to blame in less developed countries (Bray *et al*., 2012).

The National Centre for Disease Informatics and Research (NCRP) provided estimates of India's cancer burden in the ICMR 2004 report. Report of National Cancer Registry Programme) PBCR (Population-Based Cancer Registry) data (NCRP-PBCR) and the WHO-DisMod-II tool yielded 5.9 million DALYs (ICMR Report, 2020). Cancer is expected to cost India 26.7 million $DALYs_{AMI}$ (Disability Adjusted Life Years) Adjusted Mortality to Incidence ratio in 2021 and 29.8 million $DALYs_{AMI}$ in 2025.

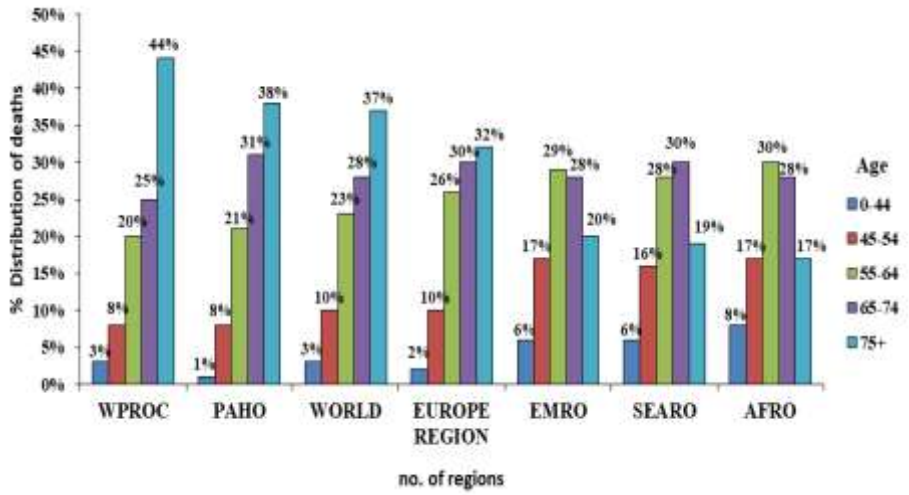


Fig. 10: (b) Graphical representation of mortality rate in percentage due to lung cancer from different regions of world

Males bore a more significant burden than females. Males contributed 14.7 million $YLLs_{AMI}$ (Years of Life Lost), 0.72 million $YLDs_{AMI}$ (Years Lived with Disability and 15.5 $DALYs_{AMI}$ from cancer in 2025, while females contributed 13.6 million $YLLs_{AMI}$,0.69 million $YLDs_{AMI}$ and 14.3 $DALYs_{AMI}$ (Kulothungan *et al.*, 2022).

As per the report of ICMR, the ratio of lung cancer increased by 17.8 - 116% $DALY_s$ per 100,000 from 2004 – 2021 in India compared to other cancers (cervix, breast, oral cavity, ovary, leukemia, lymphoma, multiple myeloma, stomach, esophagus, colon & rectum) (ICMR Report, 2006) as shown in **Fig. 11.**

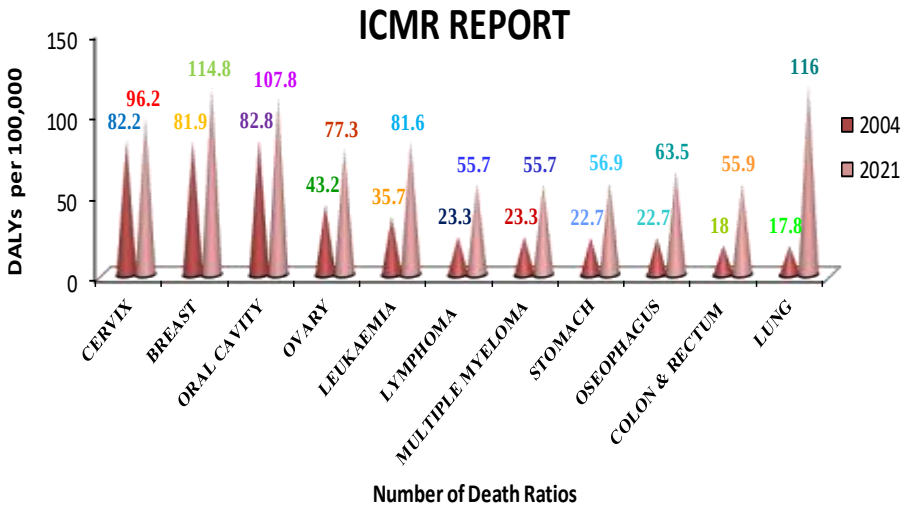


Fig. 11: Graphical representation of India's cancer burden due to different cancer types from 2004-2021

Many factors influence lung cancer survival rates, including gender, race, age, general health, and treatment used (Tas *et al*., 2013; Pinto *et al*., 2018). Starts from lungs and spreads to other body parts, such as the brain, among others. **Fig. 12** shows stages of metastasis.

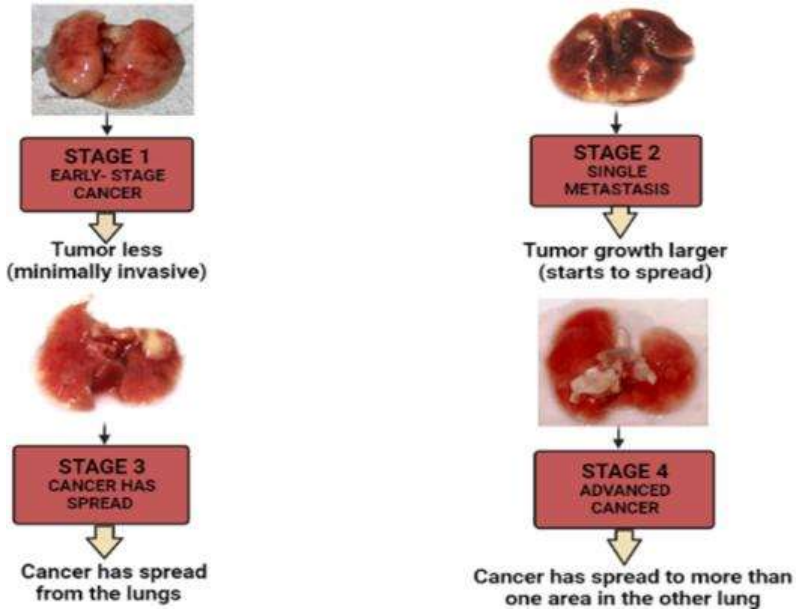


Fig. 12: Diagrammatic demonstration of different stages of lung cancer

2.2.1 Various types of lung cancer

The origin of the aberrant lung cells distinguishes a category of disorders known as lung cancer. This process is known as cell "histology." Histologically, lung cancer falls into Small cell lung cancer (15%), Non-small cell lung cancer (NSCLC) (15%), Large cell carcinoma (30%), and Adenocarcinoma (40%) are the three types of NSCLC (Li *et al*., 2022). The first two types are responsible for approximately 80% of all LCs worldwide (Hochhegger *et al*., 2022; Ekinci *et al*., 2022; Glasauer *et al*., 2014). The three main subtypes of non-small cell lung cancer are squamous-cell carcinoma, adenocarcinoma, and large-cell carcinoma (Relli *et al*., 2019) as depicted in **Fig. 13.**

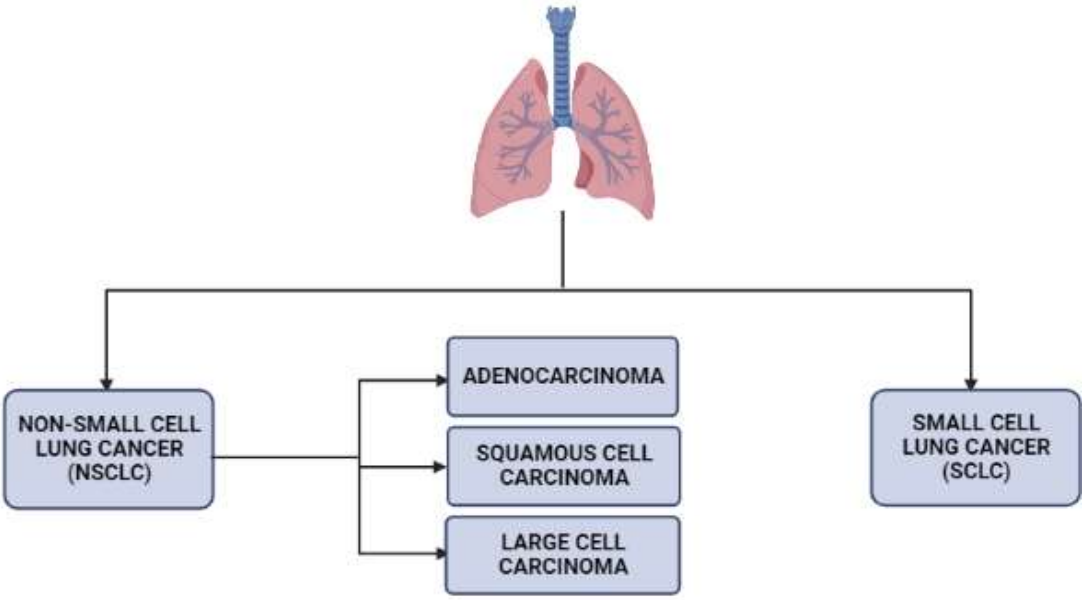


Fig. 13: Diagrammatic demonstration of various types of lung cancer based on histological (created in www.Biorender.com)

Non-small cell lung cancer (NSCLC)

It is the sort of lung cancer that is most common. NSCLC causes more than 8 out of every 10 cases. NSCLC amounts for 80 to 85%. It is a diverse class of tumors, and the genetic abnormalities that give rise to it are almost exclusively brought on by exposure to carcinogens. Most patients in this case of lung malignant lung diseases were in advanced metastatic phases at the moment of treatment (Crino *et al*., 2010). It occurs when cancer cells multiply in the lungs and form tumors. For

every megabase of DNA, there are roughly ten mutations in a tumor in a smoker; there are ten times fewer mutations in tumors in non-smokers.

Intratumor and intratumor heterogeneity can emerge from cells acquiring independent (branch) mutations. Additionally, tumors produce growth factors and other environmental adapatations to elude the immune system. NSCLC differs from SCLC in both histopathology and clinical characteristics. Adenocarcinoma, carcinoma of large cells (large-cell carcinoma), and carcinoma of squamous cells (squamous-cell carcinoma) are the three subtypes of non-small cell lung cancer (Zappa and Mousa, 2016; Torres *et al*., 2017).

Adenocarcinomas begin in glandular cells that normally secrete substances like mucus that protect against foreign particles entering the lung (Chen and Fillmore *et al*., 2014). The glands cells outside the lungs are the sites of the most prevalent type of lung cancer. In comparison this type of lung cancer, is more common in younger people, women, and nonsmokers (Islami *et al*., 2022). Some common genes are mutated that cause lung cancer (lung adenocarcinoma) in people with NSCLC such as FGFR1 (Fibroblast growth factor) = 9-20%, ALK (Anaplastic lymphoma kinase) = 3-7%, MET (Mesenchymal – epithelial transition) = 2-4%, EGFR (Epidermal growth factor receptor) = 10-35% besides others. As a result, when the changes occur to our genes, it becomes cancerous and grows out of control (McKeage *et al*., 2018; Canale *et al*., 2022). Adenocarcinoma grows slowly and is usually diagnosed until it spreads, giving it a better prognosis than most lung cancers (Hiest and Engelman, 2012; Simon, 2016).

Squamous cell carcinomas are derived from squamous cells, flat cells that line the interior of the lung's airways. They are present in the middle of the lungs, close to the main airway (bronchus), and are frequently linked to a smoking history (Hulbert *et al*., 2014). They may form cavities, hyperchromatic nuclei, and keratinization when they achieve a large size in the lung (Conde *et al*., 2013). Due to the continuous passage of fluids (lymph and blood) via the lungs, these tumors may manifest signs like bloody coughing before lungs-bordering tumors (adenocarcinoma) and, finally metastasis (Molina *et al*., 2008; Lewis *et al*., 2014).

Large cell carcinoma, also known as undifferentiated carcinoma, is a lung cancer that spreads quickly and can affect any section of the lung, making treatment more challenging (Travis, 2011). The tumor cells are larger than SCLCs. It

histologically demonstrates marked nuclear pleomorphic, a moderate amount of cytoplasm, a rosette-like structure, and the frequent presence of polygonal nuclei located in the peripheral region of the lung (Brambilla *et al*., 2004; Lewis *et al*., 2014; Zander and Farver, 2018).

About (10-15%) of lung cancers are small cell lung cancers (SCLC), commonly known as oat cell carcinoma. In comparison to NSCLC, this type of cancer is particularly severe (proliferates and spreads). It is distinguished by a high growth fraction, thin cytoplasm, rough chromatin, small, grayish & oval-shaped cells that look like under the microscope, greater propensity for early development of widespread metastases and rapid doubling time (Nicholson *et al.*, 2002; Demedts *et al.*, 2010; Kumar et al., 2017). Approximately 70% of SCLC patients will have advanced cancer when diagnosed (Carter *et al*., 2014; Saltos *et al*., 2020). About 95% of cases are caused by cigarette smoking, and of all histologic subtypes of lung cancer, SCLC has the most significant relationship with cigarette smoking (Sher *et al.*, 2008; Tan *et al.*, 2018).

The most common solid tumor that causes paraneoplastic syndromes is SCLC. Ectopic hormone production or immune-mediated tissue destruction causes paraneoplastic syndromes. Location/size in the primary tumor (SCLC) influences the signs and symptoms. Coughing, wheezing, and hemoptysis are examples of illnesses. Local intrathoracic tumor growth can affect the superior vena cava, chest wall, or esophagus, resulting in superior vena cava syndrome (Basumallik and Agarwal, 2021). SCLC-related deaths are estimated to be 2.5 lakhs per year (Devarakonda *et al*., 2019).

Smoking is strongly linked to the cancer induction in lungs, particularly SCLC. The relative incidence of SCLC has decreased, reflecting reductions in smoking prevalence, changes in cigarettes, and lower occupational hazards. Males are more likely to develop SCLC (Kasiuchnicz *et al*., 2021). However, the incidence disparity between men and women has narrowed over the last three decades (Travis, 2011). Smoking-related SCLC affects about 95% of patients, and the chances rise with daily cigarette consumption and duration of smoking. Smoking or other chemicals damage the lung epithelium, eventually leading to SCLC (Tan *et al*., 2018).

Treatment

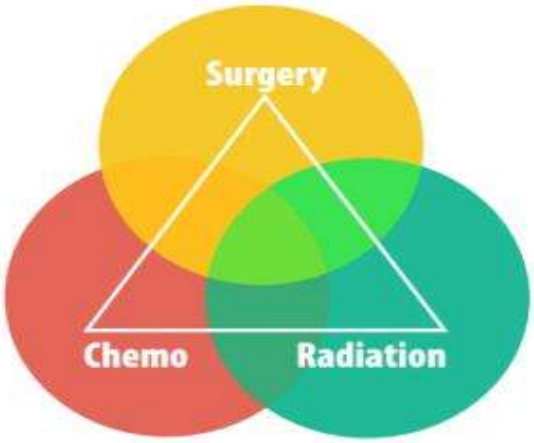


Fig. 14: (a) Diagramatic representation of different lung cancer treatment (obtained from www.google.com)

Depending on the kind and severity of the disease, there are many treatments which include surgery, chemotherapy, radiation therapy, targeted therapy, or a combination of these treatments. Small-cell lung cancer is typically treated with Palliative care, radiation therapy, immunotherapy and chemotherapy (Fitzgerald *et al*., 2020; Gesthalter and Symth, 2022; Aiad *et al*., 2022) as depicted in **Fig. 14(a).**

Lung cancer can be detected, diagnosed and staged using various techniques. There are also treatments specific to each stage (Gomez *et al*., 2019).

Chemotherapy is frequently used to prevent tumor growth before surgically removing the tumor. As a result, chemo-radiation will be used to avoid the bed cover and kill the cancer cells, followed by surgery, which will be required if the cancer has spread to the lungs (Grassberger *et al*., 2016). Significant advancements have been in using nanotechnology to treat cancer (Siddique and Chow, 2022). Compared to traditional agents, nanostructured methods benefit from being more selective. Because of these developments, nanomedicine, a cutting-edge field of cancer treatment, has emerged. New therapeutic options for lung cancer have been made possible by a variety of formulations based on nanocarriers, including dendrimers, liposomes, nanoparticles, lipids, and polymers.

The use and development of nano-agents have ushered in an exciting and challenging research era for pharmaceutical science, notably for the delivery of innovative anti-cancer therapies (Terwoord *et al*., 2022; Gholami *et al*., 2022). Researchers use new imaging methods, such as Real-time Tumor Imaging four-

dimensional computed tomography (4DCT), to enhance treatment, are being used by researchers to enhance treatment. This procedure uses a CT scanner to scan the chest constantly for roughly 30 seconds. Instead of just capturing a moment, as a typical CT does, when a person breathes, it shows where the tumor is about other organs. Using this method, clinicians could determine whether a patient is a candidate for surgery by determining whether a tumor is adhering to or attempting to penetrate significant chest tissues (Wit *et al*., 2022). Many other techniques are used for lung cancer treatment like targeting therapy drugs (sorafenib, cabozantinib, sunitinib, and vandetanib), stereotactic radiotherapy, CCRT, Immunotherapy, Palliative care etc (Patil and Mahajan *et al*., 2022) as shown in **Fig. 14(b).**

2.2.2 Lung cancer causes

Tobacco Consumption

The use of tobacco is a global public health crisis and an epidemic. Around 250 million women and 1 billion men smoke worldwide (Lando *et al*., 2010). Most industrialized nations see a decline in tobacco use, but global consumption is increasing overall, with around 5.5 trillion cigarettes smoked per year due to widespread use, financial growth and population increase (Hecht, 2003). Over the past 50 years, lung cancer's histologic characteristics have transformed in developed nations. Most human cancers, particularly lung cancer, have been linked to environmental factors like passive smoking, air pollution, smoking, exposure to alcohol consumption and carcinogenic chemicals, etc. (Boffetta, 2006; Lichtenstein *et al*., 2000; Kanwal *et al*., 2017).

In USA as well as other parts of the world where tobacco use is daily, cigarette smoking is the primary cause of all four forms of cancer related to lung (Peto *et al*., 2000; Khuder, 2001). The World Health Organisation predicts that by 2020, there would be more than 12 million annual fatalities from smoking and 15 million newly diagnosed instances of cancer due to the rising adoption of unhealthy lifestyles (WHO, 2020). Numerous compounds including nicotine and carcinogens are found in tobacco. Every year, thousands of people die from cancer around world owing to deadly to mix of nicotine addiction and toxin exposure that tobacco products bring (Hecht and Hatsukami, 2022). Multiple carcinogens, including the tobacco-specific nitrosamine (N′-nitrosonornicotine) which is probably the source of the frequently seen oral cavity malignancies in South-East Asian countries, are exposed to users of

the wide variety of smokeless tobacco products (Hecht and Hatsukami, 2022; Henly *et al*., 2022).

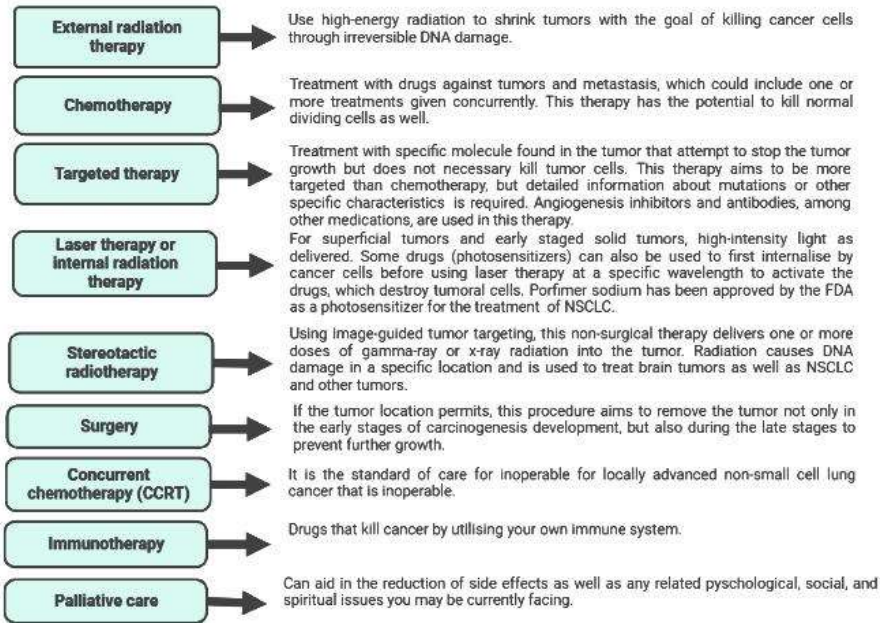


Fig. 14: (b) Illustration of various methods employed for the treatment of lung cancer prevention (created on www. Biorender.com)

The various carcinogens present in cigarette smoke create complicated alterations in essential cancer genes. Smoking continues to expose users to high levels of carcinogens like polycyclic aromatic hydrocarbons (PAHs), volatile chemical compounds, and nitrosamines unique to tobacco (Geneste *et al*., 1991; Li *et al*., 2022; Zhang *et al*., 2023). Cigarettes contain a high concentration of carcinogens, like inorganic compounds, hydrogen cyanide, metal/metalloids, aromatic amines, arsenic, aldehydes, nicotine, benzene, ammonia, lead chromium, radioactive elements (Polonium-210) (Hoffmann *et al*., 2001; Yu *et al.*, 2014; Kim *et al.*, 2020; Li *et al.*, 2022) as shown in **Table 4.**

2.2.3 Polycyclic aromatic hydrocarbons (PAHs)

Organic substances with two or more fused ring structures are referred to as PAHs (polycyclic aromatic hydrocarbons). They are very lipophilic while having poor water solubility. Low vapour pressure causes the bulk of PAHs in the air to be deposited in particles. Polycyclic aromatic hydrocarbons are created when organic materials such as tobacco, coal, petrol, oil, wood, rubbish or other organic components

burn partially. Exposure of PAHs to humans takes place through diet, tobacco use, pollution and occupational exposure.

It has immunosuppressive, genotoxic, teratogenic, mutagenic and carcinogenic effects. Previous research has shown that PAHs can disrupt key neuronal development processes like apoptosis, myelination and synaptogenesis (Nicol *et al*., 2001; Ball and Truskewycz *et al*., 2013; Lin *et al*., 2018). Besides the various health problems (eye, skin effect, nausea, vomiting, etc) orally ingested PAHs can cause toxicity and affect the pharmacokinetic properties of most therapeutic agents (Antiarrhythmic, Antimalarial, Antipsychotic, Antischizophrenic, Tricyclic antidepressant, Anesthetic, Ropivacaine Analgesic and antipyretic); illustrated in **Fig. 15.** There are many hundreds of PAHs, most of which are complicated combinations rather than single molecules.

Table 4: Different chemicals of smokeless tobacco products with the presence of carcinogens.

Smokeless Tobacco Products	Representative Carcinogens
Tobacco-specific nitrosamines	N'-nitrosonornicotinne (NNN)
	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)
Volatile N-nitrosamines	N-nitrosodiethanolamine (NDELA)
	N-nitrosodimethylamine (NDMA)
	N-nitrosomorpholine (NMOR) N-nitrosopiperidine (NPIP) N-nitrosopyrrolidine (NPYR)
Nitrosamino acids	N-nitrososareosine (NSAR)
Polycyclic aromatic hydrocarbons (PAHs)	Benz[a]anthracene (BaA)
	Benzo[a]pyrene (BaP)
	Benzo[b]fluoranthene (BbF)
	Benzo[j]fluoranthene (BjF)
	Benzo[k]fluoranthene (BkF) Dibenz[a,h]anthracene (DBahA) Dibenzo[a,i]pyrene (DBaiP) Indeno[1,2,3-cd]pyrene (IcdP)
	5-methylchrysene (5MC) Napthalene (NAP)
Aldehydes	Acetaldehyde Formaldehyde

Metals/Metalloids	Arsenic Beryllium Cadmium Cobalt Chromium VI Lead/Inorganic lead compounds Nickel compounds Polonium-21 (radioactive element)
Inorganic compounds	Nitrate (under conditions resulting in endogenous nitrosation)
Plant material	Areca nut Betel quid (with or without tobacco)
Fermentation-related compound	Ethyl carbamate (urethane)

It is produced significantly during incomplete combustion processes such as fuel combustion and cigarette smoking. Various forms of cancer are caused by a wide variety of chemical carcinogens found in tobacco products. (Hoffmann *et al*., 2001; Pfeifer *et al*., 2002) as shown in **Fig's. 10(a) and (b).**

The most significant carcinogens that have been proven in animal models and positively identified in cigarette smoke is polycyclic aromatic hydrocarbons (benzopyrene), tobacco-specific nitrosamines and aromatic amines like 4-aminobiphenyl (Stepanov *et al*., 2010; Xue *et al*., 2014; Hayakawa, 2022).

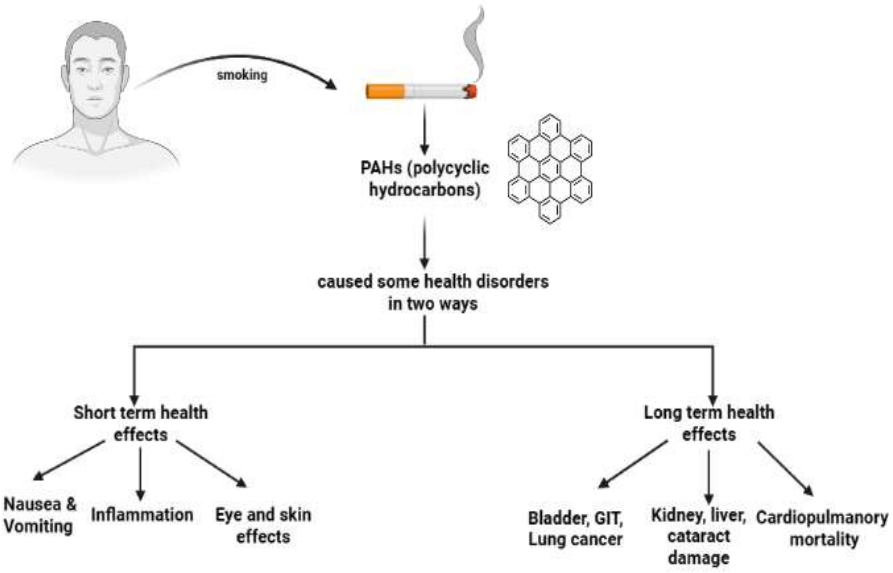


Fig. 15: Pictorial representation of polycyclic hydrocarbons (PAHs) and its effects (Prepared from www.Biorender.com)

B[a]P has been recognized as a marker of the carcinogenic potency of air pollutant mixtures, and it is frequently used as a positive control in biological assays for other PAH individuals in many studies. When estimating the potential exposure-related health effects, B[a]P has been considered more relevant than other species (Hayakawa, 2022; Stojic *et al*., 2022).

Benzo[a]pyrene($C_{20}H_{12}$) is a chemical having a polycyclic aromatic (pentacyclic, five fused benzene rings) hydrocarbon that comes from incomplete combustion of organic matter subjected to insufficient burning at temperatures ranging from 300°C to 600°C (Lehr *et al*., 1977; Wislocki *et al*., 1978) as depicted in **Fig. 16(a).**

2.2.3.1 Benzopyrene [B(a)P] (Tobacco metabolite)

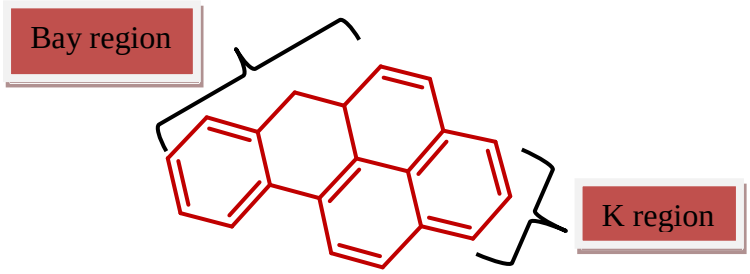


Fig. 16: (a) Structure of Benzopyrene

It is Pale-yellow in color, solid with an aromatic odor and a relative molecular mass of 252.31 (Chandrashekar, 2022). This compound is found in coal tar, car exhaust, grilled meats, oil, gas products, oil, many foods, especially charred & tobacco smoke (Hoffmann *et al*., 2001; Lee and Shim, 2007; Shi *et al*., 2017). B[a]P exposure is linked to a variety of toxic effects, including mutagenic, carcinogenic, lethal, developmental, oxidative, inflammatory, and neurological effects (Penning *et al*., 1999; Zhao *et al*., 2021).

When administered through additional routes, such as intraperitoneal, intrapulmonary, transplacental, intravenous, and subcutaneous, it can cause tumors in animals *in vivo* and *in vitro* investigations making it one of the most potent and human genotoxic carcinogens (IARC Group 1) (Liu *et al*., 2010; Poon *et al*., 2014).

After some organic substance burns, benzo[a]pyrene is created, which is then consumed and breathed. B[a]P) is metabolically generated by activated cyt-P450, and then it is successively transformed to (7,8-diol-9,10-epoxide-benzo[a]pyrene) a

cancer-causing derivative. Its diol epoxide metabolites (BPDE) react with each other and bind to DNA, causing mutations and finally cancer (Gelboin, 1980).

2.2.3.2 Benzopyrene [B(a)P] mode of action in lungs through tobacco smoke

The most potent carcinogen present in cigarettes is B(a)P, a PAH (Kasala *et al.*, 2015). It can directly bind to the agonist-acetylcholine receptor (AChR) in the cytosol, which activates it, and then move to the nucleus where it binds to DNA and induces transcription of numerous genes, such as CYP1A1, which is involved in metabolism and causes somatic mutations, DNA adduct formation, and induction of cytochrome P450 1A1 (Whitlock *et al*., 1999).

AhR directly or indirectly leads to the formation of ROS and free radicals. Cytochrome P450 pathway in the cells (Burczynski and Penning 2000; Loughran *et al.*, 2001). ROS levels are very important as its low level in utilised in redox signaling to initiate physiological processes, while high levels cause oxidative stress including DNA, lipids and protein damage (Toyokuni *et al*., 1995; Schieber *et al*., 2014).

B(a)P can also bind directly to DNA and may cause defects in those genes involved in tumor suppression thereby causing many diseases like lung cancer, genomic instability, loss of normal cellular growth mechanism and chronic inflammation (Jahangir and Sultana, 2013; Basu, 2018) as illustrated in **Fig. 16(b).**

Its diol epoxide compounds, benzo[a]pyrenediol epoxide (BPDE), react and bind to DNA, causing mutations and cancer. The primary cancer-causing substances found in tobacco smoke are carcinogenic PAHs and tobacco-specific nitrosamines, which are produced in the 900°C combustion environment at a lit cigarette tip when a smoker puffs (Sasco *et al*., 2004; Vargas and Harris, 2016).

After PAHs are bioactivated, mainly by the CYP mixed function oxidase system, their electrophilic byproducts covalently link to nucleophilic sites on macromolecules, like DNA bases, to create clumpy chemical oxidative DNA damage.

The pro-carcinogen B[a]P and its significant DNA adduct r7,t8,t9-trihydroxy-c-10 (BPdG) are shown in **Fig. 16(b)** (Alexandrov *et al*., 2010; Johnson *et al*., 2021). Because DNA replication of damaged DNA results in mutations at the locations where DNA adducts are produced, the formation of PAH-DNA adducts may lead to TP53 (G-T transversions).

Smokers lung tumors frequently contain mutation at-the-codon in TP53 (G-T transversions) (a hotspot for mutation induction), whereas non-smokers do not (Wood *et al.,* 2018; Barnes *et al.,* 2018; Davies *et al.*, 2020). There is a strong correlation between lung carcinogenesis and cigarette-smoke-induced chronic inflammation of airways (alveoli).

ROS levels in lung epithelial cells are altered and various signaling pathways associated with onset and progression, and overproduction of ROS in the presence of environmental toxins like tobacco smoke, invading growth factors, genetic instability and induced DNA damage as shown in **Fig. 16(b).**

2.4 Chemoprevention

Utilizing chemicals to prevent and postpone the development of a tumor (Surh *et al*., 2003; De flora and Ferguson, 2005; Ma *et al*., 2021). The best example of chemoprevention is lung carcinogenesis, detected most frequently in elderly people and has a sluggish growth and development rate as illustrated in **Fig. 11** and **Table 5(a) and (b).**

Chemoprevention is a rising area of cancer research, emphasizing diverse interventions such as biological, pharmacological, and dietary therapies (Bonovas *et al*., 2008).

Depending on the stage at which they act, chemoprevention involves one of three different strategies: primary, secondary or tertiary (Swetha *et al*., 2022; Gezici, 2022). Primary chemopreventives are designed to include interventions intended to prevent tumor formation in healthy individuals, such as genetic predisposition in a vulnerable population. Secondary chemoprevention is primarily used to treat premalignant tumors to stop tumorigenesis (e.g. lung metaplasia, colon polyps, cervical dysplasia etc).

Tertiary chemoprevention reduces the likelihood of tumor recurrence after a successful surgical and chemotherapeutic intervention. Despite significant advances in lung cancer therapy, such as chemotherapy, radiotherapy, targeted therapy and immunotherapy, the average survival rate in lung cancer is the lowest (Tang *et al*., 2022; Lahiri *et al*., 2023).

These agents also also inhibited angiogenesis; scavenged free radicals, reduced proliferation, and enhanced PCD, slowed down cell invasion/progression, retard certain cell signal transduction pathways, and metastasis (Kuroda and Hara, 1999; Phan *et al*., 2008; Yang *et al*., 2009; Sharma *et al*., 2019). At least 40% of alternative treatments such as herbal medicine, were employed in the US to treat or prevent disease (Gordaliza, 2007; Ma *et al*., 2022).

More than half of currently accessible pharmaceuticals are natural remedies or are linked to them and historically medicinal botanicals have served as an inspiration for the discovery of innovative pharmacological agents as part of complementary medicine in USA.

Majority of the licensed anticancer medicines, including podophyllotoxin and retinoids appear to be natural substances or natural product derivatives produced from herbal medicine, according to significant epidemiological evidence.

Additionally, a variety of dietary and botanical natural products with the capacity to control physiological processes such as alkaloids, phenolics, carotenoids, flavonoids, organosulfur compounds and gingerols have been demonstrated to constrain carcinogenesis (Vallinas *et al*., 2013; Hosseini and Ghorbani, 2015; Sharma and Goyal, 2015).

Almost 11 million patients worldwide are provided with anticancer doses each year and 6.7 million of them pass away as a result. Globally lung, stomach and liver cancers have the greatest fatality rates of any malignancy whereas breast, lung and colorectal cancers are the most often diagnosed types.

A different and unique herbal way of treating cancer is the need of hour considering the drawbacks of modern cancer treatments such as the decline in patient quality of life, elevated mortality rate and disease severity as a result of therapy (Brustle and Heidecker, 2017; Deng *et al*., 2021).

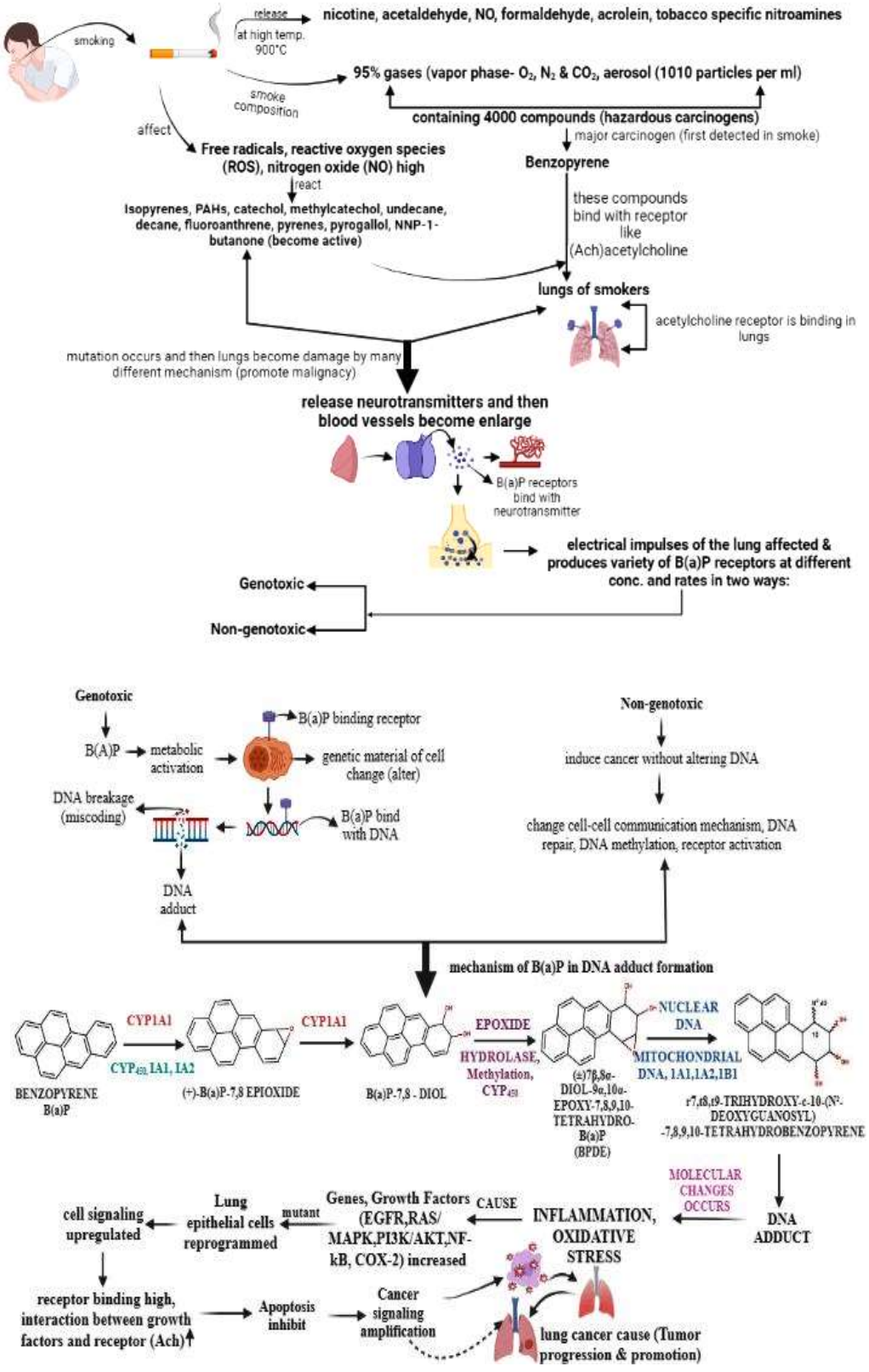


Fig. 16: (b) Illustration of the Mechanism of Benzopyrene in Smoking (created in www.biorender.com)

Recently, the topic of cancer chemoprevention has gained attention in these regimens. Natural or artificial substances are used to reduce, stabilize and reverse precancerous lesions as depicted in the **Fig. 17.** Numerous dietary phytochemicals obtained from food, vegetables, fruits, and tea may show promising results in cancer prevention (Shukla and Pal, 2004; Probst *et al.*, 2017; George *et al.*, 2021; Paskeh *et al.*, 2021). Natural products, in general have been the most significant source of drugs in science, and for cancer, it is not different. They contain very good amount of compound having application in the number of grounds like medicine, biochemistry and pharmacy (Karikas, 2010; George *et al*., 2021; Ma *et al*., 2022). These agents may be natural or made in a laboratory (synthetic) as shown in **Table 5(a) and (b).** A doctor uses chemoprevention to lower cancer developing risk (Surh, 2003; George *et al*., 2021; Ma *et al*., 2022) e.g:

Table 5(a) Synthetic agents (drugs) and their effect on different types of cancer.

Drug Name (Chemopreventive Agent)	Type of Cancer
Tamoxifen (Soltamox)	reduced breast cancer
Raloxifene	Reduction in tumor incidence (Breast cancer)
Aspirin	Reduction of risk factor in different types of cancers
Non-steroidal anti-inflammatory drugs	Reduction of risk factor in different types of cancers

Certain foods or minerals, other nutrients, and vitamins, they contain may increase or diminish the cancer risk (Surh, 2003; George *et al*., 2021; Ma *et al*., 2022) in the **Table 5(b)** e.g

Table 5(b) Natural agents (phytochemicals) and their effect on different types of cancer.

Plant-Based Foods (Chemopreventive Agent)	Type of Cancer
Carotenoids or carotene (yellow, orange, red, and some dark-green vegetables	Lower the risk of head, neck, esophagus, stomach, lung, pancreatic, prostate, mouth & throat cancer
Polyphenols (herbs, chocolate, tea, spices, nuts, apples, onion, berries, and other plants)	Lower the risk of neck, esophagus, head & stomach cancer
Allium (Chives, leeks, garlic, and onions)	Lower the risk of neck, esophagus, head &stomach cancer
Green tea extract	Lower the risk of breast cancer

The importance of dietary phytochemicals with chemopreventive properties is significantly increased. Several plant phytochemicals have anticancer activity against B(a)P-induced pulmonary cancer while targeting the cancer-related pathways (Garg *et al*., 2008; Rajendran *et al*., 2008) as shown in **Fig. 17.**

The polyphenols in black tea affect multiple targets, which may explain why it has cancer-fighting properties. The table lists various dietary phytochemicals/whole extracts and beverages that act as chemopreventive agents against lung tumorigenesis as seen in **Table 5(a) and (b).**

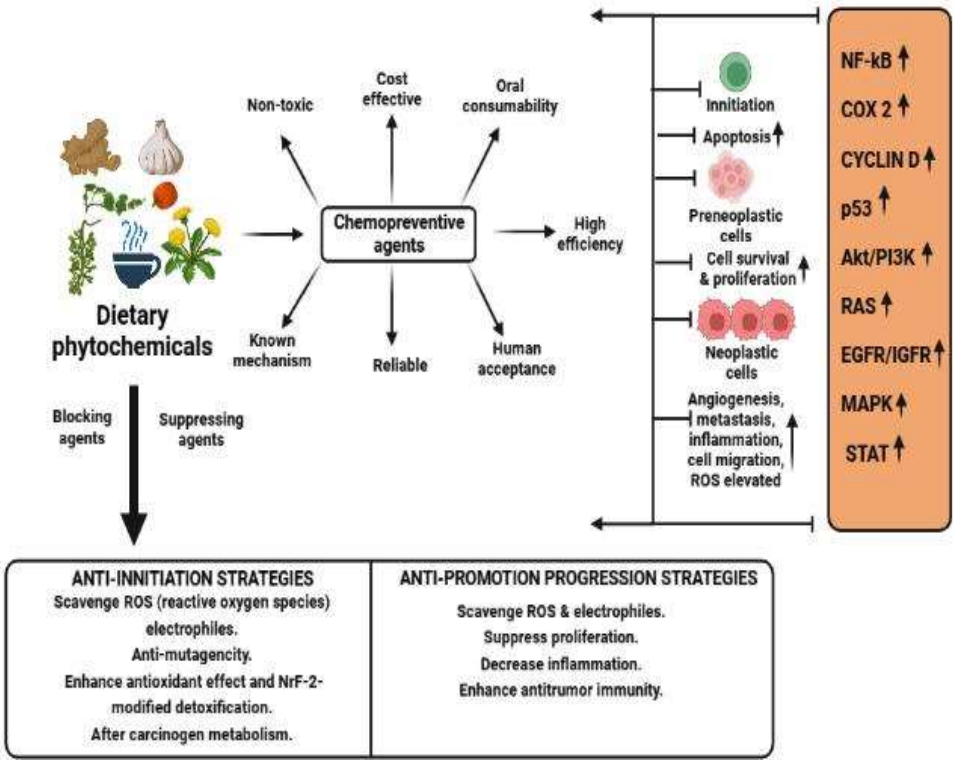


Fig. 17: Pictorial representation of chemopreventive properties of dietary phytochemicals and their role

2.5 Tea and its manufacturing steps

The most popular beverage on the planet, tea (*Camellia sinensis*, Family: Theaceae), is served in various ways (Wei *et al*., 2018; Abudureheman *et al*., 2022). Main types of tea include Assamese variety *Camellia sinensis assamica* and the Chinese variety *C.s. sinensis* (Labbe *et al*., 2006; Tounekti *et al*., 2013; Kumar *et al*., 2018). It can reach a height of 30 feet in acidic soil at 2460 m and a temperature range of 13 to 29 degrees Celsius (Singh *et al*., 2017). Consuming without milk or sugar is energizing, quenching, relaxed, natural, and almost calorie-free. Tea trees first appeared 60–70 million years ago in southern China.

It became a refreshing drink for the first-time during China's ShenNong period (about 2737 BC). The most consumed and least cost artificial non-alcoholic liquid refreshment worldwide, excluding plain water, is tisane. As a result, tea promotes global economic growth. The value of the worldwide tea market was predicted to be close in 2020 (USD 200 billion) and to surpass by 2025 (USD 318 billion) (Pan *et al*., 2022).

Table 6: Animal models were used to study the effects of dietary phytochemicals/whole extracts/beverages or other herbs on B(a)P or another chemical-induced lung cancer.

Dietary phytochemical/whole extract	Animal species/age/ duration of time	Drug name	Dose of phytochemical& drug/administration route	The method of action	Reference
GT & BT infusion (aqueous)	**Species:** Female A/J mice **Age:** 8 weeks **Time:** 16 weeks	NDEA, NNK	**Dose:** 10mg/kg- NDEA 103mg/kg- NNK GT – 1.25% BT – 0.6% **Route of administration:** Orally	Revealed inhibitory action against tumor multiplicity in mice lungs.	Wang *et al.*, 1992
BT phytoconstituentsTFs (aqueous)	**Species:** Female A/J mice **Age:** 6 weeks **Time:**	NNK, BrDU	**Dose:** 103mg/kg b.wt. TF – 0.3% **Route of administration:** Intraperitoneal	Multiplication suppression in lung adenomas.	Yang *et al.*, 1997
GT & BT (aqueous) extracts	**Species:** Female A/J mice **Age:** 6 weeks **Time:** 60 weeks	Spontaneous tumors	**Dose:** 0.5% BT & 1% GT in distilled water **Route of administration:** Orally	BT showed inhibition in the formation of rhabdomyosarcomas and lung tumors.	Landau *et al.*, 1998
EGCG & caffeine (aqueous) – GT and BT	**Species:** A/J mouse and (F344) rat **Age:** 6-8 weeks old **Time:** 18 weeks	NNK	**Dose:** 11.7mg/kg b.wt. BT & OG – 2% Caffeine – 680 ppm **Route of administration:** Chronic	The active components of GT (EGCG), BT (TF) & caffeine expressed chemopreventive potential	Chung, 1999

				against lung cancer.	
BT(aqueous) extract	**Species:** Swiss Albino mice **Age:** 6-8 weeks old **Time:** 28 weeks	DEN	**Dose:** 20mg/kg b.wt. in 0.2 ml distilled water BT – 1,2 % 4% **Route of administration:** Oral intubation	Reduction in DEN-induced tumor development	Shukla *et al.,* 2002
BTPs (TF)& EGCG	**Species:** Newborn strain B mice **Age:** 24-48h old **Time:** 26 weeks	BrDU, B(a)P	**Dose:** 0.2mg/newborn mouse BT – 1.5% **Route of administration:** 1% aqueous gelatin solution was injected subcutaneously	Expressed inhibition against B(a)P TF& EGCG can play a protective role against lung carcinogenesis .	Banerjee *et al.*, 2006
PBPs& DBTE	**Species:** A/J - male mice **Age:** 6-8 weeks old **Time:** 2 weeks	GTA, B(a)P	**Dose:** B(a)P (3µM/0.1ml)/glyceryl trioctanoate PBPs – 1% DBTE – 2.5% **Route of administration:** Intraperitoneal injection	Significant depletion in liver and lung CYP1A1, CYP1A2 enzyme protein levels that are caused by B(a)P.	Krishan *et al.,* 2005
BTP Polyphenon B	**Species:** Male Wistar rats **Age:** 6-8 weeks **Time:** 26 weeks	MNNG	**Dose:** 0.05% polyphenon-B + MNNG (150mg/kg b.wt.) Polyphenon B – 0.05% **Route of administration:** Intragastric	Showed chemopreventive effects with normalization of cellular redox status, decreased angiogenesis, cell	Murugan *et al.*, 2007

			intubation	proliferation & induced apoptosis.	
Hesperidin	**Species:** Swiss albino mice **Age:** 6-8 weeks **Time:** 16 weeks	B(a)P	**Dose:** B(a)P – 50mg/kg b.wt. Hesperidin – 25mg/kg b.wt. **Route of administration:** Orally	Hesperidin revealed its antiproliferative and chemopreventive effect against lung cancer in mice.	Kamaraj *et al.*, 2009
Fisetin	**Species:** Swiss albino mice **Age:** 6-8 weeks **Time:** 16 weeks	B(a)P	**Dose:** B(a)P – 50mg/kg b.wt. Fisetin – 25mg/kg b.wt. **Route of administration:** Orally	Fisetin significantly reduced the histological lesions, benzopyrene effect and depicts as a chemopreventive- lung cancer	Ravichandran *et al.*, 2011
BTPs & Res	**Species:** Male, Balb/c mice **Age:** 6-8 weeks **Time:** 26 weeks	TPA, DMBA	**Dose:** TPA- 5µg/animal DMBA- 52µg/animal BTPs – 0.1% Res – 25 µM/ animal **Route of administration:** dorsal skin-interscapular region (i.p)	Resveratrol& BTP expressed synergistically reduction in the cumulative numbers of tumors in the lungs.	George *et al.*, 2011
BT&Curcumin	**Species:** Sprague Dawley rats **Age:** 7 weeks **Time:** 90 days	AFB	**Dose:** 750mg/kg b.wt. BT – 2% Curcumin – 200mg/kg b.wt. **Route of administration:**	BT&Curcumin in combination inhibit biliary proliferation and repair distorted	Eldeen *et al.*, 2015

			Intragastrically through a stomach tube	hepatic lobules in the liver.	

GT = Green Tea, BT = Black Tea, NDEA = N-nitrosodiethylamine, NNK = 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone, TF = Theaflavins, BrDU = Bromodeoxyuridine, EGCG = Epgallocatechingallate, DEN = Diethylnitrosoamine, BTP = Black tea Polyphenols, PBP = Polymeric Black tea Polyphenols, DBTE = Decaffinated Black tea, MNNG = N-methyl-N'- nitro-N-nitrosoguanidine, Res = Resveratrol, TPA = Tetradecanoylphorbol 13-acetate, DMBA = 7,12-dimethylbenz[a]anthracene, AFB = Alfatoxin B

Tea is now popular as liquor among over 3 billion people in 160 countries and regions. Today, there are hundreds of different types of tea, with many countries constantly developed tea plants and processing techniques (Saberi, 2010; Li *et al*., 2022). Based on their production process, tea is traditionally classified into six categories: white, oolong, green, yellow and black tea (Saberi, 2010; Zhang *et al*., 2019; Li *et al*., 2022). This classification relies on the number of polyphenols, physical attributes and organoleptic qualities as a result of chemical modifications produced to polyphenols during processing (Roshanak *et al*., 2016; Wong *et al*., 2022). Lipids, carbohydrates, minerals, theobromine, theophylline, caffeine (alkaloids) and flavonoids, catechins (polyphenols) are known to present in tea as illustrated in **Fig. 18** and **Table 7**. The quality, richness, taste, flavour, and health advantages of different teas are aided by these ingredients (Kumar *et al*., 2018; Wang *et al*., 2019; Chaudhari *et al*., 2020; Wong *et al*., 2022). Tea flavor developments have shifted towards time-saving, portability, real-time monitoring, and visualization to better understand how different processing methods affect the formation and changes of flavor compounds, particularly desired and off-flavor compounds present in (ultra) trace amounts in tea products (Zhai *et al*., 2022).

Catechins and theaflavins are main secondary metabolite present in tea. These two compounds possess good antioxidant properties. Subsequently, these compounds help to counter harmful radicals present in the body, which ultimately reduces oxidative stress related ailments like diabetes, cardiovascular problems, various malignancies, etc, as shown in **Table 6.** Besides, it also has various health promoting benefits such as weight management, better dental health, anticarcinogenic, arteriosclerotic and better bone health (Adnan *et al*., 2013; Tang *et al*., 2019; Prasanth *et al*., 2019; Pan *et al*., 2022).

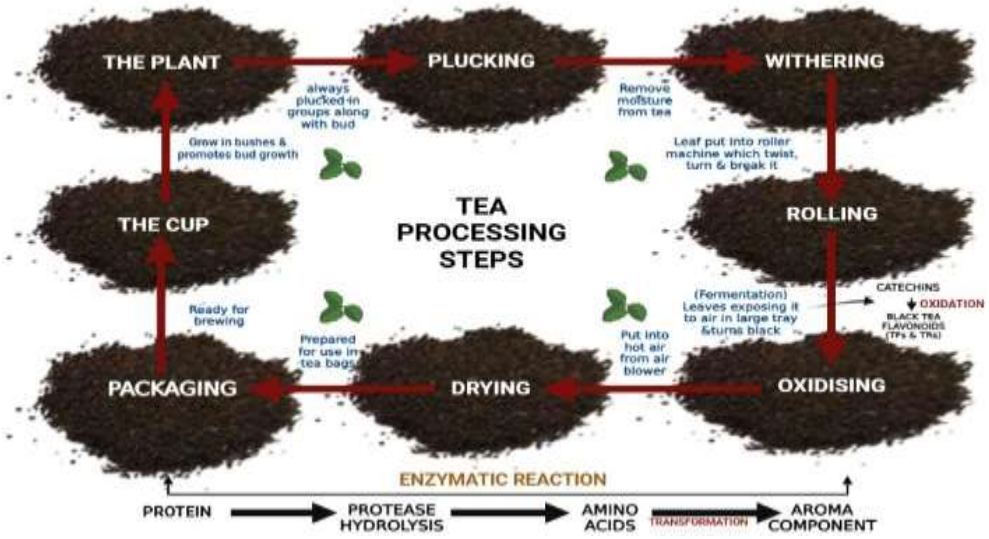


Fig. 18: Pictorial representation of different steps involved in manufacturing of Tea (created in www.Biorender.com)

Interest in tea research has risen significantly recently because of its possible health advantages (Khan and Mukhtar, 2018). This classification is influenced by the distinctive characteristics of each tea (color, flavour etc.) and the modifications made to the materials during the tea processing. The different processes/methods of manufacturing give the various teas their characteristic colors and flavors, as illustrated **Fig. 18 and Table 7.**

Table 7: Tea and its cultivation &manufacturing process in different steps.

Methods of Tea Processing	Working Technique
THE PLANT	If left unattended, the huge fields of tea plants can reach heights of 20 meters (65 feet), however, the bushes are typically clipped during hand-picking to encourage bud growth (Smith, 2016; Karlson *et al.*,2022; Pan *et al.*, 2022).
PLUCKING	The bud that forms at the stem's end as well as any individual leaves are always plucked off the plant in groups of one, two, or three leaves (Takeo *et al*., 1992; Hazra *et al*., 2019; Lin *et al*., 2013).
WITHERING	Freshly harvested leaves are spread out for 14 to 18 hours

	(the ideal time) to wither on huge troughs or shelves. Air is frequently passed through to aid in moisture removal, and the leaves appear wilted after withering (Deb and Jolvis Pou, 2016; Tehsome, 2019).
ROLLING	The leaves are disintegrated, and enzymes are released to get ready for oxidation. There are two rolling techniques: traditional, where leaves are gently broken by rollers, and CTC-cut, tear, curl, when leaves are mechanically cut (Hilal and Engelhardt 2007).
OXIDIZING/FERMENTATION	The rolled, withered leaves are left outside for a few hours to oxidize, which means they start fermenting when they come into contact with oxygen. Chemical reactions break down the leaves to some extent (Teshome, 2019; Long *et al.*, 2023).
DRYING	The oxidation processes are then stopped just in time with drying, giving the tea its ideal flavor. To remove any excess moisture, the oxidized leaves are gently heated (Sharma *et al.*, 2018). In general, drying black tea at 110°C with a dryer speed of 1.5 rpm results in high-quality tea (Teshome, 2019).
PACKAGING	The size of the dried leaf determines the grade of the tea after drying. Smaller leaves are prepared for use in tea bags, while larger leaves are offered loose for tea. Tea should be wrapped in food-safe materials (Rana, 2022).
THE CUP (READY TO DRINK)	The tea is now ready to be brewed. Tea leaves that have been dried impart a subtle flavor to hot water that is determined by the growing environment and painstaking preparation method. Start the kettle (Wong *et al.*, 2022).

The tea plant is known to be grown in almost thrity countries and the total amount for human consumption and produced is black (80%), green (20%), and oolong tea (2%) (Sinha *et al*., 2010; Qasim *et al*., 2017; Feng *et al*., 2019; Wong *et al*., 2022).

2.5.1 Black Tea

Black tea is mostly produced and consumed in India. The fresh young fragile *C. sinensis assamica* leaves are harvested, withered, macerated (rolled), fermented and dried to create the most well-known refreshing beverage (Pincemaille *et al*., 2014; Guleria *et al*., 2022) as depicted in **Fig. 6 and Table 7.** The most popular type of tea on earth is black tea, which comes in a variety of flavors (Ruxton, 2008; Basu, 2010; Guleria and Sehgal, 2022). The leaves cell structure is damaged during withering because the leaves take on a form that facilitates the maceration process. The brewing process then begins. Tea leaves contain catechins which are transformed into theaflavins and thearubigins by an enzyme process that involves oxidation and polymerization to produce a complex combination of important secondary polyphenols (Takino *et al*., 1964; Nonaka *et al*., 1984; Abeywickrama *et al*., 2011; Qi *et al*., 2021), theasinensins (Kusano *et al*., 2008; Li *et al*., 2010), oolongtheanins (Finger, 1994; Kuhnert *et al*., 2010; Tanaka and Matsuo, 2020) that are mainly responsible for the astringent taste and coppery color of black tea (Guleria and Sehgal, 2022; Abudureheman *et al*., 2022) as illustrated in **Fig. 18 and Table 8**.

2.5.2 Chemical constituents

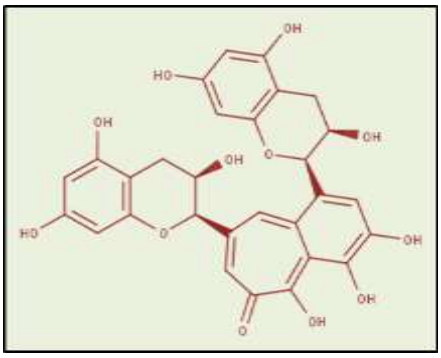
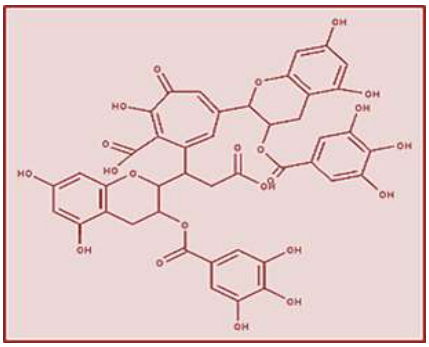
Black tea contains various chemical compounds such as carbohydrates, proteins, amino acids, vitamins, polyphenols (flavonoids, catechins, theaflavins and flavonols) and alkaloids (caffeine) as shown in the **Table 8.** Young tea shoots are high in polyphenolic compounds and catechins (most abundant and responsible for black tea pigment formation). Regarding black tea attributes, theaflavins (TF) is unique in predicting tea quality and account for 0.3 to 1.8% of the dry weight (Takino *et al*., 1964). TF (orange-red compounds) is formed during fermentation via polymerization of polyphenols, undertaken by the enzyme oxidase that gives the tea liquor its distinct briskness, color and strength (Obanda, 1997; Takino *et al*., 1964; Nonaka *et al*., 1983; Abeywickrama *et al*., 2011; Qi *et al*., 2021). Thearubigins (red-brown color) is a pigment derived from the oxidative degradation of theaflavins and catechins account for (10- 20% dry weight) of tea, are responsible for the color and the strength of the

beverage's flavor (Haslam, 2003; Bond and Derbyshire, 2020; Zhu *et al*., 2021) as illustrated in **Table 8.**

2.5.3 Health-promoting benefits

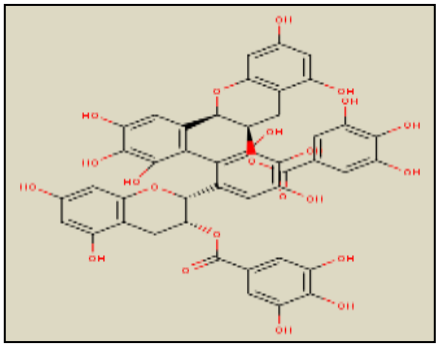
Polyphenols like theaflavins/thearubugins contain antioxidant properties thereby helping in number of ailments such as decreased cardiovascular diseases, inflammation, inhibiting the obesity by suppressing absorption and digestion of lipid and complex sugars, the elevation of lipolysis and down-regulated the lipid accumulation by reducing the accumulation of fat cells.

Table 8: Chemical constituents of Black tea.

Black tea composition	Structure
Theaflavins Theaflavin and its esters are the main sources of red color in black tea, which have various health promoting benefits, like glucose-lowering abilities and fat-reducing in nature. Moreover, it has been found to show anti-inflammatory, antibacterial, anti-obesity, anti-cancer, anti-dental, anti-osteoporotic, anti-atherosclerotic and antiviral properties.	
Thearubigins All high molecular weight polyphenols with a reddish-brown color and good water solubility are together referred to as "**thearubigins**" (TRs). It contains various therapeutic effects like antimutagenic, anti-cancer, and antioxidant effects on skeletal health, gastrointestinal motility and mitochondrial activation.	

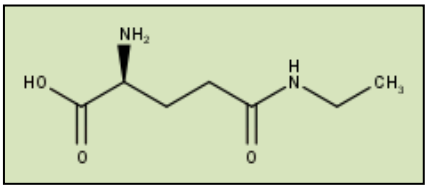
Thesinensins (TS)

Theasinensin A and B is polyphenol flavonoids or dimers of tea catechin which are produced from black tea (*Camellia sinensis*) and Oolong tea during the processing/fermentation-oxidation of epigallocatechin gallate.



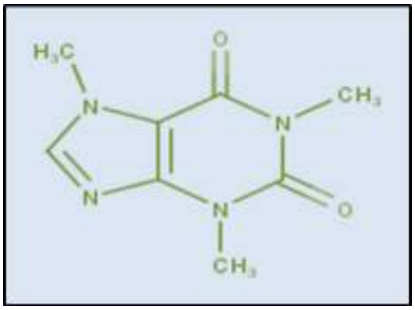
Oolongtheanins (Theanine)

Tea also contains unique amino acids which are called **oolongtheanins (L-theanine)** that contribute to its taste and may influence a variety of human brain functions and contains various health-promoting effects like reducing stress/hypertension, hepatoprotective effects and prevention of vascular diseases etc.



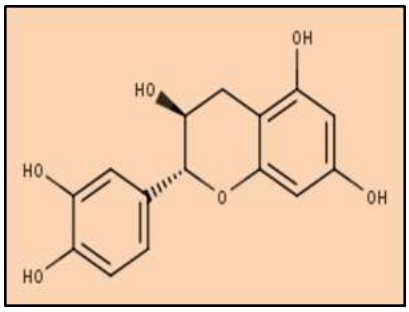
Caffeine

It is a natural chemical that regulates blood pressure by stimulating the muscles, heart and CNS and acts as a "water pill" that increases water flow. **The high content of catechin** is present in black tea.



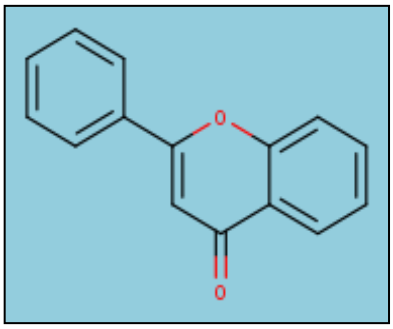
Catechins

Catechins are a type of phenolic compound and secondary metabolite that provides antioxidant roles in plants. **EGCG, EC, EGC and ECG** are found in tea.



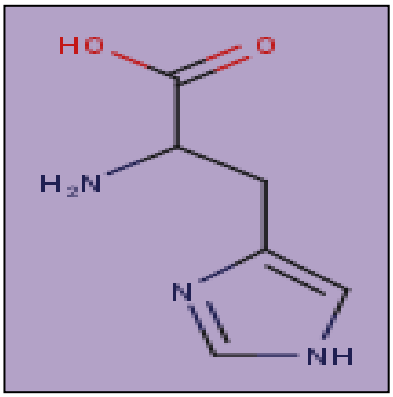
Flavonoids (flavons)

It is a class of organic compounds with varying phenolic structures that are present in grains, flower stems, etc. **70% of thearubigins** (flavonoids) present in black tea.



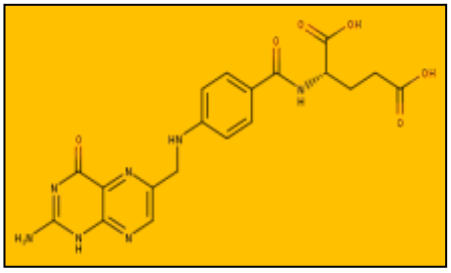
Histidine

Organic compounds that contains both carboxylic & amino functional groups. They help in nutrient absorption as well as tissue repair throughout the body. Black tea contains a higher amount of **aspargine, theanine and glutamate**.



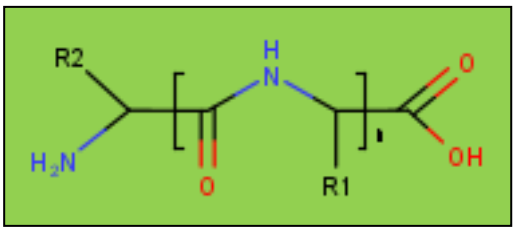
Nutrients (Folate)

Tea contains approx. **5-7% phosphorus, magnesium, calcium and mainly potassium**.



Proteins

Tea accommodates high amounts of around **21-28% protein**.

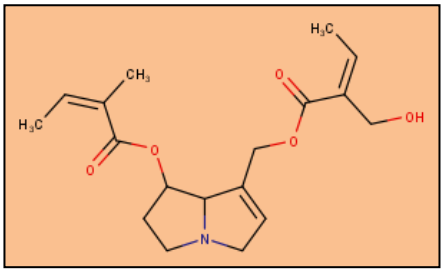


Carbohydrates

Stachyose, fructose, sucrose, raffinose & glucose (free sugars) are found in tea.

Alkaloids

Caffeine and theobromine were identified in tea.



Literature documented that drinking 2-3 cups of black tea on the daily shows the risk of stroke reduction and prevents unregulated cell communication and signaling pathways like epidermal growth factors, caspases etc (Hodgson *et al*., 2012; Rasheed, 2019; Wang *et al*., 2022) as illustrated in **Fig. 19.**

2.5.4 Anti-tumor and chemopreventive mode of action of Black tea and its bioactive compounds

Free radicals are known for their reactivity and potential for cell damage. They are created when an electron, a tiny negatively charged particle found in atoms, is gained or lost by an atom or molecule (a compound made up of two or more atoms) (Phaniendra *et al*., 2015; Charlesby, 2016; Alkadi, 2020; Unsal *et al*., 2021).

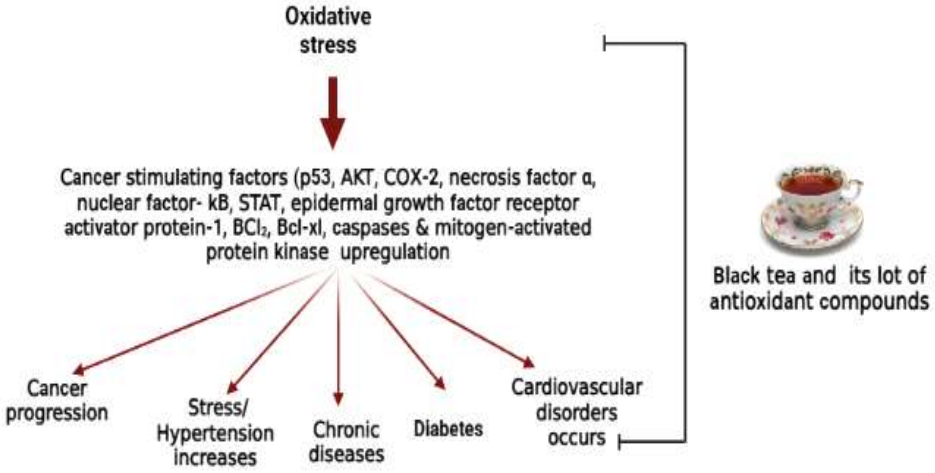


Fig. 19: Pharmacological and therapeutical properties of black tea and its mode of action (created on www.Biorender.com)

The body naturally produces free radicals crucial to some regular cellular functions. Ionizing radiation exposure, as well as other environmental contaminants (cigarette smoke, certain metals), can result in excessively high levels of radicals (oxidative stress), which harm all significant components of cells, including cell membranes, DNA and proteins (Suryadinata and Wirjatmadi, 2020; Hasan *et al*., 2022; Rudrapal *et al*., 2022). ROS damage the cells and contribute to cancer development and other conditions like vision loss, ailments to the heart, some chronic diseases, etc. Chemicals called antioxidants (free radical scavengers) neutralize these to stop them from harming (Singh *et al*., 2017; Parcheta *et al*., 2021; Zahra *et al*., 2021; Vinci *et al*., 2022).

To neutralise ROS, the body produces several endogenous antioxidants. The rest of the antioxidants the body needs, however, come from outside (exogenous) sources, chiefly the diet. Dietary antioxidants are another name for exogenous antioxidants (Zahra *et al*., 2021; Parcheta *et al*., 2021). Dietary antioxidants are abundant in grains, fruits, beta-carotene, lycopene, vitamins A, C and E and vegetables. Some dietary antioxidants can also be obtained as supplements. Any tissue or organ that naturally loses cells develops cancer, which is characterised by uncontrolled growth (Arias *et al*., 2022; Souri *et al*., 2022). Accumulating ROS harms the body's defense mechanisms, including enzymatic balance and DNA structure, triggering the cancer pathogenesis. Numerous animal and laboratory studies have demonstrated that raising exogenous antioxidant levels can halt the types of free radical damage that lead to the growth of cancer.

To prevent and advance cancer, antioxidants found in tea, fruits and vegetables are helpful. As a result, researchers have looked at whether consuming dietary antioxidant supplements can lower the likelihood that people will acquire cancer or pass away from it. Since their use has no adverse side effects, natural health product research and their molecular-level health advantages have gained widespread acceptance worldwide over the past 20 years (Qasim *et al*., 2017; Rasheed, 2019). According to earlier studies, black and green tea show effectiveness (Initiation, Progression and Metastases) in cancer (Mates and Jimenez, 2000; Pan *et al*., 2013; Ganguly *et al*., 2016; Rasheed, 2019). Aside from water, one of our most popular and consumed beverages, black tea is now available in markets all over the world in both unblended forms such as Darjeeling and Assam tea or blended with various other plant products to obtain different flavors like Chai, Bigelow and Earl Grey (Davies *et*

al., 2003; Chan *et al*., 2011; Singh *et al*., 2017). Numerous cancers are said to be prevented by good amound of bioactive compounds present in black tea, such as volatile chemicals, flavonoids, phenolics and alkaloids. These compounds control oxidative damage to biomolecules, endogenous antioxidants, as well as various pathways of mutagen (elevated transcription factors like Akt, EGFR, STAT, JNK etc.) and transcription of the antioxidant gene (Baibado *et al*., 2011; Singh *et al*., 2017; Ihsan *et al*., 2018).

According to Singh *et al.*, 2017 regular drinking of black increases the protective antioxidant enzymes as well as suppressed several cell signaling pathways incremation which is responsible for developing cancer and blocking Wnt/β-catechin, cyclin D, molecular targets like COX-2, STAT, EGFR, Nf-kB, p27, FOX O1 and JNK that contribute to inducing arrest of cell cycle. Inflammation is significantly influenced by the inducible enzymes cyclooxygenase-2/nitric oxide synthase. Its activity is allied with proliferation and PCD prevention. Additionally, it is critical for cancer development in the lungs and may serve as a biomarker for tumor development (Subbaramaiah and Dannenberg, 2003).

There are several theories that tea has anti-cancer properties, including the fact that theaflavins' gallate structure is crucial for the chemicals' ability to inhibit proliferation (Bode and Ann, 2003; Steele *et al*., 2010). The following administration methods and dosages were used to assess anticancerous properties of black tea and its various constituents: 0.6-4% (BTE), diet (0.05% - polyphenon-B), caffeine (0.044-0.24%), TFs (360 ppm) and 0.02 mg (TFs) given intravenously (Kumar *et al.*, 2010). It should be emphasized that anticancerous activity was noticed at doses (per kg BW) far higher than those typically consumed by people (Yang *et al*., 2000; Krishnan *et al*., 2006; Kumar *et al*., 2010).

Apoptosis- critical in removing the mutated hyperproliferating cells from the system. Apoptosis which involves many complex pathways and enzymes, is ideal function for cell survival and proliferation. Carcinogenesis is closely linked to the proliferation of abandoned cells due to the absence of PCD (Gusman *et al*., 2001). Thus, inducing apoptosis in tumor cells may be regarded as a defense barrier against cancer development and progression. Polyphenols and catechins found in black tea and green tea, respectively documented to inhibit tumor growth *in vitro*/*in vivo* (Yang *et al*., 1999; Lu *et al*., 1997; Ahmad *et al*., 1997; Yang *et al*., 2000; Halder *et al.*,

2008). Theaflavins and thearubigins are black tea's most exclusive polyphenols. Although there aren't numerous research studies that show black tea (theaflavins) and green tea (epigallocatechin gallate, epicatechingallate and epicatechin) extracts (hot aqueous) suppress neoplastic transformation in human lung tumour epithelial cells, rat tracheal epithelial cells, mouse mammary organ cultures, and other tissues (Steele *et al.,* 2000). The integrity of our DNA is crucial to our health, yet ROS can harm it. Guanine bases are the most susceptible of the DNA bases. Each human cell's DNA is thought to undergo 10,000 oxidations daily, and *in vitro* studies have discovered over 35 different forms of oxidized bases in DNA.

It is believed that some of the damaged DNA accumulates over time and some of it escapes repair, resulting in permanent damage. Carcinogenesis is heavily reliant on damaged DNA. Damaged DNA is vital in carcinogenesis because it affects gene expression, mitochondria/cytoplasm communication, and the cell cycle. This damage done to the DNA is known as Genotoxicity. Hydrogen peroxide (H_2O_2) is a common reactive oxygen intermediate produced by oxidative stress. H_2O_2 one of the most prevalent ROS that damage DNA in a range of cell types. H_2O_2 can directly interact with DNA, modulate transcription and suppress genomic repair pathways.

Numerous studies have shown that natural phenolic antioxidants can prevent oxidative DNA damage in two different ways: first, by scavenging the •OH radical before DNA damage occurs, and second, by preventing the DNA radicals that are created as a result of the •OH radical attack (Anderson *et al.,* 2001; Liu *et al*., 2017; Romero *et al*., 2018; Dar and Sehgal, 2020). Black tea showed maximum antigenotoxic activity as compared to OG, OS, OC & St while BTOGSt expressed the highest effect of antigenotoxicity as compared to other combinations (Guleria and Sehgal, 2022). Another study found that black tea's monomeric and dimeric flavonols (TFs and TRs) protected lymphocyte DNA against Trp-P-2-induced damage (Dhawan *et al.,* 2002). Hence, it was found that black tea polyphenols (TFs and TRs) and phytochemical constituents act as oncostatin roles in different cancer stages of development as well as owing to mutant cells accompanying an inflammation microenvironment, gene mutations, proteinase secretion, epigenetic mutations, oxidative stress, suppression of apoptosis, inflammatory-induced cell proliferation, angiogenesis or expression of adhesion molecules which drive the malignant transformation whereas, tea consumption also blocked a varied range of diseases-atherosclerosis, stomach ulceration and cardiovascular diseases as depicted in **Fig. 20**

(Gardner *et al*., 2007; Pan *et al*., 2008; Sil *et al*., 2010; Hayat *et al*., 2015; Singh *et al*., 2017; Yun *et al*., 2021; Chen *et al*., 2022).

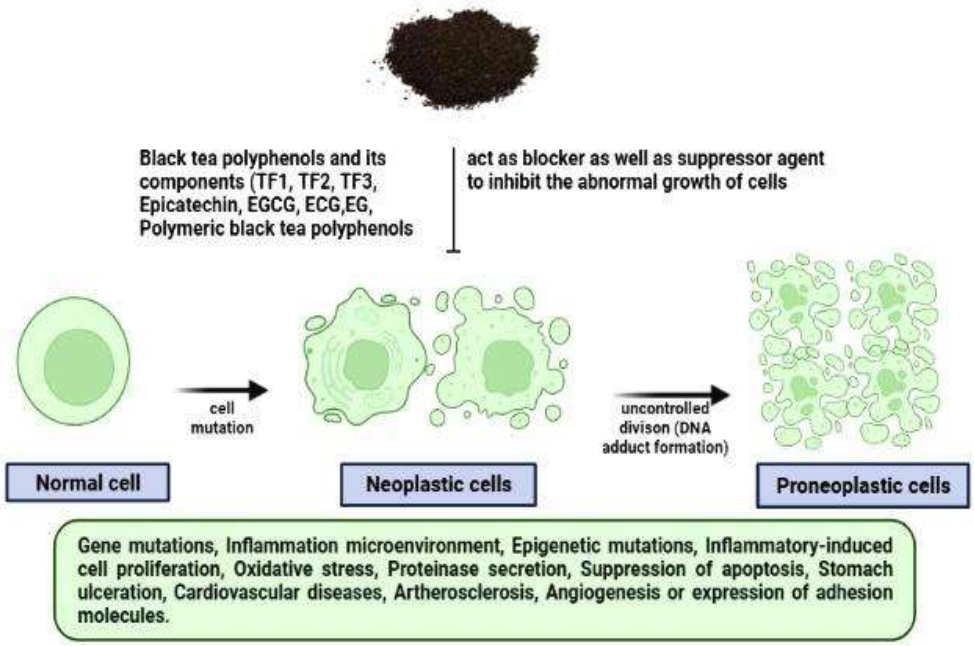


Fig. 20: Diagrammatic representation of chemopreventive action of black tea phytoconstituents (created in www.Biorender.com)

2.5.4.1 Animal/Clinical studies

Tea and its chemical constituents have been researched for their potential to fight cancer by utilising various animal-based models. Most animal research on tea and cancer that has been published in the literature concentrates on green tea's effects. Researchers have examined black tea's potential as a carcinogenesis inhibitor, even though it makes up more than 75% of all tea produced worldwide. Black tea's chemical composition is very different from green tea's due to the considerable oxidation of catechins during manufacture. Thearubigins, theaflavins and other oxidation products of black tea exhibit antioxidant properties, signifying that black may avert lung cancer brought on by NNK. Bioassays in A/J mice showed that drinking black tea prevented the growth of lung cancer brought on by NNK therapy (Cao *et al*., 1996; Yang *et al*., 1997; Chung *et al*., 2003). Black, green tea and its polyphenols, EGCG and theaflavins, were given to hamsters, rats and mice for inhibition in the cancer in lung brought on by the chemical 4 (methylnitrosamino) 1 (3 pyridyl) 1 butanone (NNK) (Wang *et al*., 1992; Xu *et al*., 1992; Yang *et al*., 1997; Chung *et al*., 1999; Mimoto *et al*., 2000; Liao *et al*., 2004; Schuller *et al.*, 2004; Liu

and Huang, 2015; Sun *et al*., 2018). The phosphorylated transcription factors JUN, ERK1, and ERK2 were shown to be at lower concentrations in adenocarcinomas after coffee and PPE (Polyphenon E) therapy, which prevented increased apoptosis and cell proliferation. However, neither treatment significantly changed the amount of cell death or growth in healthy lung tissue.

At 60 weeks, giving green or black tea to A/J mice, they also prevented lung tumors from developing independently (Landau *et al*., 1998; Chung *et al*., 2003). Green tea (decaffeinated) and dehydrated black tea solids decreased lung tumor multiplicity at 16 weeks following the administration of [DGT (0.6%) or DBT (0.6 g)] of tea solids reconstituted in 100 ml of warm water). -(methylnitrosamino)3-pyridyl -1-dosage of -1-butanone (NNK) (Katiyar *et al*., 1993). Black tea polyphenols significantly lowered volume of tumor, hyperproliferation of bronchiolar epithelial cells and cell proliferation, in a different model (Yang *et al.,* 1998). It has also been documented that in different animal model experiments, administering black and green tea orally reduced chromosome damage (micronuclei) in the peripheral blood as well as potent mutagen's tumor-initiating potency of mice given benzopyrene (Kuroda and Hara, 1999; Muto *et al*., 1999; Le *et al*., 2019). Decaffeinated black and green tea significantly decreased the growth of liver tumors and lung malignancies in male C3H mice, according to Cao *et al*., 1996.

Black tea prevented lung adenoma in A/J mice from developing into adenocarcinoma and decreased the incidence and number of spontaneously produced rhabdomyosarcomas (Clark and You, 2006). Findings from more than a decade of cell culture and tumor bioassays in animal model research indicate the significance of tea in cancer prevention. Epidemiological studies on tea and cancer has produced conflicting results, with some suggesting a preventive effect and others showing no link or even an increase in the incidence of specific malignancies. Animal studies and population-based studies differ in meaningful ways. Black tea contains several bioactive substances that have antiradical effects. Several researchers have found that the chemopreventive benefits of black tea polyphenols, in either a directly or indirectly by inhibiting tumor promoters. While few human intervention trials show regular drinking of black tea, black tea, has chemopreventive potential, and clinical research has been conducted on green tea. Recent epidemiologic research has produced conflicting findings about the correlation between drinking tea and the risk of acquiring lung cancer, particularly in case-control and cohort studies. In the people

of Moscow, drinking black tea was linked to a lower chance of rectal carcinogenesis. A pooled analysis of 13 prospective studies (n = 1480 cases) on renal cell carcinoma was conducted. Black tea had more significant impact on women than on men, probably as a result of men's higher alcohol consumption. In contrast to the findings of the previous case-control study, black tea consumption (3 cups per day) lowers risk of cancer development (15%) when compared to fewer than 1 cup/day (Khan and Mukhtar, 2013).

In the present study, current and former smokers (993) with primary incident lung cancer and hospital controls with non-neoplastic illnesses (986) were coordinated for age, gender, and smoking to investigate the effects of black tea and coffee ingestion on lung cancer risk. The findings demonstrated that people who regularly drank black tea (2 cups/day) had a decreased incidence of developing lung cancer than those who did not (non-smokers) (Baker *et al*., 2005). Another case-control study discovered a protective effect for non-smoking women who drank black tea often, daily, or several times per week (Kubik *et al.*, 2004).

2.6 Meta-analysis

A systematic method (meta-analysis) is based on combining data from multiple scientific studies rather than individual study results. As a result, it is regarded as a more precise and reliable approach to reviewing research than other qualitative approaches (Vermeire *et al*., 2001). 22 papers that offered data on the relationship between black tea, green tea, or both consumers and the chance for lung cancer development were included in this meta-analysis. With a 95% confidence range, the summary relative risk (RR = 0.82, green tea) revealed a marginally significant connection between the maximum green tea ingestion and a decreased 18% risk of developing lung cancer (CI = 0.71–0.96). Furthermore, 2 cups/day of black tea showed increased lung tumor generation (95% CI = 0.65-1.03, RR = 0.82) through the meta-analysis (Tang *et al*., 2009). Another study was conducted on 1130 cases and 1484 controls using information from one of 18 hospitals in the Montreal area and Canadian people aged 35 to 75 who were recruited between 1996 and 2001. They established a connection between adenocarcinoma risk and high black tea drinking for 50 years or longer compared to no consumption (Pasquet, 2013). There was no conclusive link between black tea use and lung cancer risk in this analysis of 22 research studies (Tang *et al*., 2009). Another case-control and cohort study revealed

that both black and green tea significantly lowers chances of lung tumor development (Wang *et al*., 2014). The Netherlands cohort study expressed no evidence of a substantial cancer preventive effect of black tea on breast, colorectal, stomach or lung cancers (Gardener *et al*., 2007). However, epidemiological studies have not always found a connection between black tea's cancer-fighting qualities and decreased incidence of cancer. Contrarily, black tea did not appear to be a material that fosters cancer development (Mohan *et al*., 2005; Singh *et al*., 2017).

Combining black tea with medicinal plants in herbal teas or tisanes enhances black teas potential health benefits. Herbal tea is also known as herbal infusions since it is made from the decoction of herbs and other plant material in hot water (Killedar *et al*., 2017). Many people believe that herbal tea is not tea, even though it looks like tea and is brewed similarly to tea. This is because they are not derived from the *Camellia Sinensis* bush, the source of all teas. Herbal teas are widely consumed worldwide and are employed as medicinal tools in conventional medicine (Ravikumar *et al*., 2014; Erasmus *et al*., 2017). The various clinical evidence suggests that to achieve a healthy diet; individuals should prioritize plant materials such as vegetables, fruit, grains, oils and nuts while reducing their intake of red and processed sugary drinks as well as meat (McMacken and Shah, 2017; Bvenura *et al*., 2017; Wallace *et al*., 2020). In China and India, herbal teas are used in the traditional medicinal systems.

Due to the low cost of *in vitro* research, there is a wealth of preclinical information about phytoconstituents and their pharmacology is available (Colalto, 2010). However, additional research on humans is required to fully understand the advantages of drinking herbal beverages and the efficacy of production techniques like fermentation, which gives the tea its distinctive flavor and may boost biological activity. Herbal tea consumption is beneficial in nutritional-physiological, maintaining and promoting health, particularly in terms of public health and especially in terms of cardiovascular disease prevention. They are simple and effective methods for reaping the herbs aroma and health benefits (Killedar and Pawar, 2017). It is very well known nowadays that there is positive link between herbal tea consumption and its preventuion of various health problems. The pharmacological effects of drugs, functional foods or botanical dietary supplements are frequently altered by interactions between phytochemical components (Lila *et al*., 2005; Gurley, 2012; Shareena and Kumar, 2022). These interactions can either enhance or inhibit the

activity of bioactive phytochemicals. There could be synergistic, additive or antagonistic interactions between various plant-based diets that change their biological features. The anti-cancer, metabolic disorders, anti-inflammation, and chemoprevention of much oxidative stress are antioxidant synergistic interactions demonstrated in *in vitro* studies using mixes of pure bioactive chemicals or phytochemical-containing plant extracts (Lila *et al*., 2005; Muhammad *et al*., 2017). The chemopreventive potential of illnesses associated with redox imbalance can be increased by the antioxidant synergistic interactions between teas and herbal infusions (Phan *et al*., 2022). At various antioxidant assays, a binary/ternary decoction of black tea with ginger, tulsi, and black pepper recorded the synergistic, additive and antagonistic interactions at a 1:1:1 ratio (Gupta *et al*., 2014). An additive scavenging capability was demonstrated by a binary combination of black tea (*Camellia sinensis assamica*), and tulsi (*O. gratissimum*) (Guleria *et al*., 2022). To enhance black tea's medicinal properties and flavor. Green cardamom, tulsi leaves, ginger, and other medicinal herbs can be added (Guleria *et al*., 2022).

Thus, in the present study, herbal medicinal plants examined, such as *O. gratissimum* (Vana tulsi), *O. sanctum* (Rama tulsi), *O. canum* (Dulal tulsi), rich in antioxidants and commonly used by people as herbal drinks for herbal therapies from ancient times. We are making a refreshing tea to include stevia leaves (*S. rebaudiana*) used as an additive that contains high antioxidant as well as anticancerous properties to improve or enhance the cancer potential property of black tea, shown in **Fig. 21.**

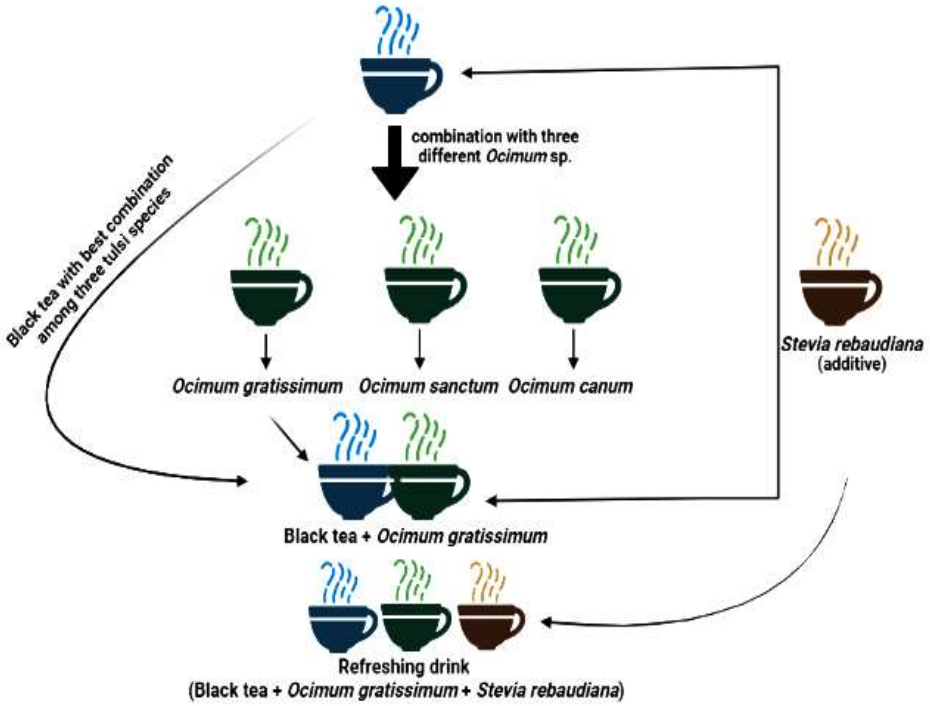


Fig. 21: Diagrammatic representation of research design (created on www.Biorender.com)

2.7 Remedial plants used in the research study

2.7.1 Ocimum gratissimum

O. gratissimum, also known as scent leaf, Vana tulsi, clove basil, African basil, or wild basil is one of the most recently found plants for medicinal purposes, that has the potential to be used as a new drug that has been around since the ancient times and utilized for treating various illnesses, including anemia, diabetes, inflammation, cancer, diarrhea, pains, fungal and bacterial infections.

It is a common perennial herbaceous plant that is economically successful and has a potent aroma (Prabhu *et al*., 2009; Monga *et al*., 2017; Bhavani *et al*., 2019; Nganteng *et al*., 2022). It is a member of the Lamiaceae and is known to occur in South America, Asia and Africa. The classification is as given below in **Fig. 22(a).**

Kingdom	Plantae
Division	Tracheophyta
Class	Magnoliopsida
Order	Lamiales
Family	Lamiaceae
Genus	*Ocimum*
Species	*gratissimum*

Fig. 22: (a) Taxonomic position of *O. gratissimum*

2.7.1.1 Botanical features

Perennial herbeacous, aromatic, erect stem with 1-3 height, much branched, round-quadrangular, woody at the base, glabrous or pubescent, leaves are opposite, blade elliptical to ovate, membranaceous or apex cute. On the leaves, glandular trichomes are seen, the calyx is 2-lipped and 2-3 mm long, the corolla is campanulate 3.5-5 mm long, an ovary is superior, consisting of two carpels, pubescent outside, greenish-white in color and inflorescence – verticillaster (Prabhu et al., 2009; Monga et al., 2017; Valsan *et al.,* 2022) as illustrated in **Fig. 22(a) and (b).**

Fig. 22: (b) External morphology of *O. gratissimum*

2.7.1.2 Health-promoting benefits

It has long history of use in traditional system of medicine to cure many ailments, including anemia, inflammation, diarrhea, diabetes, cancer, protect heart health, and bacterial or fungal infections. Its leaves also act as a repellent for

mosquitoes or insects. Fresh scent leaves contain magnesium, cinnamic acid and protein.

Scent leaf tea has traditionally been used to treat stomach disorders such as gastroenteritis. Cold and flu symptoms can be relieved by chewing on the leaves (Benelli *et al*., 2019; Okeke *et al*., 2023). Leaves boiled with ginger or honey to treat cough, bronchitis, asthma, influenza, and cold. The phytochemical testing reported that it contains some important secondary metabolites thereby showing various pharmacological activities such as antioxidant, hepatoprotective, immuno-suppressive, anti-inflammatory, antidiarrhoeal, antihypertensive and antimicrobial (Mohammad *et al*., 2007; Monga *et al*., 2017; Valsan *et al*., 2022).

2.7.2 Ocimum sanctum

For more than 2000 years, *O. sanctum* has been acknowledged as one of the most adaptable medicinal herbs, with a diverse spectrum of biological activity. In the Indian subcontinent, it is a well-known religious herb also known as tulsi, Shyma, holy basil, or Krishna tulsi. Tulsi "Queen of Herbs", is one of the Orient's health-giving and the most revered of many healing herbs (Gupta *et al*., 2002; Singh *et al*., 2007). It is an aromatic shrub endemic to the eastern hemisphere tropic and is assumed to have originated in north-central India. It belongs to the basil family Lamiaceae. Tulsi is renowned for its potent scent and occasionally bitter, astringent flavor. It can also have flowery and peppery aromas because it is linked to the culinary basil family. Some tulsi kind's smell and taste like cloves, while others have a crisp, lemony flavor (Singh *et al*., 2007; Mondal *et al*., 2009; Cohen, 2014; Khatoon *et al*., 2022). The taxonomic information is shown below in **Fig. 23(a).**

Kingdom	**Plantae**
Division	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Family	Lamiaceae
Genus	*Ocimum*
Species	*sanctum*

Fig. 23: (a) Taxonomic position of *O. sanctum*

2.7.2.1 Botanical features

It is a heavily branched sub-shrub that is 30 to 60 cm tall, upright, with opposing, highly hairy purple or green leaves and aromatic stems. Oval leaves with up to 5 cm long petioles are present. It is a fragrant bushy perennial that can reach a height of 1.5 cm. Its flowers appear in compact whorls on long racemes with white blooms and somewhat purplish-tinted foliage. It is grown for essential oil production as well as for religious and therapeutic purposes (Singh *et al*., 2007; Mondal *et al*., 2009; Cohen, 2014; Kumar *et al*., 2022) as shown in **Fig. 23 (a) and (b).**

Fig. 23: (b) External morphology of *O. sanctum*

2.7.2.2 Heath-promoting effects

It reduces psychological stress by improving memory and cognitive function, acting as an anxiolytic and antidepressant, and metabolic stress by restoring average blood glucose, blood pressure, and lipid levels to normal. It cures many skin disorders, insect bites, or itching.

Tulsi is a rejuvenator, reducing stress, relaxes the body, and improves memory. In Ayurvedic treatments, Tulsi extracts treats stomach disorders, common colds, inflammation, heart disease, malaria, various poisonings, and headaches. It has a wide range of therapeutic uses, including catarrhal fever, cardiopathy, haemopathy, gastropathy, leucoderma, asthma, vomiting, ringworm, genitourinary disorders, verminous, and bronchitis. It has also been suggested to possess analgesic, anticancer, antifungal, antifertility, hepatoprotective, antidiabetic, antispasmodic, cardioprotective, antimicrobial, diaphoretic and Adaptogenic activity. *O. sanctum* is traditionally consumed in various forms, including dried powder, fresh leaf and herbal tea (Singh *et al*., 2007; Pattanayak *et al*., 2010; Cohen, 2014; Kumar *et al*., 2022).

2.7.3 Ocimum canum

O. canum Sims is a traditional medicinal plant that grows throughout Tamil Nadu and is commonly referred to as NaiTulasi in Tamil. Goes by the name “hoary basil” and “Dulal tulsi”. A powerful antioxidant polyphenol known locally as eme or akokobesa (Ghana), rosmarinic acid (RA) found in *O. canum* (Nascimento *et al*., 2011; Ngassoum *et al*., 2004; Manjudevi *et al*., 2022) as shown in **Fig. 24.**

Fig. 24: External morphology of *O. canum*

2.7.3.1 Botanical features

This annual plant is native to Africa and can reach 2 feet. It is also known as African basil and has hairy leaves, scented flowers and a distinct mint flavor. The plant branches out from the base with angled stems and open foliage.

It has a pungent, aromatic flavor and is commonly cultivated for culinary purposes. Flowers are white, pubescent, 7.5 to 20 cm long, with a 3 mm long calyx and a 4 mm long corolla. The petioles are slender and hairy and range from 1.3 to 2.5 cm. There is a short pedicel. The leaves are elliptic-lanceolate, sharp at both ends, gland-dotted, virtually glabrous, and have edges that are whole or shallowly serrated (Selvi *et al*., 2015; Bhattacharjya *et al*., 2019; Gaurav *et al*., 2022) as depicted in **Fig. 24.**

2.7.3.2 Health-promoting benefits

O. canum is explicitly used for treating various diseases, lowering blood glucose levels, and treating colds, fevers, joint inflammation, headaches, and parasitic infestations on the body. The plant has a spicy, aromatic flavour and is widely grown

for culinary purposes. Traditional medicine recognized its utility in treating fevers, tooth problems and dysentery (Aluko *et al*., 2012; Pandey *et al*., 2014). It was used as an insect repellent to prevent insect damage after harvest. Colds, fevers, parasitic infestations on the body, joint inflammation and headaches can all be treated with this herb. It has therapeutic and pharmacological effects like anti-depressant, sedative and anxiolytic, antihypertensive, cardioprotective, antioxidant, antidiabetic, anti-inflammatory, antinociceptive, antimicrobial, antitumor, and chemopreventive properties. The leaves are used to flavor food. Linalool is found in the oil of *O. canum*. The seeds could provide dietary fiber or help with constipation. Magnesium, also present in basil, helps blood flow by relaxing the muscles and blood vessels (Bhattacharjya *et al*., 2019; Gaurav *et al*., 2022).

2.7.4 Stevia rebaudiana

S. rebaudiana Bertoni, (asteraceae), is a perennial herb/shrub grown commercially worldwide for the natural sweetener steviol. Steviol glycosides are incredibly sweet compounds found in the leaves of the stevia plant and are used as a sugar substitute in the food and beverage industries for sweetener extraction and flavor enhancement (Savita *et al*., 2004; Goyal *et al*., 2010). Steviol glycosides extracted from stevia leaves are intensely sweet and 100–300 times sweeter than table sugar (sucrose); it is devoid of zero artificial chemicals, low-calorie and carbs (Goyal *et al*., 2010; Karagoz, 2022). Not everyone finds it to their taste. Some people find it bitter, while others believe it tastes like menthol (Tadhani *et al*., 2007; Ahmad *et al*., 2020). Obesity rise is on peak and the diabetes epidemic, low-calorie sugar alternatives (stevioside) have flooded the market around the globe. The leaves of the plant *S. rebaudiana* are used to make the sweet herb stevia (Yadav *et al*., 2011; Gupta *et al*., 2013; Karagoz, 2022). Taxonomic classification is given below in **Fig. 25(a).**

Kingdom	Plantae
Division	Tracheophyta
Class	Magnoliopsida
Order	Asterales
Family	Asteraceae
Genus	*Stevia*
Species	*rebaudiana*

Fig. 25: (a) Taxonomic position of *S. rebaudiana*

2.7.4.1 Botanical features

It is a small seasonal plant that grows up to 1-2 feet tall and is typically grown for its leaves, from which a sweetener is made. It has elongated leaves that grow along the stems and are arranged in rows. Generally, flowers are pruned to improve the flavor of the leaves. Tubular flowers in terminal clusters are white and light purple accents. Cut off the flowers to get better-tasting leaves. Steviol glycosides, a group of sweet-tasting compounds found in the leaves, are used for sweetening foods or beverages and are also industrially processed to create powdered non-caloric sweeteners. Along the stems, the fragrant, oblong leaves are arranged in an opposing pattern and measure 2.5 cm (1 inch) in length (Yadav *et al*., 2011; Gantait *et al*., 2018; Karagoz, 2022) as illustrated in **Fig. 25 (a) and (b).**

Fig. 25: (b) External morphology of *S. rebaudiana* (obtained from www.google.com)

2.7.4.2 Health-promoting effects

S. rebaudiana extract and its glycosides have anti-cancer, anti-hypertensive, anti-obesity, anti-hypertensive, anti-diabetic, antioxidant, low blood pressure, anti-tumor, anti-inflammatory and antimicrobial effects and improved kidney function. Proteins, carbohydrates, oils, lipids, phenolic compounds, dietary fibers and vitamins are all found in stevia leaves (Marcinek *et al*., 2016; Sukhmani *et al*., 2018; Kurek and Krejpcio, 2019; Khilar *et al*., 2022).

2.8 Interactions between teas/herbs/phytochemicals/drugs (antioxidant interactions) and their effect on their biological properties (bioactive compounds)

The interactions between different phytoconstituents may impact the total antioxidant capacity of the combination. These interactions are categorized into synergistic, additive and antagonistic (Skroza *et al*., 2022; Liu *et al*., 2022; Guleria and Sehgal, 2022). Different teas mixed with herbs having different bioactive constituents can produce antagonistic or synergistic bioactive impacts (Boyer and Liu, 2004; Malongane *et al*., 2017). Additionally, combining many plant extracts lowers the dose of each plant that is needed, preventing the negative consequences of utilizing high doses of each plant extract. Combining different nutrients and phytochemicals provides more robust protection because nutrients are more effective when combined than if taken alone. According to the earlier study, herbs are known to include a variety of bioactive constituents with antioxidant effects. Antioxidants present in combinations vary in quantity and ratio, which impacts how they interact (Chou, 2006; Guleria *et al*., 2022).

Recently, it has been discovered that distinct bioactive molecules such as enzymes, refined compounds (vitamins and phytochemicals), synthesized antioxidants, and crude extracts can interact to reveal numerous antioxidant interactions (additional, antagonistic and synergistic). Adding *O. gratissimum* to black tea resulted in an additive interaction and the highest antioxidant potential, whereas supplementation with *O. sanctum* or *O. canum* resulted in an antagonistic interaction and lower radical scavenging ability (Guleria and Sehgal, 2022). An additive interaction occurs when the concentration of phytochemicals responsible for antioxidant potential increases, whereas an antagonistic interaction occurs when the structural characteristics of phytochemicals change, potentially decreasing the functional properties of a mixture (Muhammad *et al*., 2017; Farooq and Sehgal, 2019).

Pure compounds that have been isolated may or may not behave in the same way as the original food component, losing biological functions, complexity of whole foods, synergistic interactions and dietary patterns. While isolated phytochemicals may have specific properties or functions, they may not replicate the same health benefits as consuming a balanced diet rich in whole foods are believed to play a crucial role in promoting health and preventing disease as well and two or more mixtures affect antioxidant, anti-lipid peroxidation and anti-cancerous properties (Hidalgo *et al*., 2010; Muhammad *et al*., 2017). There have been reports of synergistic antioxidant interactions between kaempferol and cyanidine-3-glucoside, kaempferol and delphinidine-3-glucoside, cyanidine-3-glucoside and myricetin-3-glucoside. Extracts of black and green tea along with ascorbic acid's; interactions with antioxidant activity, ranged from antagonistic to additive in different proportions (10:1, 5:1, 2:1, 1:1, 1:2, and 1:10). Combining various tea varieties may have a synergistic impact that improves health results (Enko and Swigło, 2015).

Clinical investigations have shown several physiological reactions to tea, and these responses may have significant impact on the management, prevention, and treatment of some long-term illnesses. With the addition of each herb, black tea's antioxidant activity increased (lemon, bergamot, clove and cinnamon). Because polyphenolic substances prevent vascular illnesses frequently in diabetes (type 2), the combination's synergistic antioxidant action may indirectly benefit the body (Buyukbalci and Nehir, 2008). Using various cancer chemopreventive agents combined with various action pathways may be a viable methodology for maximizing efficiency while minimizing toxicities (Hong and Sporn, 1997; Sporn, 1980).

Treatment with a single compound, such as polyphenon E or atorvastatin, was ineffective in reducing lung carcinogenesis, whereas combined treatment with these two compounds significantly reduced tumor number and tumor load (Lu *et al*., 2008). The binary combinations of phytochemicals also show a reduction in antioxidant potential. For example, the combination of alpha-tocopherol with ferulic or caffeic acid showed synergism against lipid peroxidation but antagonism with chlorogenic acid which may be due to chlorogenic acid, steric structure, which prevents it from attaching with alpha-tocopherol (Neunert *et al*., 2015). Tumor cell growth is inhibited by polyphenols found in tea by influencing many molecular targets that are involved in proliferation.

There have been numerous studies on cancer-prevention properties of rooibos, black, and green tea (Marnewick *et al*., 2005; Phan *et al*., 2013). Nevertheless, an increase in gren tea drinking did lessen the incidence of lung cancer but not black tea consumption, according to met-analysis study (Tang *et al.*, 2009). A prospective cohort study found similar results, indicating that drinking black tea had no defense action against lung, breast and colorectal cancer (Blot *et al*., 1997). These findings don't mean that black tea can't be used for the treatment of other cancer types like bladder/ovarian in women (Wu *et al.*, 2013; Singh *et al.*, 2017). Combining black tea with other types of teas may possese anticancerous properties (George *et al*., 2011). It has been documented by (George *et al*., 2011) that while combining black tea with resveratrol has synergistic suppression on the skin tumors growth and subsequently lead to reduction in volume of tumor and cell number compared to alone. Combined polyphenol mixtures are more effective in treating human cancer than a single polyphenol. A highly malignant B16 melanoma F10 cell line (B16M-F10) was treated with quercetin and pterostilbene in a way that increased the number of cancer cells in the G0/G1 phase and reduced the risk of metastasis (Fantini *et al*., 2015). Additionally, when the polyphenol thearubigin and the isoflavone genistein from black tea were combined there was a higher inhibition of prostate cancer cell proliferation *in vitro* (Sakamoto, 2000).

The advantages of poly-herbal formulation for illness management and prevention help manage disease and lessen the adverse effects of pharmaceutical medications (Chawla *et al*., 2013; Aleesha *et al*., 2020). Although herbal infusions are beneficial, caution should be exercised due to potential contamination and inherent side effects (Fakeye *et al*., 2009; Jordon *et al*., 2010). Individual plant compounds, herbs, and tea all have synergistic effects that may increase the benefits of plants (Malongane *et al*., 2017). Different tea polyphenols work synergistically with herbal treatments or pharmaceuticals to boost their antimicrobial, antioxidant, anti-cancer and anti-diabetic activities (Nikmaram *et al*., 2017; Malongane *et al*., 2017).

The combinations have multiple beneficial effects, including cancer cells reduction, generation of inflammatory cytokines and proteasome activity, inducing apoptosis and affecting key enzymes like glutamine synthetase and superoxide dismutase to control epigenetic regulation (Chistiakov *et al*., 2017; Rahman *et al*., 2022).

Tea polyphenols may improve the therapeutic effects of medications that treat infectious diseases, cancer and diabetes mellitus (Malongane *et al*., 2017; Rana *et al*., 2022). Additionally, because the combination reduces the number of individual pharmaceuticals, the beneficial effect may result in lower levels of most prescribed drugs.

Combinations have also been shown to optimize individual teas phytochemical constituents, financial value and sensory properties of individual teas. Consumption of black, herbal and green tea is linked to minimizing the risk of diabetes and heart stroke (Chatterjee *et al*., 2012; Kokubo *et al*., 2013; Hayat *et al*., 2015; Yamagata *et al*., 2015; Yu *et al*., 2020) as shown below in **Fig. 26.**

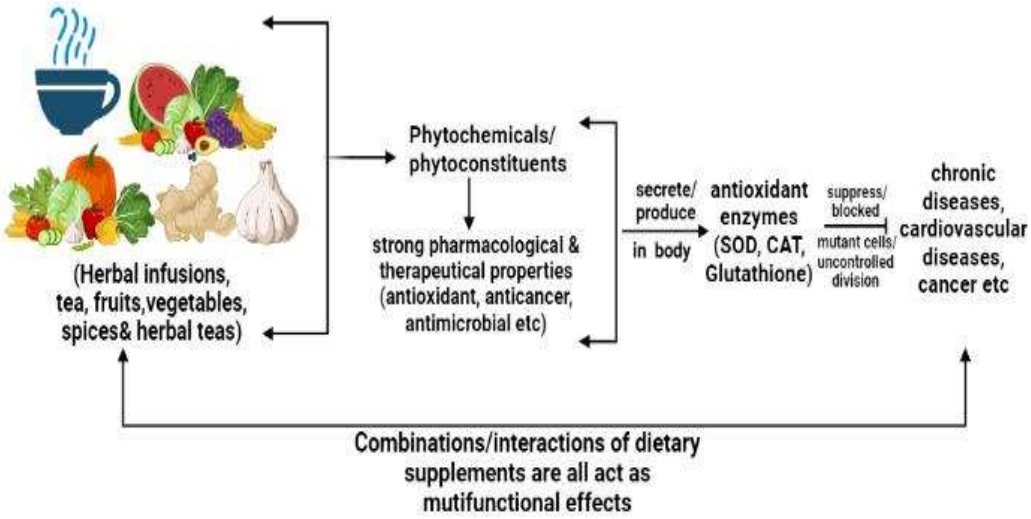


Fig. 26: Diagram showing the effect of dietary supplement (herbs/teas) phytoconstuients on the various ailments when taken in combination (created on www.Biorender.com)

CHAPTER 3

HYPOTHESIS

HYPOTHESIS

Cancer is ranked second worldwide after cardiovascular diseases as the major killer (WHO, 2018). Among the different cancer types, lung cancer is the leading cause of mortality in both sexes (Bray *et al*., 2018). It has been proven difficult to deal with using surgical and therapeutic techniques. The side effects and long-term sequelae of anti-cancer chemotherapy continue to be a significant cause of concern for both patients and clinicians (Nurgali *et al*., 2018; Smith *et al*., 2020; Rahman *et al*., 2022). Existing treatments for chemotherapy-related side effects are generally ineffective, routinely ignore potential long-term consequences, or can cause additional side effects that only make patients feel worse (Chui, 2019; Kuderer *et al*., 2022). There is an urgent need to find new agents for cancer prevention and treatment (Chikara *et al*., 2018).

The use of natural and dietary agents that can retard, suppress, reverse, or block the process of cancer development is gaining interest among the scientific community and the general public (Pan *et al*., 2008; Cragg *et al*., 2016; Garg *et al*., 2023). The numerous plant extracts or their phytochemicals have illustrated chemopreventive effects on cancer (Dillard *et al*., 2000; Rizeq *et al*., 2020; Muhammad *et al*., 2022). From the ancient era, teas made from the *Camellia sinensis* have been used to promote health and manage conditions like cancer, inflammation and headache (Mahdi *et al*., 2020; Brimson *et al*., 2022). There are many different kinds of tea including white, black, oolong and green tea, but black tea is one of the most consumed beverages globally (Wong *et al*., 2022; Brimson *et al*., 2022). Various *in vitro* and *in vivo* reports showed the anticancer properties of black tea. The studies on humans showed contradictory results, some studies reported that regular consumption of black tea may help in preventing cancer (Baker *et al*., 2007; Nei *et al*., 2014; Wang *et al*., 2014; Singh *et al*., 2017) whereas others found no clear evidence of its anticancer properties (Gardner *et al*., 2007; Ganmaa *et al*., 2008; Tang *et al*., 2009; Nie *et al*., 2014).

Combining Black Tea with other medicinal plants can enhance its health-promoting ability. When two or more plant extracts are mixed, they can interact in a synergistic, additive or antagonistic manner (Gawlik, 2012). It is also observed that the proportion of the combined phytochemicals or the plant extracts can influence the biological properties. In India, tulsi leaves are generally added to black tea for flavor

and medicinal properties. It has a strong-smelling aromatic flavor. Although, tulsi is commonly used as an additive in black tea, the scientific literature about the interaction of black tea with different *Ocimum* species is limited. Due to the rise of obesity and diabetes epidemic, different beverages are being flooded in the market which contains low calorie. Interest is being generated for stevia whole leaf extract obtained from *Stevia rebaudina* as it acts as a sweetener and contains phytochemicals that have antioxidant and anticancer properties. The present study will combine black tea granules with three different *Ocimum* spp. (*O. gratissimum*/*O. sanctum*/*O. canum*) to explore or identify the possible synergistic or additive interactions between Black Tea and respective *Ocimum* spp., and the binary combination with the highest efficacy will be chosen for same parameters testing at five different proportions (3:1, 2:1, 1:1, 1;2 & 1:3). The proportion of black tea and *Ocimum* spp. demonstrating maximum antioxidant and antigenotoxic potential will be combined with stevia leaves. These combinations will be further tested for cytotoxicity against A549 lung cancer cell line and in vivo using B(a)P- induced lung carcinogenesis in mice as a model as depicted in **Fig. 27.**

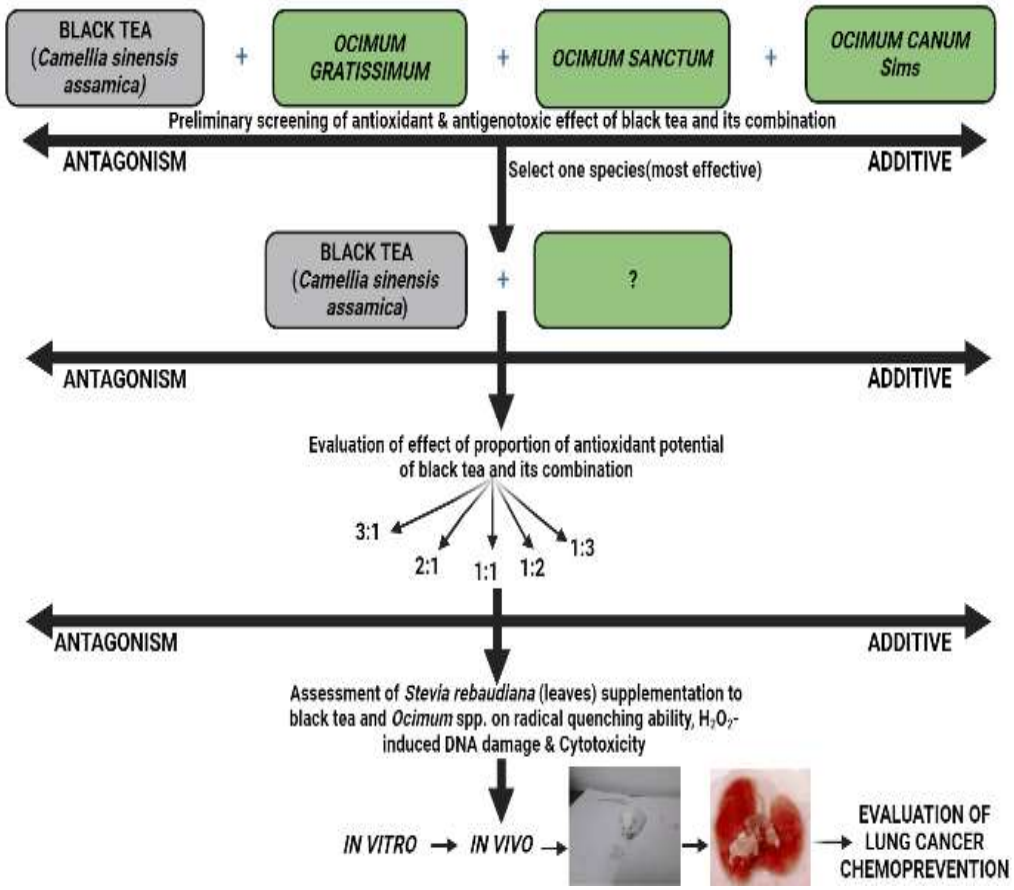


Fig. 27: Diagrammatic representation of hypothesis

CHAPTER 4

OBJECTIVES

OBJECTIVES

Screening of radical scavenging, anti-lipid peroxidation, anti-hemolytic and anti-genotoxic potential of binary combination of Black Tea granules with three different species of *Ocimum* (tulsi).

Selection of most active Black Tea and *Ocimum* spp. combination for further determining the effect of their proportion on antioxidant and anti-genotoxic potential**.**

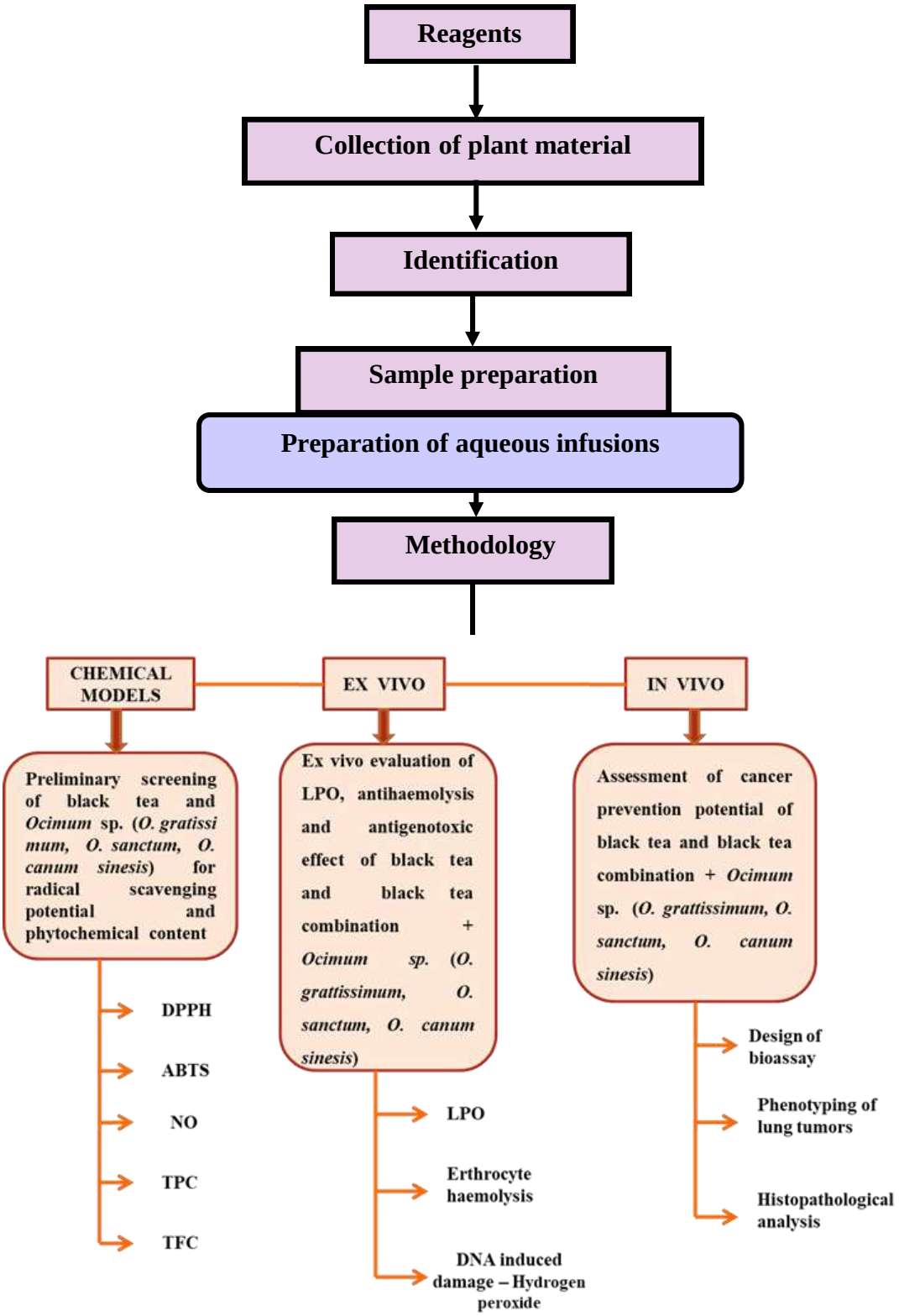
Assessment of *Stevia rebaudiana* (leaves) supplementation to black tea and *Ocimum* spp. on radical quenching ability and H_2O_2-induced DNA damage.

Appraisal of cancer chemo preventive potential of ternary combination of Black Tea, Tulsi and Stevia against benzo(a)pyrene [B(a)P]-induced lung carcinogenesis in mice model.

CHAPTER 5

MATERIAL & METHODS

Material and Methods (design outline)



5.1 Chemicals and reagents

The chemicals utilized during the course of this study are as: DPPH (2,2-diphenyl-1-picryl hydrazyl), ascorbic acid, ABTS [2,2'-azino-bis(ethylbenzthiazoline-6-sulfonic acid)] (HiMedia), Sodium persulfate (HiMedia), Methanol (HiMedia), Ammonium persulfate (HiMedia), NEDA (Napthylethylenediaminedihydrochloride) (HiMedia), Sodium nitroprusside (HiMedia), TCA (trichloro acetic acid) (HiMedia), TBA (thiobarbituric acid) (HiMedia), Ferrous sulphate ($FeSO_4$) (HiMedia), Hydrochloric acid (HCl) (HiMedia), Sodium carbonate (Na_2CO_3) (HiMedia), Butylated hydroxyl toluene (BHT) (HiMedia), Gallic acid (HiMedia), Follin-Ciocalteu's reagent (HiMedia), Quercetin (HiMedia), Aluminium chloride ($AlCl_3$) (HiMedia), Ethidium bromide (HiMedia), Eosine (HiMedia), Hematoxylin (HiMedia), DNA sodium salt (HiMedia), Phosphate buffer (HiMedia), Ferrous chloride ($FeCl_3$) (HiMedia), Ascorbic acid (HiMedia), Sodium hydroxide (NaOH) (HiMedia), Trizme base (HiMedia), RPMI 1640 medium (10% fetal bovine serum with 2mM L-glutamine) (HiMedia), Sulforhodamine (SRB) (HiMedia), Benzo(a)pyrene [M/s SRL Chemicals (Mumbai, India)], A549 human cancer cell line were purchased from ATCC (Middlesex, UK).

5.2 Collection of Plant Material

Samples of the *O. gratissimum* L., *O. sanctum* L. and *O. canum* were procured from the Herbal Garden, at Lovely Professional University (LPU). The black tea granules (Taj Mahal brand, India) were bought from Brooke-Bond, India. The dried stevia leaves were obtained from Green Valley Stevia (Batch no. HL50/1304), Punjab, India)

5.2.1 Identification

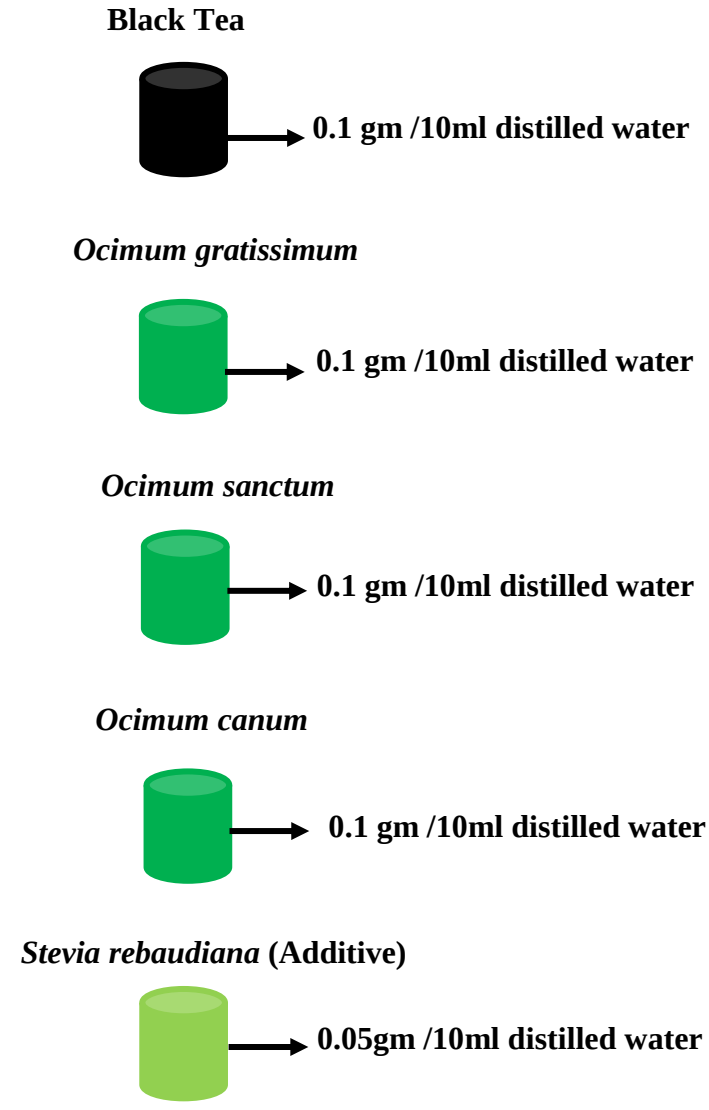
The *Ocimum* spp. was anthenticated by Dr. Sumeet Gairola, Plant Sciences Division, CSIR- Indian Institute of Integrative Medicine (IIIM) and the voucher specimen was submitted to herbarium of IIIM, Jammu, bearing voucher specimen number (*O. gratissimum*- 23391, *O. sanctum*- 23392 and *O. canum* - 23390) for future reference.

5.2.2 Sample Preparation

5.2.2.1 Preparation of aqueous infusions

The aqueous infusion of black tea granules (1%), tulsi leaves (1%) and stevia (0.5%) as per the manufacturer's instruction) alone and in combination (2%) were steeped at 90-100ºC for five minutes (Islam *et al.*, 2018). These infusions were filtered using Whatman's filter paper, and the filterate was stored at 4ºC till further investigation. The composition of different infusions is shown below.

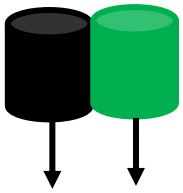
Alone Preparation



Binary mixture preparation

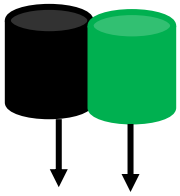
Black Tea + *Ocimum gratissimum* (BTOG)

(Best Combination)



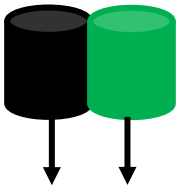
0.1 gm + 0.1 gm in 10ml distilled water

Black Tea + *Ocimum sanctum* (BTOS)



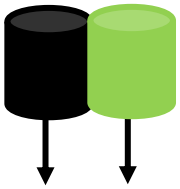
0.1 gm + 0.1 gm in 10ml distilled water

Black Tea + *Ocimum canum* (BTOC)



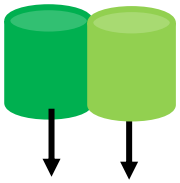
0.1 gm + 0.1 gm in 10ml distilled water

Black Tea + *Stevia rebaudiana* (BTSt)



0.1 gm + 0.05 gm in 10ml distilled water

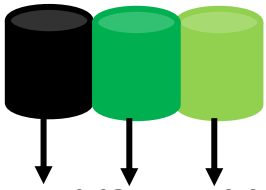
***Ocimum gratissimum* + *Stevia rebaudiana* (OGSt)**



0.1 gm + 0.05 gm in 10ml distilled water

Ternary Mixture Preparation: (Best Combination)

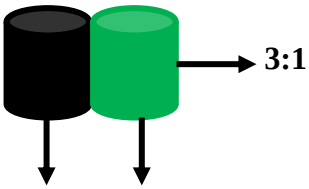
Black Tea + *Ocimum gratissimum* + *Stevia rebaudiana* ((BTOGSt)



0.075 gm + 0.025 gm+ 0.05 gm in 10ml distilled water

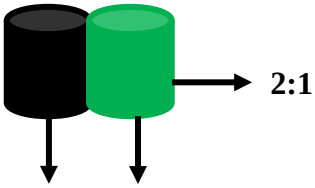
Ratios Preparation: (Best Combination)

Black Tea + *Ocimum gratissimum* (BTOG)



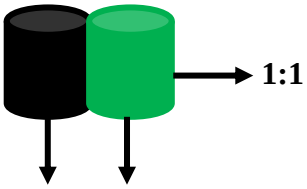
0.075gm + 0.025 gm in 10ml distilled water

Black Tea + *Ocimum gratissimum* (BTOG)



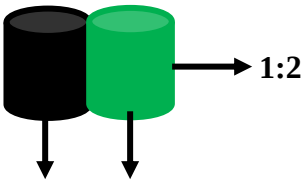
0.066gm + 0.033gm in 10ml distilled water

Black Tea + *Ocimum gratissimum* (BTOG)



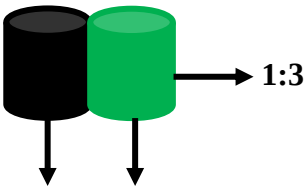
0.050gm + 0.050gm in 10ml distilled water

Black Tea + *Ocimum gratissimum* (BTOG)



0.033gm + 0.066gm in 10ml distilled water

Black Tea + *Ocimum gratissimum* (BTOG)



0.025gm + 0.075gm in 10ml distilled water

5.3 Methodology

Preliminary screening of Black Tea and tulsi species (*O. gratissimum, O. sanctum* and *O. canum)* for radical scavenging potential, total phenolic and flavonoid content.

DPPH, ABTS and NO quenching ability tests were used to evaluate the radical scavenging capacity of aqueous infusions. The total polyphenolic content (phenolic and flavonoid) was also assessed.

5.3.1 DPPH (2,2-diphenyl-1-picryl hydrazyl) assay

DPPH is a colored, stable free radical molecule. The antioxidant compound donates hydrogen to reduce DPPH (Mensor *et al*., 2001). This can be measured spectrophotometrically. A freshly prepared DPPH solution (0.011gm in 50 ml of methanol) was used for spectrophotometric analysis. Methanol was used to dilute the

DPPH solution further, and the optical density (OD) was set at 0.8-1. Every 2 ml of DPPH solution received an addition of an extract with a different concentration. 30 minutes into the incubation. When purple turns yellow, use a visible spectrophotometer to measure absorbance at 520 nm. DPPH (2 ml) was used as a control, while methanol was used as a blank. The experiment was carried out in triplicates. The following relationship was used to calculate radical scavenging activity:

% scavenging activity = (Ac-At)/Ac x 100, where: At and Ac are the respective absorbance of test samples and DPPH. The activities were measured in terms of EC_{50}; the lower the EC_{50} (µg/ml) higher is the antioxidant potential.

5.3.2 ABTS [2,2'-azino-bis (ethylbenzthiazoline-6-sulfonic acid)] assay

This test measures a substance's ability to scavenge 2,2'-azino-bis (ethylbenzthiazoline-6-sulfonic acid). Antioxidants prevent ABTS from oxidizing and lower the level of monocation (Re *et al*., 1999). The radical cation was produced by mixing ABTS (7mM) solution with ammonium persulfate (3mM). The mixture was kept in a dark place for 16 hours at room temperature. The blue color is produced after 16 hours. Methanol further dilutes the ABTS solution, and the optical density (OD) was set between 0.8-1. A different concentration of extracts was added to every ABTS (2 ml) solution. Take a reading of the absorbance of each sample at 745 nm after 30 minutes of incubation. The following equation was used to compute the percentage of scavenging activity:

% scavenging activity = (Ac-At)/Ac x 100, where: At and Ac are the respective absorbance of test samples and ABTS. The activities were measured in terms of EC_{50}; the lower the EC_{50} (µg/ml) higher is the antioxidant potential.

5.3.3 NO (Nitric oxide scavenging activity) assay

Take (10mM) sodium nitroprusside and dissolved it in phosphate buffer saline (PBS). Then add sodium nitroprusside (2 ml) and PBS (0.9 ml) in different concentrations of aqueous extract of samples that were incubated at 37ºC for 2 hours. After incubation, Griess reagent 0.5 ml (sulphamide (1%), o-phosphoric acid (2%), and NEDA (0.1%) were added. Then, measure the absorbance of chromophore formed during diazotization showing pink color and read at 546 nm (Shirwaikar *et al*., 2006). Take PBS as a blank in this experiment.

% scavenging activity = (Ac-At)/Ac x 100, where: At and Ac are the respective absorbance of test samples and NO. The activities were measured in terms of EC_{50}; the lower the EC_{50} (µg/ml) higher is the antioxidant potential.

5.3.4 Determination of Total Phenolic Content

Take 50 µl of infusions (diluted) in test tubes and add 1:10 v/v Folin's-Ciocalteu's reagent (2.5 ml). After 5 minutes of equilibration, 2.0 ml of sodium carbonate (7.5%) was added to each tube. Before taking an optical density reading at 760 nm, the tubes were incubated at 37ºC in a water bath (90 minutes). Milligrams of gallic acid equivalents (mg GAE/g) were used to express the results (Gani *et al*., 2015).

5.3.5 Total Flavonoid Content Estimation

Take extract (0.5 ml) and mix it with methanol (1.5 ml). After waiting for 5 minutes, add 0.1 ml of 10% $AlCl_3$. After another 5 minutes, add potassium acetate (9.8 gm in 100 ml). Finally, add 2.9 ml of distilled water water was added to determine the total flavonoid concentration using quercetin as the reference. For 30 minutes, the reaction mixture was heated in a water bath at 37ºC. At 415 nm, the mixture's absorbance was measured against a prepared reagent blank. The standard was prepared by different concentrations of quercetin in methanol (Nabavi *et al*., 2008).

Ex-vivo evaluation of antilipid peroxidation, antihemolysis, and antigenotoxic effect of Black Tea and Black Tea combination with *Ocimum* species (*O. gratissimum, O. sanctum and O. canum*)

5.4 Lipid Peroxidation (LPO) assay

The fresh chicken liver was collected from the slaughterhouse, stored in chilled or cold PBS, and preserved at 4ºC until usage. To estimate lipid peroxidation, 10% liver homogenate (10%) was made (Ohkawa *et al*., 1979). Different concentrations of extracts were added. Then add 0.5 ml of the liver homogenate to these samples. Wait for 5 min. at 37ºC. Add 100 µl of 15mM $FeSO_4$ solution. Again, wait for 30 min. at 37ºC. After incubation add the mixture of TBA (1): TCA (1): HCl (1) ratio in each test tube. The completed combination was boiled (30 minutes) at

100ºC in a water bath. Centrifugation for 10 minutes at 2000 rpm to cool it off. Consider the supernatant absorbance at 532 nm.

% scavenging activity = (Ac-At)/Ac x 100, where: At and Ac are the respective absorbance of test samples and LPO. The activities were measured in terms of EC_{50}; the lower the EC_{50} (µg/ml) higher is the antioxidant potential.

5.5 Erythrocyte hemolysis

The blood was collected from healthy volunteers after obtaining informed consent and delivered into heparinized tubes (Okoko and Ere, 2012). To achieve the target hematocrit level, whole blood was resuspended in the same buffer after being centrifuged at 4000 rpm (10 minutes) at 4°C. The test tube was filled with a fraction of erythrocytes (200 µl), test samples, and 100 µl of hydrogen peroxide (100 M), which was added to cause hemolysis. The mixture (200µl) and the entire content were incubated for 3h at 37ºC. After incubation, 2 ml of PBS was added, and the solution was centrifuged at 3000 rpm for 10 minutes. The absorbance was measured at 540nm.

5.6 Evaluation of hydroxyl radical-induced DNA damage (Genotoxicity) assay

Take different aqueous infusions (black tea, *O. gratissimum*, *O. sanctum*, *O. canum*, *S. rebaudiana* alone and its binary (BTOG, BTOS, BTOC, BTOG (3:1), BTOG (2:1), BTOG (1:1), BTOG (1:2), BTOG (1:3), BTSt, OGSt) & ternary combination (BTOGSt) in the tubes, phosphate buffer (300 µl) (0.2 M, pH 7.4) was added to the sample residue. Subsequently, (50 µl) DNA sodium -10 mg/ml, (75 µl) H_2O_2 - 33.6 mM, (50 µl) $FeCl_3$ - 3.2 mM, and 100 µl of Na_2EDTA - 0.5 mM solution was added. 1.2 mM of ascorbic acid was added to 75 µl to start the reaction. After 20 minutes, the reaction was stopped by adding 250 L of 10% w/w trichloroacetic acid. of incubation (55°C) in a water bath. After that, the color was created by adding 150 L of TBA (5%, in 1.25% NaOH aqueous solution) and heating it for 15 minutes at 105 °C in a small water bath (Li *et al.*, 2016). After cooling the combination, the absorbance at 530 nm was measured in comparison to the buffer (used as the blank). The inhibition percentage for •OH is expressed as follows:

$$\text{Protective effect \% - } A_0 - A/A_0 \times 100\%$$

Where A0 is the absorbance at 530 nm of the control without sample, and A is the absorbance at 530 nm of the reaction mixture with sample.

5.7 Cytotoxicity (Sulforhodamine) SRB assay

RPMI 1640 medium (10% fetal bovine serum with 2mM L-glutamine) was used to grow the cell line (A549 - Human cancer cell line). 96-well microtiter plates with cells should be used. Incubate at 37ºC, CO_2 (5%), air (95%) & relative humidity (100%) for 24 hours (add experimental drugs) – water solvent. Addition of drugs in different concentrations on plates. Incubate (48 hours) under normal conditions by adding cold TCA; the assay was terminated. Cells *in situ* (fixed) (10%, 30% TCA add). Again incubate (60 minutes) at 4ºC. Supernatant discarded. Plates washed (5 times) with tap water and air dried. Each well received 1% acetic acid and sulphorhodamine solution, and the plates were incubated for 20 minutes. Unbound dye was removed upon staining, and any dye that was still present was removed five times in 1% acetic acid before being air-dried (Skehan *et al*., 1990; Vichi *et al*., 2006). Then, 10mM trizme base was used to elute the bound pigment. The plate reader measured the absorbance at a wavelength of 540 nm using a reference wavelength of 690 nm.

The percentage increase in growth for test wells in compared to control wells was calculated on a plate-by-plate basis. By dividing the average absorbance of the test well by the average absorbance of the control wells and multiplying the result by 100, the percentage of growth was obtained.

To calculate the percentage growth at each of the four drug concentration levels, the six absorbance measurements (time zero (Tz), control growth (C), and test growth in the presence of the drug at the 4 levels of concentration (Ti)) were used. Calculating the percentage of growth inhibition was as:

$$[Ti/C] \times 100\ \%$$

5.7.1 Assessment of cancer chemoprevention potential of ternary combination of black tea (BT), stevia and tulsi (*O. grattissimum*) combination against benzo(a)pyrene [B(a)P]-induced lung carcinogenesis in mice model

5.7.2 Infusions preparation

Alone preparation: Black tea, *Ocimum gratissimum* – 0.1gm in 10ml distilled water (1%). *Stevia rebaudiana* (St) – 0.05gm in 10ml distilled water.

Binary combination preparation: Black tea + *Ocimum gratissimum* (BTOG) – 0.2gm in 10ml distilled water (2%).

Ternary combination preparation: BT (0.075gm) + OG (0.025gm) (3:1) – in 10ml distilled water. BT (0.1gm) + St (0.05gm) – in 10ml distilled water, BT (0.1gm) + OG (0.1gm) + St (0.05gm) – in 10ml distilled water.

5.7.2.2 Preparation of drug (benzopyrene)

Benzopyrene [B(a)P] (50mg/kg b.wt. dissolved in corn oil, orally) twice a week for 4 successive weeks to induce lung cancer by 16^{th} week.

5.7.2.3 Care taken for animal ethical point of view

Mice were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences Old campus, Hisar (Haryana). The animals were acclimatized for 15 days before the start of the experiment and provided with a standard diet and water. The chemopreventive effects of black tea (BT), *Ocimum* spp., and *S. rebaudiana* (St) alone or in combination with benzo(a)pyrene-induced lung tumorigenesis in mice were studied (Kamaraj *et al.*, 2007; Ravichandran *et al.*, 2011) by following preparation steps as shown below.

5.7.2.4 Preparation of mice experiment step by step: (anticancer study)

A. Select the species of animal (mice/rat) and acclimatized the animal for 15 days before starting the experiment**.**

B. After starting the experiment, firstly clean the cage of the mice.

C. Put the fresh husk in the cage.

D. Mice food.

E. Measure the food weight (acc. to your protocol) before giving it to the mice.

F. Prepare & measure the drink (acc. to your protocol).

G. The drink is ready and filters through the funnel.

H. Give a drink to the mice.

I. Measure the weight of the mice.

J. Prepare the drug (according to protocol) which will be given to the mice.

K. Inject the drug into the mice through oral administration (Orally).

L. After completing the experiment period cleaning the equipment is used in the dissection of mice.

M. Preparing the alcohols and PBS and sterilizing them before using them in the dissection of mice.

N. Fix the body parts of mice like (feet & hands) with pins on a dissection pad.

Start the dissection of mice. The organ part of the mice on which one must research and see the result, take it out of its body (lung, etc.). After completing the dissection, place the mice cages on the cage stand after cleaning them first as shown in **Fig. 28.**

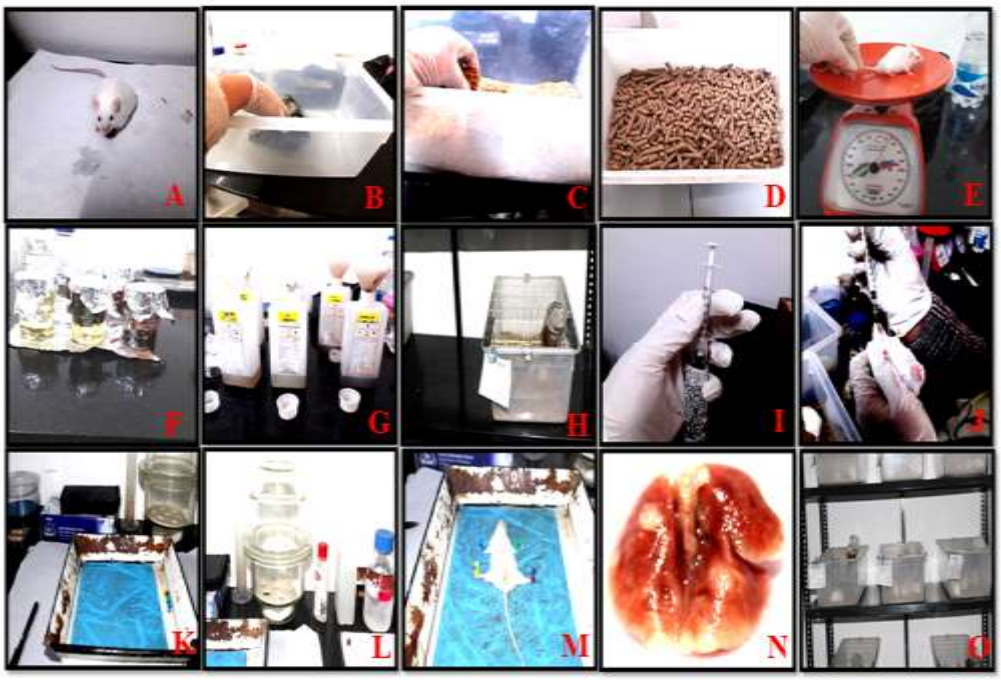


Fig. 28: Preparation of mice experiment step by step

5.7.2.5 Animal grouping

The mice were divided into 8 groups with 6 mice in each group and a total of 48 animals. All the mice except group I were given B(a)P (50mg/kg b.wt.) from the 8th day, week (twice) for 4 successive weeks to induce lung cancer by the 16th week (Kamaraj *et al*., 2007; Ravichandran *et al*., 2011). The animals of groups I and II was supplied drinking water whereas of groups III, IV, V, VI, VII, and VIII were administered an aqueous infusion of black tea (BT, 1% w/v), *O. gratissimum* (OG, 1% w/v), *Stevia rebaudiana* (St, 0.05% w/v), BT (1% w/v) + St (0.05% w/v), BT + OG (1% w/v, 3:1) and [BT + OG] (1% w/v, 3:1) + St (0.05% w/v) respectively, throughout the study (16 weeks) as shown in **Table 9.**

Body weight, water and infusion consumption were monitored in mice of different groups. All animals were sacrificed after 16 weeks by using sodium pentobarbital (250 mg/kg, orally). We investigated the chemopreventive potential of black tea, tulsi, and stevia against benzo(a)pyrene [B(a)P]-induced lung carcinogenesis in mice. The design of the bioassay is shown in **Table 9.**

5.7.2.6 Design of bioassay

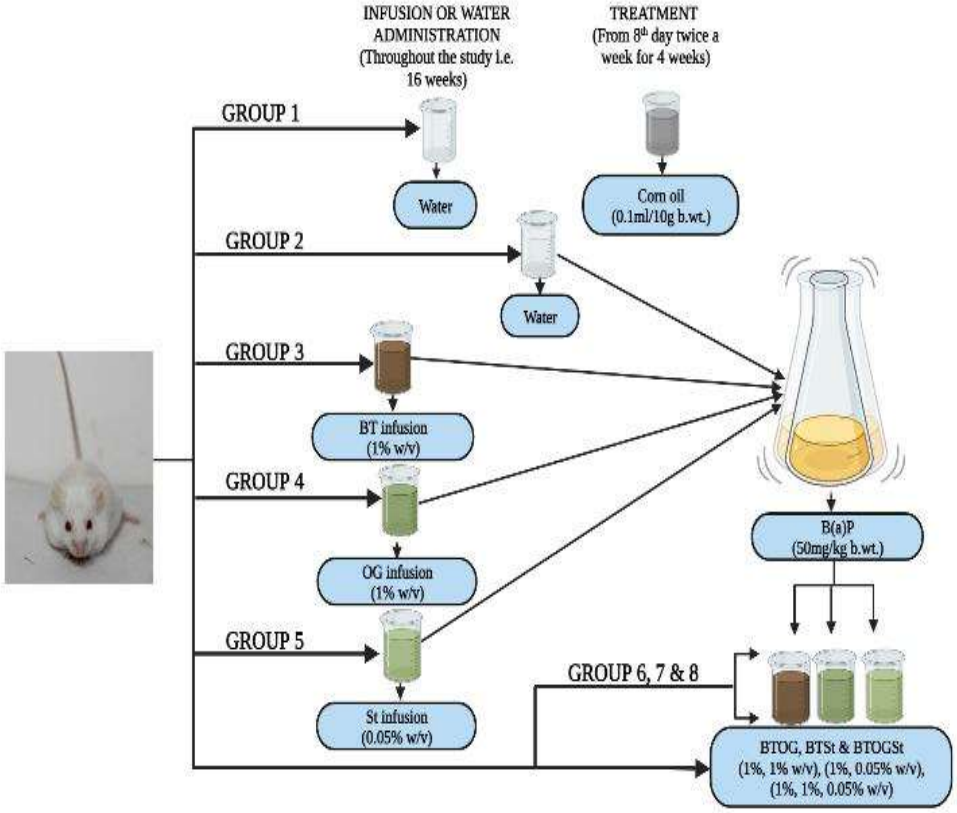


Fig. 29: The experimental design of mice treatment

The design of the bioassay is in **Fig. 29**. The mice were divided into eight groups, with six mice in each group and a total of 48 mice. All the mice except group I were given B(a)P (50mg/kg b.wt.) from the 8th day, twice a week for 4 successive weeks, to induce lung cancer by the 16th week.

Table 9: Systematic representation of treatment regime (Feeding of 1% BT (black tea), 1% OG (*O. gratissimum*), 0.5% Stevia, and BT +OG + Stevia in drinking water).

S.no.	Groups	Infusion or Water Administration (Throughout the study i.e. 16 weeks)	Treatment (From 8th day twice a week for 4 weeks)	Number of Animals
1.	**Group-I**	Water	Corn oil (0.1 ml/10g b.wt.)	6
2.	**Group-II**	Water	B(a)P (50mg/kg b.wt.)	6
3.	**Group-III**	BT infusion (1% w/v)	B(a)P (50mg/kg b.wt.)	6
4.	**Group-IV**	OG infusion (1% w/v)	B(a)P (50mg/kg b.wt.)	6
5.	**Group-V**	St (0.05% w/v)	B(a)P (50mg/kg b.wt.)	6
6.	**Group-VI**	BT (1% w/v) + St (0.05% w/v)	B(a)P (50mg/kg b.wt.)	6
7.	**Group-VII**	BT + OG (1% w/v, 3:1)	B(a)P (50mg/kg b.wt.)	6
8.	**Group-VIII**	[BT + OG] (1% w/v, 3:1) + St (0.05% w/v)	B(a)P (50mg/kg b.wt.)	6

BT- Black Tea, OG – *Ocimum gratissimum*, St- *Stevia rebaudiana*, B(a) P: Benzo(alpha)pyrene.

5.8 Histopathological analysis

After sacrificing, mice lungs were removed and kept in ice cold phosphate buffer. Lungs were fixed in 10% buffered formalin, followed by histological sectioning, processing, and staining (with hematoxylin and eosin) for microscopic examination.

5.9 Statistical analysis

The results were presented as mean S.D. for readings taken in triplicate. The statistical comparison was performed by one-way ANNOVA followed by Tukey's honestly significant difference test using SPSS software (version 18). If the p-values are 0.05 or less, the results were considered statistically significant. The interaction between binary combinations was calculated by combination index and isobologram using Compusyn software. EC_{50} of various infusions alone or in combination were calculated for radical scavenging ability (% inhibition), anti-lipid peroxidation activity, antigenotoxic potential, and antihemolytic activity applying Compusyn software (version 1.0).

CHAPTER 5

RESULTS

RESULTS

6.1 Screening of radical scavenging, anti-lipid peroxidation and antihemolytic potential of the binary combination of black tea granules with three different species of *Ocimum* (Tulsi).

6.1.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

It is used as a chemical model to determine the radical scavenging capability of plant extracts. The activities were measured in terms of EC_{50}; the lower the EC_{50} (µg/ml) higher is the antioxidant potential. It was observed that black tea (BT) showed the highest percentage inhibition with the lowest effective concentration (EC_{50} = 80.35 µg/ml), followed by *O. gratissimum* (OG) (EC_{50} = 91.55 µg/ml), *O. sanctum* (OS) (EC_{50} = 147.58 µg/ml) and *O. canum* (OC) (EC_{50} = 202.055 µg/ml). While in combination, black tea with with *OG* demonstrated the maximum DPPH radical quenching potency and less effective concentration (EC_{50} = 96.49 µg/ml) than BT with OS (BTOS) (EC_{50} = 138.98 µg/ml) and BT with OC (BTOC) (EC_{50} = 212.41 µg/ml) as shown in **Fig. 30** and **Table 10**.

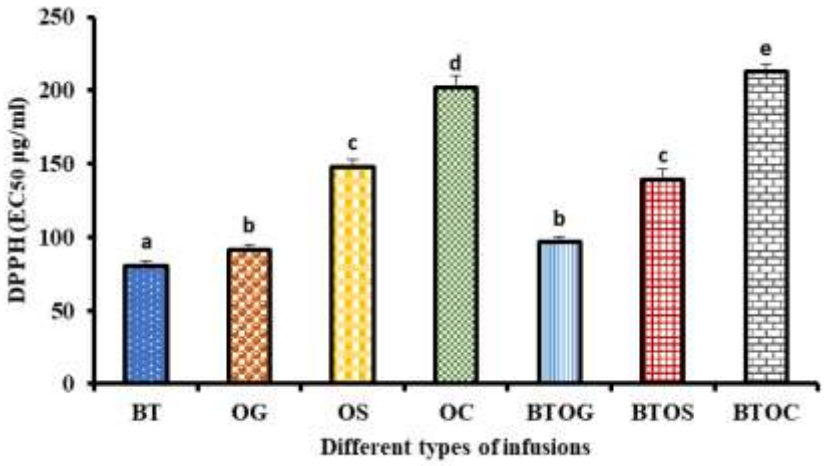


Fig. 30: DPPH scavenging activity of Black tea (BT), *O. gratissimum* (OG), *O. sanctum* (OS), *O. canum* (OC) and different infusion types. The data are shown as MEAN ± S.D of 3 independent tests (each with triplicates for each test point). EC_{50} defined as (Effective concentration causing 50% scavenging activity). The dissimilar alphabets illustrated significant difference ($p \leq 0.05$) among the EC_{50} of different infusion types. Values in parenthesis are arcsine transformed values; a-d represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

Table 10: EC_{50} values values Of DPPH scavenging activity of different types of infusions alone and in combination.

Sample Extract	EC_{50} (µg/ml)
BT	80.35[a]
OG	91.55[b]
OS	147.58[c]
OC	202.05[d]
BTOG	96.49[b]
BTOS	138.98[c]
BTOC	212.41[e]

Values are represented as MEAN ± S.D, n = 3; replicates. EC_{50} indicates (Effective concentration causing 50% scavenging activity). The dissimilar alphabets illustrated significant difference ($p \leq 0.05$) among the EC_{50} of different infusion types- (BT – black tea, OG – *O. gratissimum*, OS – *O. sanctum* and OC – *O. canum*, BTOG – black tea + *O. gratissimum*, BTOS – black tea + *O. sanctum*, BTOC – black tea + *O. canum* for an antioxidant parameter (DPPH – 2,2- diphenyl-1-picryl hydrazyl). Values in parenthesis are arcsine transformed values; a-d represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

6.1.2 ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay

ABTS is a radical cation decolorization assay used as a model for the determination of antioxidant properties. The activities were measured in terms of EC_{50}; the lower the EC_{50} (µg/ml) higher is the antioxidant potential. The highest percentage inhibition of radical scavenging efficacy of black tea alone and infusion was in the following order: BT (EC_{50} = 38.03µg/ml) > OG (EC_{50} = 44.87 µg/ml) > OS (EC_{50} = 139.50 µg/ml) > OC (EC_{50} = 139.97 µg/ml) whereas, in combination the highest activity was observed in BTOG infusion (EC_{50} = 47.45 µg/ml), > BTOS (EC_{50} = 130.27 µg/ml) > BTOC (EC_{50} = 139.97 µg/ml) as shown in **Fig. 31** and **Table 11**.

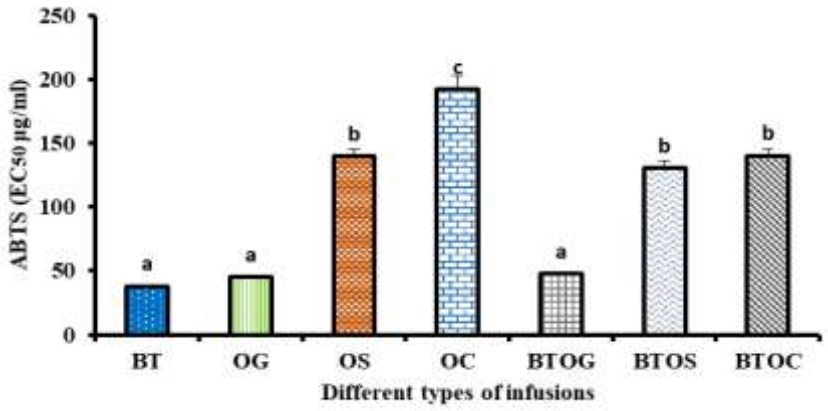


Fig. 31: ABTS scavenging activity of Black Tea and Basil species (*O. gratissimum, O. sanctum and O. canum*) and different infusion types. Values are represented as MEAN ± S.D (n = 3 replicates). Letters above a column followed by dissimilar letters above other columns are significantly different at p ≤ 0.05. The dissimilar alphabets illustrated significant difference (*p ≤ 0.05*) among the EC_{50} of different infusion types. Values in parenthesis are arcsine transformed values; a-c represents the levels of treatments: 'a' = highest effect and 'c' = less effect.

Table 11: EC_{50} values of ABTS scavenging activity of different infusions alone and in combination.

Sample Extract	EC_{50} (µg/ml)
BT	38.03[a]
OG	44.87[a]
OS	139.50[b]
OC	191.86[c]
BTOG	47.45[a]
BTOS	130.27[b]
BTOC	139.97[b]

Values are represented as MEAN ± S.D, n = 3; replicates. EC_{50} indicates (Effective concentration causing 50% scavenging activity). The dissimilar alphabets illustrated significant difference (p ≤ 0.05) among the EC_{50} of different infusion types- (BT – black tea, OG – *O. gratissimum*, OS – *O. sanctum* and OC – *O. canum*, BTOG – black tea + *O. gratissimum*, BTOS – black tea + *O. sanctum*, BTOC – black tea + *O. canum* for an antioxidant parameter (ABTS- 2,2'-azino-bis(ethylbenzthiazoline-6-sulfonic acid). Values in parenthesis are arcsine transformed values; a-c represents the levels of treatments: 'a' = highest effect and 'c' = less effect.

6.1.3 Nitric Oxide Scavenging Activity (NO) assay

To ascertain the free radical scavenging capacity of plant extracts, it serves as a chemical model. The activities were measured in terms of EC_{50}; the lower the EC_{50} (µg/ml), the higher the antioxidant potential. The percentage scavenging effect of BT showed maximum with the lowest EC_{50} value (EC_{50} = 43.3 µg/ml) as compared to OG (EC_{50} = 47.35 µg/ml), OS (EC_{50} = 145.11 µg/ml), OC (EC_{50} = 183.71 µg/ml). Although, BT along with OG combination (EC_{50} = 47.09 µg/ml) expressed more effect than BTOS (EC_{50} = 102.23 µg/ml), and BTOC (EC_{50} = 134.38 µg/ml) as shown in **Fig. 32** and **Table 12**.

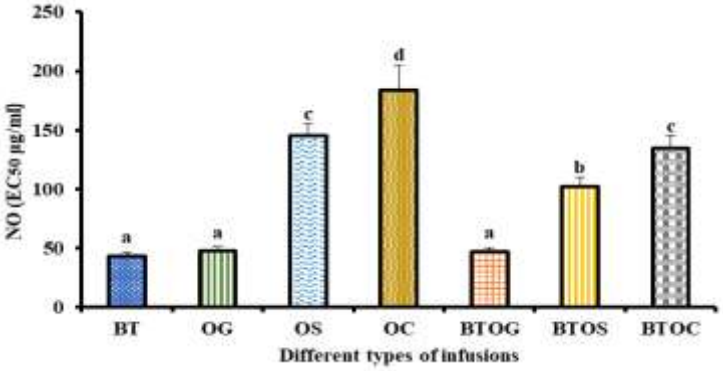


Fig. 32: NO scavenging activity of Black Tea and Basil species (*O. gratissimum, O. sanctum and O. canum*) and different infusion types. Values are represented as MEAN ± S.D (n = 3 replicates). Letters above a column followed by dissimilar letters above other columns are significantly different at p ≤ 0.05. The dissimilar alphabets illustrated significant difference (*p ≤ 0.05*) among the EC_{50} of different infusion types. Values in parenthesis are arcsine transformed values; a-d represents the levels of treatments: 'a' = highest effect and 'd' = less effect.

Table 12: EC_{50} values of NO scavenging activity of infusions alone and in combination.

Sample Extract	EC_{50} (µg/ml)
BT	43.30[a]
OG	47.35[a]
OS	145.11[c]
OC	183.71[d]
BTOG	47.09[a]
BTOS	102.23[b]
BTOC	134.38[c]

Values are represented as MEAN ± S.D, n = 3; replicates. EC_{50} indicates (Effective concentration causing 50% scavenging activity). The dissimilar alphabets illustrated significant difference (p ≤ 0.05) among the EC_{50} of different infusion types- (BT – black tea, OG – *O. gratissimum*, OS – *O. sanctum* and OC – *O. canum*, BTOG – black tea + *O. gratissimum*, BTOS – black tea + *O. sanctum*, BTOC – black tea + *O. canum* for an antioxidant parameter NO – nitric oxide scavenging activity). Values in parenthesis are arcsine transformed values; a-d represents the levels of treatments: 'a' = highest effect and 'd' = less effect.

6.1.4 Lipid Peroxidation Activity (LPO) assay

Free radicals "steal" electrons during the oxidative breakdown of polyunsaturated fatty acids with any number of carbon double bonds during lipid peroxidation, which results in membrane damage. The activities were measured in terms of EC_{50}; the lower the EC_{50} (µg/ml), the higher the antioxidant potential. The maximum potency was seen in the aqueous extract of black tea's inhibitory effect against $FeSO_4$-induced lipid peroxidation in chick liver with (EC_{50} = 50.34) µg/ml followed by OG (EC_{50} = 55.12 µg/ml), OS (EC_{50} = 136.21 µg/ml), OC (EC_{50} = 163.08 µg/ml) respectively, while, BTOG infusion showed maximum anti-lipid peroxidation activity (EC_{50} = 55.24 µg/ml) followed by BTOS (EC_{50} = 97.63 µg/ml), BTOC (EC_{50} = 111.8 µg/ml) as shown in **Fig. 33** and **Table 13.**

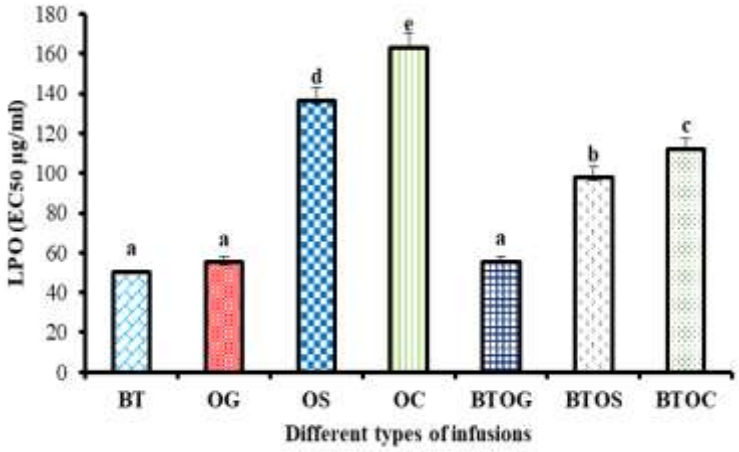


Fig. 33: LPO scavenging activity of Black Tea and Basil species (*O. gratissimum, O. sanctum and O. canum*) and different infusion types. Values are represented as MEAN ± S.D (n = 3 replicates). Letters above a column followed by dissimilar letters above other columns are significantly different at p ≤ 0.05. The dissimilar alphabets illustrated significant difference (*p ≤ 0.05*) among the EC_{50} of different infusion types. Values in parenthesis are arcsine transformed values; a-e represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

Table 13: EC_{50} values of Lipid Peroxidation scavenging activity of different infusions alone and in combinations.

Sample Extract	EC_{50} (µg/ml)
BT	50.34^{a}
OG	55.12^{a}
OS	136.21^{d}
OC	163.08^{e}
BTOG	55.24^{a}
BTOS	97.63^{b}
BTOC	111.80^{c}

Values are represented as MEAN ± S.D, n = 3; replicates. EC_{50} indicates (Effective concentration causing 50% scavenging activity). The dissimilar alphabets illustrated significant difference ($p \leq 0.05$) among the EC_{50} of different infusion types- (BT – black tea, OG – *O. gratissimum*, OS – *O. sanctum* and OC – *O. canum*, BTOG – black tea + *O. gratissimum*, BTOS – black tea + *O. sanctum*, BTOC – black tea + *O. canum* for an antioxidant parameter LPO – lipid peroxidation scavenging activity. Values in parenthesis are arcsine transformed values; a-d represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

6.1.5 Hemolysis assay

This test looks for red blood cells that are weaker than usual. To estimate the free radical scavenging capacity of plant extracts; it serves as a chemical model. The activities were measured in terms of EC_{50}; the lower the EC_{50} (µg/ml) higher is the antioxidant potential. The percentage scavenging effect of BT showed maximum with the lowest EC_{50} value (EC_{50} = 83.70 µg/ml) as compared to OG (EC_{50} = 89.38 µg/ml), OS (EC_{50} = 94.31 µg/ml), OC (EC_{50} = 146.10 µg/ml). Although, BT along with OG combination (EC_{50} = 98.00 µg/ml) expressed more effect than BTOS (EC_{50} = 162 µg/ml), and BTOC (EC_{50} = 277 µg/ml) as shown in **Fig. 34** and **Table 14.**

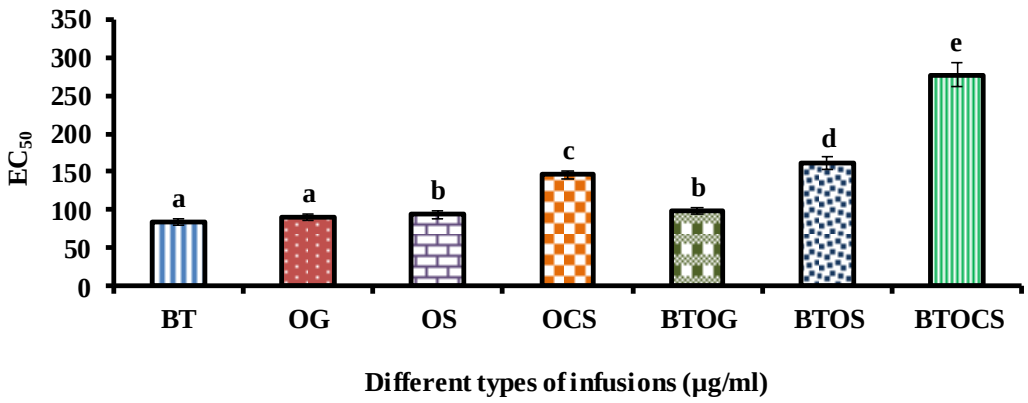


Fig. 34: Anti-haemolytic scavenging activity of Black Tea and Basil species (*O. gratissimum, O. sanctum and O. canum*) and different infusion types. Values are represented as MEAN ± S.D (n = 3 replicates). Letters above a column followed by dissimilar letters above other columns are significantly different at p ≤ 0.05. The dissimilar alphabets illustrated significant difference (*p ≤ 0.05*) among the EC_{50} of different infusion types. Values in parenthesis are arcsine transformed values; a-d represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

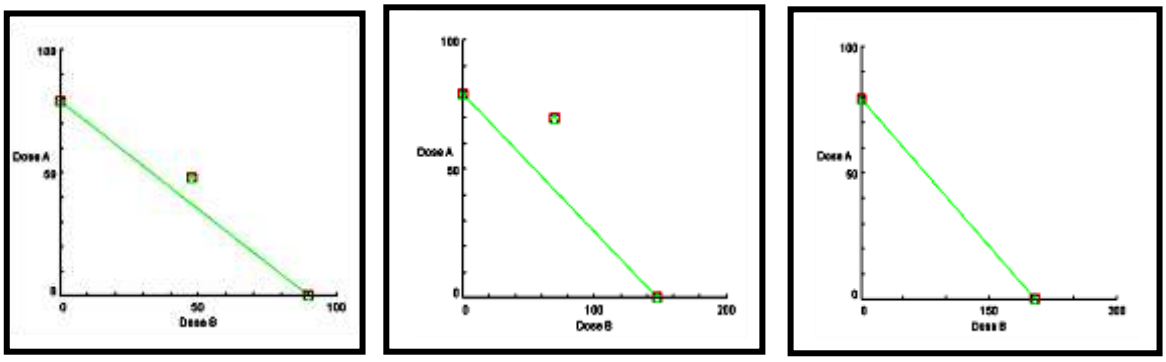
Table 14: EC_{50} values of Anti-hemolytic scavenging activity of different infusions alone and in combinations.

Sample Extract	EC_{50} (µg/ml)
BT	83.70[a]
OG	89.38[a]
OS	94.31[b]
OC	146.10[c]
BTOG	98[b]
BTOS	162[d]
BTOC	277[e]

Values are represented as MEAN ± S.D, n = 3; replicates. EC*50* indicates (Effective concentration causing 50% scavenging activity). The dissimilar alphabets illustrated significant difference (p ≤ 0.05) among the EC_{50} of different infusion types- (BT – black tea, OG – *O. gratissimum*, OS – *O. sanctum* and OC – *O. canum*, BTOG – black tea + *O. gratissimum*, BTOS – black tea + *O. sanctum*, BTOC – black tea + *O. canum* for an antioxidant parameter anti - hemolytic scavenging activity. Values in parenthesis are arcsine transformed values; a-d represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

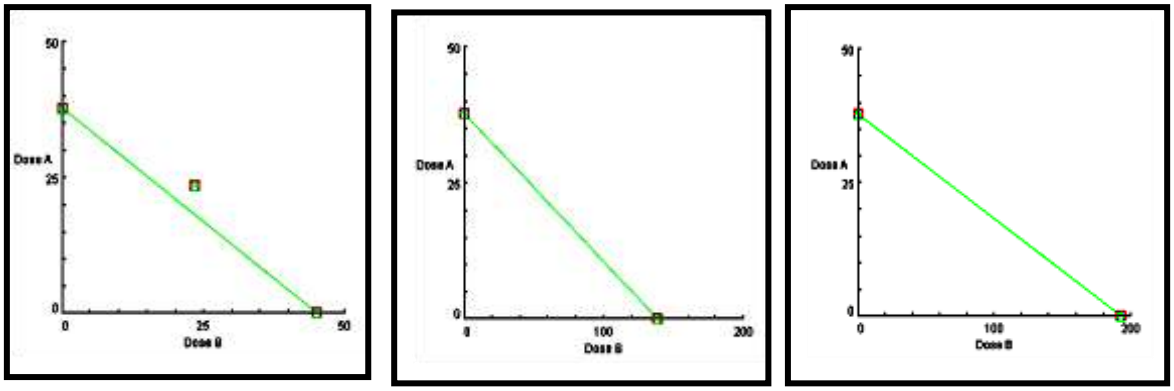
6.2 Antioxidant interactions of Black Tea and its combination is done in two ways

6.2.1 Isobologram of various combinations in different assays



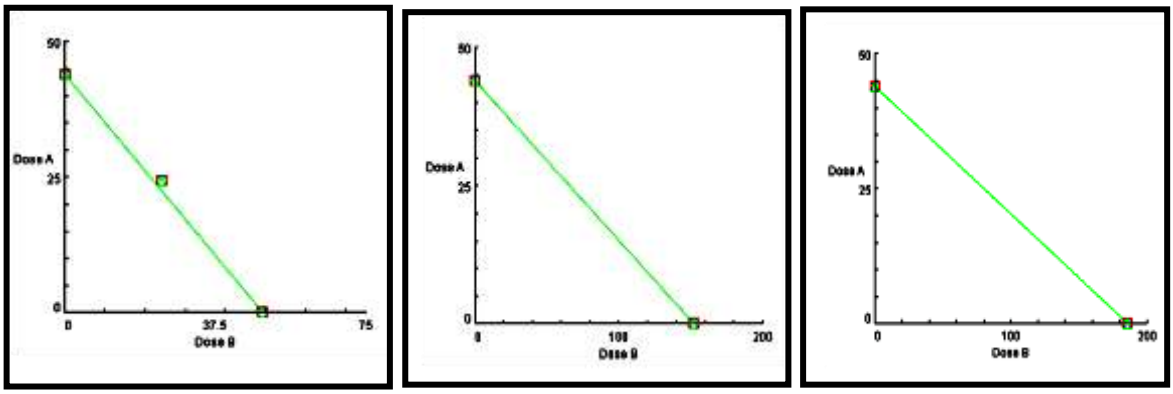
(A) **(B)** **(C)**

Fig. 35: Isobolographic plot of Black Tea with *O. gratissimum* [BTOG (A)], Black Tea with *O. sanctum* [BTOS (B)], and Black Tea with *O. canum* [BTOC (C)] at 1:1 ratio in DPPH assay. It is a graphical representation used in pharmacology to analyze mixture effects. Dose A represents the EC_{50} (µg/ml) of BT in each graph and Dose B stands for the EC_{50} of OG or OS or OC. The straight line (additive line) is made by Dose A and B (EC_{50} value). If the square symbol lies below the line of additivity it indicates synergistic interaction. When it is plotted on this line, it shows an additive effect. If drawn above the line, it is directed towards antagonistic interaction.



(D) **(E)** **(F)**

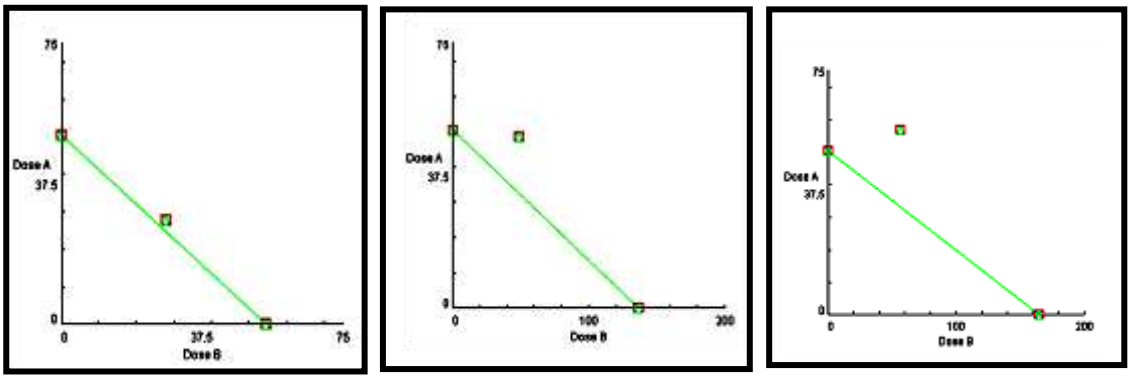
Fig. 36: Isobolographic plot of Black tea BT with *O. gratissimum* [BTOG (D)], Black tea BT with *O. sanctum* [BTOS (E)], and Black tea BT with *O. canum* [BTOC (F)] at 1:1 ratio in ABTS assay. It is a graphical representation used in pharmacology to analyze mixture effects. Dose A represents the EC_{50} (µg/ml) of BT in each graph and Dose B stands for the EC_{50} of OG or OS or OC. The straight line (additive line) is made by Dose A and B (EC_{50} value). If the square symbol lies below the line of additivity it indicates synergistic interaction. When it is plotted on this line, it shows an additive effect. If drawn above the line, it is directed towards antagonistic interaction.



(G) **(H)** **(I)**

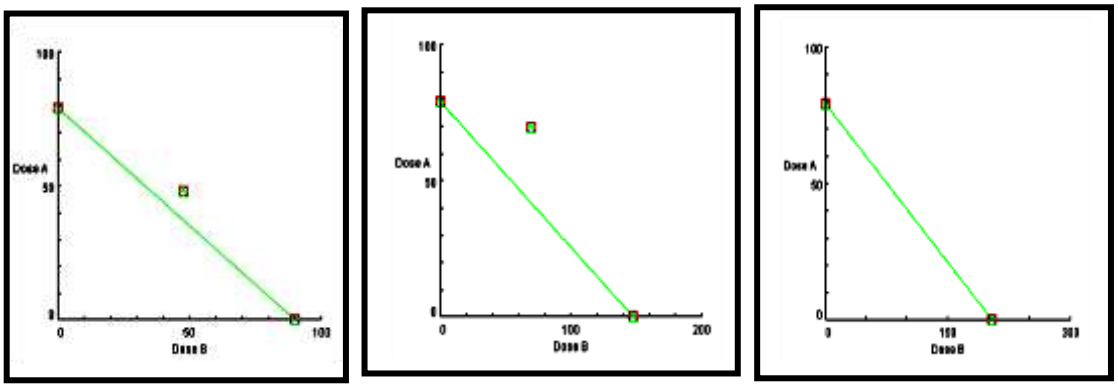
Fig. 37: Isobolographic plot of Black Tea with *O. grattissimum* [BTOG (G)], Black tea BT with *O. sanctum* [BTOS (H)], and Black tea BT with *O. canum* Sims [BTOC (I)] at 1:1 ratio in NO assay.

It is a graphical representation used in pharmacology to analyze mixture effects. Dose A represents the EC_{50} (µg/ml) of BT in each graph and Dose B stands for the EC_{50} of OG or OS or OC. The straight line (additive line) is made by Dose A and B (EC_{50} value). If the square symbol lies below the line of additivity it indicates synergistic interaction. When it is plotted on this line, it shows an additive effect. If drawn above the line, it is directed towards antagonistic interaction.



(J) **(K)** **(L)**

Fig. 38: Isobolographic plot of Black Tea with *O. gratissimum* [BTOG (J)], Black tea BT with *O. sanctum* [BTOS (K)], and Black tea BT with *O. canum* [BTOC (L)] at 1:1 ratio in LPO assay. It is a graphical representation used in pharmacology to analyze mixture effects. Dose A represents the EC_{50} (µg/ml) of BT in each graph and Dose B stands for the EC_{50} of OG or OS or OC. The straight line (additive line) is made by Dose A and B (EC_{50} value). If the square symbol lies below the line of additivity it indicates synergistic interaction. When it is plotted on this line, it shows an additive effect. If drawn above the line, it is directed towards antagonistic interaction.



(M) **(N)** **(O)**

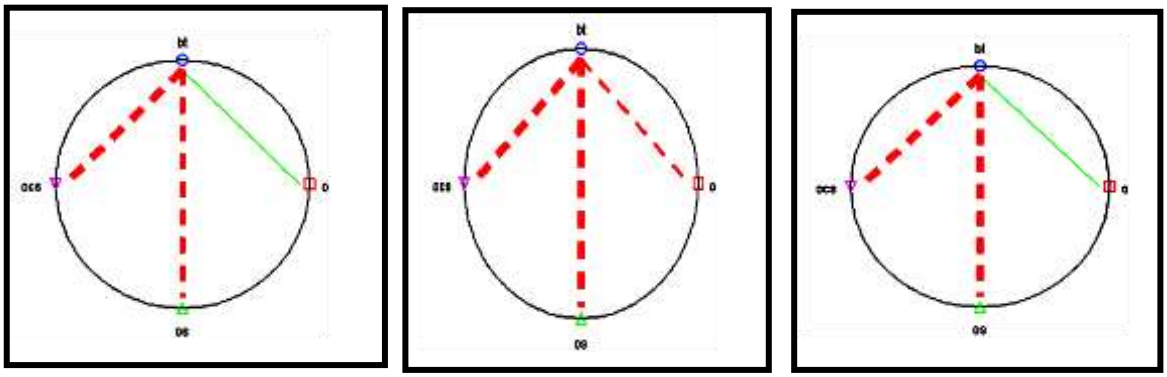
Fig. 39: Isobolographic plot of Black Tea with *O. grattissimum* [BTOG (M)], Black tea with *O. sanctum* [BTOS (N)], and Black tea with *O. canum* [BTOC (O)] at 1:1 ratio in Hemolytic assay. It is a graphical representation used in pharmacology to analyze mixture effects. Dose A represents the EC_{50} (µg/ml) of BT in each graph and Dose B stands for the EC_{50} of OG or OS or OC. The

straight line (additive line) is made by Dose A and B (EC_{50} value). If the square symbol lies below the line of additivity it indicates synergistic interaction. When it is plotted on this line, it shows an additive effect. If drawn above the line, it is directed towards antagonistic interaction.

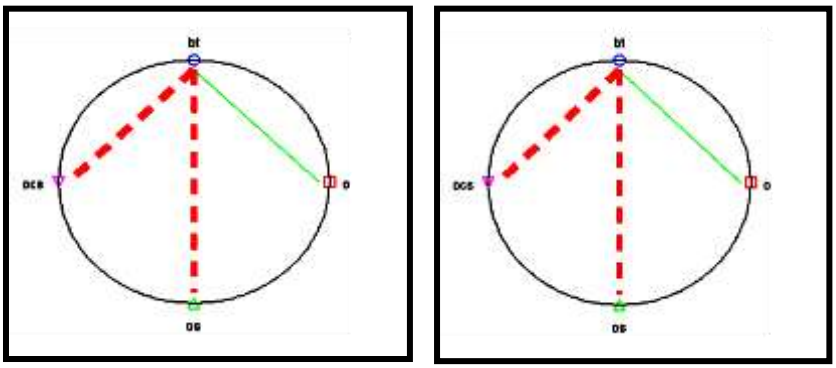
7.2.2 Polygonogram of various combinations in different assays

In the DPPH test, BTOG (CI=1.13) showed nearly additive (green line, less thickness), BTOS (CI=1.35) - moderate antagonism (red line, more thickness), and BTOC (CI=1.84) – antagonism (red line, more thickness) at EC_{50} as shown in **Fig. 40** and **Table 15**.

BTOG (CI=1.13) displayed also slight antagonism (red line, less thickness), BTOS (CI=2.18) – moderate (red line, more thickness), and BTOC (CI=2.21) – antagonism interaction (red line, more thickness) in the ABTS model at EC_{50}. In the NO test, BTOG (CI=1.04) showed nearly additive (green line, less thickness), BTOS (CI=1.54) – antagonism (red line, more thickness), and BTOC (CI=1.84) - antagonism interaction (red line, more thickness). For lipid peroxidation, nearly additive interaction (green line, less thickness) was found in BTOG (CI=1.06), moderate antagonism (red line, more thickness) in BTOS (CI=1.32), and strong antagonism interaction (red line, more thickness) was found in BTOC (CI=1.47). Whereas, in the anti-hemolytic test, BTOG (CI=1.06) expressed nearly additive (green line, less thickness), BTOS (CI=1.45) – moderate antagonism (red line, less thickness), and BTOC (CI=2.59) – antagonism interaction (red line, more thickness) respectively as depicted as shown in **Fig. 40** and **Table 15**.



P **Q** **R**



S T

Fig. 40: Polygonogram representation of Black Tea with *O. grattissimum* (BTOG), Black tea with *O. sanctum* (BTOS), and Black tea with *O. canum* Sims (BTOC) at 1:1 ratio in DPPH (P), ABTS (Q), NO (R), LPO (S) & hemolytic assay (T).

6.2.3 Phytochemical analysis of Black Tea (BT), *O. gratissimum* (OG), *O. sanctum* (OS) and *O. canum* (OC)

6.2.3.1 Estimation of phenolic compounds

OG contained a high amount of phenolic content 166.44 (mg/100 ml) GAE as compared to (OS) – 131.55 (mg/100ml) GAE, BT – 112.72 (mg/100ml) GAE and OC – 34.55 (mg/100ml) GAE, respectively.

BTOG expressed a maximum quantity of phenolic compounds 252.38 (mg/100ml) GAE than BTOS – 207.83 (mg/100ml) GAE, and BTOC - 201.61 (mg/100ml) GAE as depicted in **Fig's. 39(a), (b)** and **Table 16**.

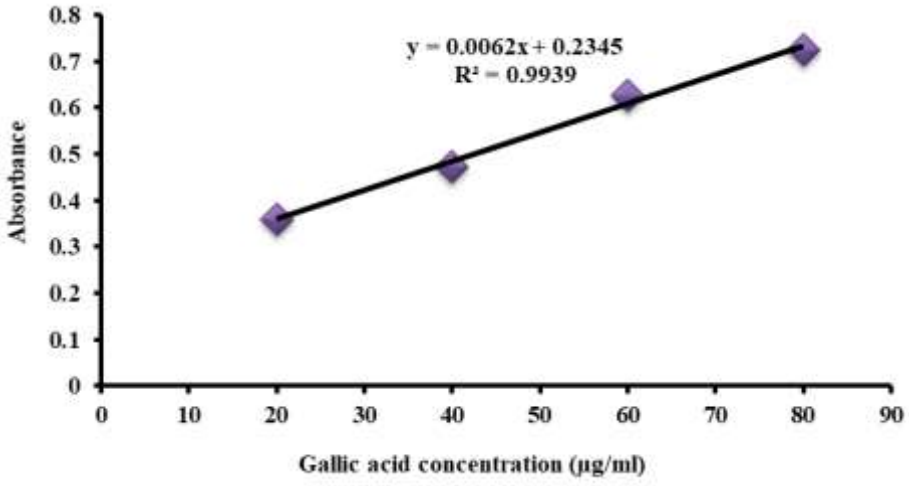


Fig. 41: (a) Standard calibration curve of gallic acid is measured by GAE (gallic acid equivalent)

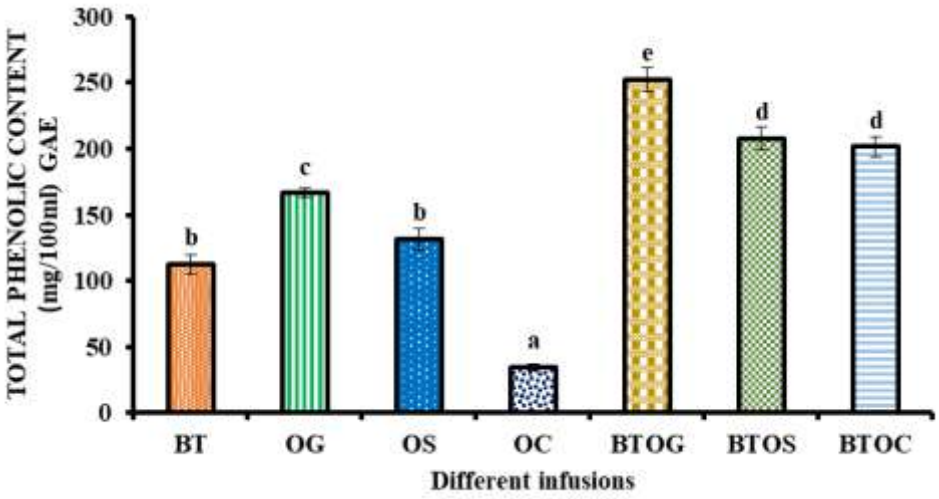


Fig. 41: (b) TPC of Black Tea (BT), *O. gratissimum* (OG), *O. sanctum* (OS), and *O. canum* (OC) and different infusion types. Values are represented as MEAN ± S.D (n = 3 replicates). The dissimilar alphabets illustrated significant difference ($p \leq 0.05$). Values in parenthesis are arcsine transformed values; a-d represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

Table 15: Antioxidant activity and type of interaction of Black Tea and *Ocimum* spp. (*O. gratissimum, O. sanctum, and O. canum*) in various assays.

Assay	Binary combinations	EC_{50} (µg/ml)	CI at EC_{50}	Type of interaction
DPPH	BTOG	95±3.78[a]	1.08	Nearly additive
	BTOS	139±7.88[b]	1.35	Moderate antagonism
	BTOC	210±5.21[c]	1.84	Antagonism
ABTS	BTOG	46±1.81[a]	1.03	Nearly additive
	BTOS	129±5.69[b]	2.18	Antagonism
	BTOC	140±5.22[c]	2.21	Antagonism
NO	BTOG	48±2.77[a]	1.04	Nearly additive
	BTOS	105±7.84[b]	1.54	Antagonism
	BTOC	131±10.97[c]	1.84	Antagonism

LPO	BTOG	55±2.88[a]	1.06	Nearly additive
	BTOS	97±5.43[b]	1.32	Moderate Antagonism
	BTOC	113±5.50[c]	1.47	Antagonism
Anti-hemolytic activity	BTOG	98±4.16[a]	1.06	Nearly additive
	BTOS	162±8.70[b]	1.45	Moderate Antagonism
	BTOC	277±16.14[c]	2.59	Antagonism

Values are represented as MEAN ± S.D; n = 3 replicates. Different alphabets on the column show a significant difference ($p \leq 0.05$) between different infusions (BT-Black Tea, OG-*O. gratissimum*, OS-*O. sanctum*, and OC-*O. canum*. BTOG- Black Tea + *O. gratissimum*, BTOS- Black Tea + *O. sanctum*, BTOC- Black Tea + *O. canum*) for an antioxidant parameter (DPPH, ABTS, NO and LPO). Values in parenthesis are arcsine transformed values; a-c represents the levels of treatments: 'a' = highest effect and 'c' = less effect. EC_{50} defined as (Effective concentration causing 50% scavenging activity); CI (Combination index). The range of CI value from 0.90–1.10 indicates (nearly additive), 1.20–1.45 (moderate antagonism), and 1.45–3.3 (antagonism).

Table 16: The total phenolic concentration was identified in various aqueous infusions of black tea (BT), *O. gratissimum* (OG), *O. sanctum* (OS), and *O. canum* (OC), both individually and in combination.

Sample Extract	Total Phenolic Content (mg GAE/g) per 100ml
BT	112.72 ± 6.99[b]
OG	166.44 ± 3.29[c]
OS	131.55 ± 8.32[b]
OC	34.55 ± 2.82[a]
BTOG	252.38 ± 9.02[e]
BTOS	207.83 ± 8.72[d]
BTOC	201.61 ± 7.93[d]

Values are represented as MEAN ± S.D; n = 3 replicates. Different alphabets on the column show a significant difference ($p \leq 0.05$) between different infusions for the determination of phenolic content (BT-Black Tea, OG-*O. gratissimum*, OS-*O. sanctum*, and OC-*O. canum*. BTOG- Black Tea + *O. gratissimum*, BTOS- Black Tea + *O. sanctum*, BTOC- Black Tea + *O. canum*). Values in parenthesis are arcsine transformed values; a-e represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

6.2.3.2 Estimation of flavonoid compounds

BT contained a high amount of flavonoid content 80.45 (mg/100 ml) QAE as compared to OG – 34.66 (mg/100ml) QAE, OS – 32.57 (mg/100ml) QAE, and OC – 11.32 (mg/100ml) QAE, respectively.

BTOG expressed a maximum quantity of flavonoid 199.28 (mg/100ml) QAE than BTOS – 140.94 (mg/100ml) QAE, and BTOC - 88.87 (mg/100ml) QAE as depicted in **Fig's. 42(a), (b) and Table 17**.

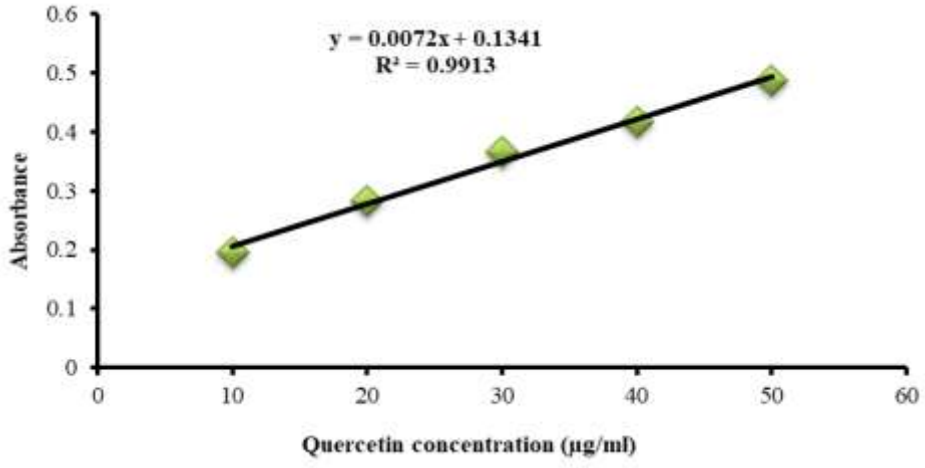


Fig. 42: (a) Standard calibration curve of quercetin measured by QE (quercetin equivalent)

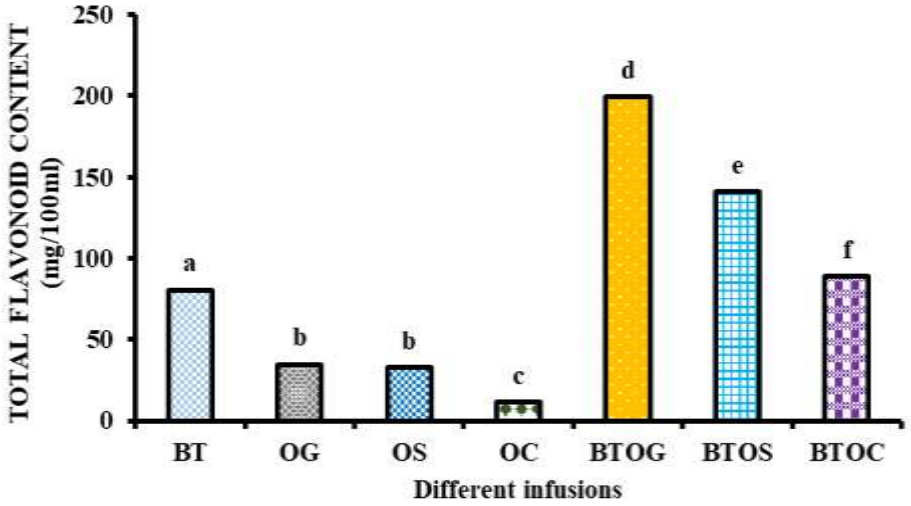


Fig. 42: (b) TFC of Black Tea (BT), *O. gratissimum* (OG), *O. sanctum* (OS), and *O. canum* (OC) and different infusion types. Values are represented as MEAN ± S.D (n = 3 replicates). The dissimilar alphabets illustrated significant difference ($p \leq 0.05$). Values in parenthesis are arcsine

transformed values; a-f represents the levels of treatments: 'a' = highest effect and 'f' = less effect.

Table 17: The total Flavonoid content was identified in various aqueous infusions of black tea (BT), *O. gratissimum* (OG), *O. sanctum* (OS), and *O. canum* (OC), both individually and in combination.

Samples	Total Flavonoid Content (mg QAE/g) per 100ml
BT	80.45 ± 0.16^{a}
OG	34.66 ± 0.26^{b}
OS	32.57 ± 0.26^{b}
OC	11.32 ± 0.40^{c}
BTOG	199.28 ± 0.19^{d}
BTOS	140.94 ± 0.40^{e}
BTOC	88.87 ± 0.30^{f}

Values are represented as MEAN ± S.D; n = 3 replicates. Different alphabets on the column show a significant difference ($p \leq 0.05$) between different infusions for the determination of flavonoid content - (BT-Black Tea, OG-*O. gratissimum*, OS-*O. sanctum*, and OC-*O. canum*. BTOG- Black Tea + *O. gratissimum*, BTOS- Black Tea + *O. sanctum*, BTOC- Black Tea + *O. canum*). Values in parenthesis are arcsine transformed values; a-f represents the levels of treatments: 'a' = highest effect and 'f' = less effect.

Table 18: Correlation between phenolic level and EC_{50} values of aqueous infusions, both alone and in combination.

SAMPLE EXTRACT	TPC	DPPH	correlation	ABTS	correlation	NO	correlation	LPO	correlation	Anti - haemolysis	correlation
BT	112.72	79.54		37.86		43.97		50.48		83	
OG	166.44	89.93	-0.798	45.53	-0.752	49.21	-0.697	54.48	-0.704	89	-0.881
OS	131.55	147.24		138.47		152.52		136.49		94	
OC	34.55	207.92		192.99		186.19		164.97		146	
BTOG	252.38	47.83		23.45		24.31		27.86		98	
BTOS	207.83	69.72	-0.853	64.86	-0.999	52.69	-0.980	48.61	-0.986	162	-0.839
BTOC	201.61	105.35		70.03		65.68		56.87		277	

Values are represented as MEAN ± S.D, n = 3 replicates. Different alphabetical letters showed significant differences ($P \leq 0.05$) between different infusions of BT – Black Tea, OG – *O. gratissimum*, OS – *O. sanctum*, OC – *O. canum*. BTOG – Black Tea + *O. gratissimum*, BTOS – Black Tea + *O. sanctum*, BTOC – Black Tea + *O. canum* for looking into correlation of phenolics with EC_{50} values of DPPH, NO, LPO and Anti-hemolysis. (TPC – Total Phenolic Content, DPPH – 2,2 – diphenyl 1-1, picryl hydrazyl, ABTS - 2,2'-azino-bis (ethylbenzthiazoline-6-sulfonic acid, NO – Nitric Oxide scavenging activity, LPO – Lipid Peroxidation scavenging activity).

A correlation between phenolic or flavonoid with EC_{50} of antioxidant assays (DPPH, ABTS, NO, and LPO) was expressed with Pearson's correlation coefficient (r). A high negative correlation was found between EC_{50} and total phenolic content [hemolysis (r = -0.881), DPPH (r = -0.789), ABTS (r = -0.752), LPO (r = -0.704), NO (r = -0.697)] or flavonoid content [DPPH (r = -0.810), ABTS (r = -0.789), NO (r = -0.768), LPO (r = -0.769) and hemolysis (r = -0.750)] for individual infusions.

Table 19: Correlation between flavonoid level and EC_{50} values of aqueous infusions, both alone and in combination.

SAMPLE EXTRACT	TFC	DPPH	correlation	ABTS	correlation	NO	correlation	LPO	correlation	Anti-hemolysis	correlation
BT	80.84	79.54		37.86		43.97		50.48		83	
OG	34.66	89.93	-0.810	45.53	-0.789	49.21	-0.768	54.48	-0.769	89	-0.750
OS	32.57	147.24		138.47		152.52		136.49		94	
OC	11.32	207.92		192.99		186.19		164.97		146	
BTOG	199.28	47.83		23.45		24.31		27.86		98	
BTOS	140.94	69.72	-0.985	64.86	-0.925	52.69	-0.984	48.61	-0.977	162	-0.980
BTOC	88.87	105.35		70.03		65.68		56.87		277	

Values are represented as MEAN ± S.D, n = 3 replicates. Different alphabetical letters showed significant differences ($P \leq 0.05$) between different infusions of BT – Black Tea, OG – *O. gratissimum*, OS – *O. sanctum*, OC – *O. canum*. BTOG – Black Tea + *O. gratissimum*, BTOS – Black Tea + *O. sanctum*, BTOC – Black Tea + *O. canum* for looking into correlation of flavonoids with EC_{50} values of DPPH, NO, LPO and Anti-hemolysis. (TPC – Total Flavonoid Content, DPPH – 2,2 – diphenyl 1-1, picryl hydrazyl, ABTS - 2,2'-azino-bis (ethylbenzthiazoline-6-sulfonic acid, NO – Nitric Oxide scavenging activity, LPO – Lipid Peroxidation scavenging activity).

Similarly, a strong and negative correlation was found (BTOG, BTOS, and BTOC) between EC_{50} and total phenolic content [ABTS (r = -0.999), NO (r = -0.980), LPO (r = -0.986), hemolysis (r= -0.839), and DPPH (r = -0.853)] or total flavonoid content [DPPH (r = -0.985), NO (r = -0.984), LPO (r = -0.977), hemolysis (r = -0.980), and ABTS (r = -0.925)] in binary combinations, respectively as illustrated in **Table 18** and **19.** These results indicated that the antioxidant activity of black tea (BT) and different *Ocimum* spp. (*O. gratissimum*, *O. sanctum* and *O. canum*) alone or in binary combinations (BTOG, BTOS, and BTOC) are highly contributed by phenolics or flavonoids.

Table 20: Antioxidant potential of individual and combined infusion in comparison to Black Tea.

Infusion type	% Change in DPPH activity	% Change in ABTS activity	% Change in NO activity	% Change in LPO activity	% Change in anti-haemolytic activity	The average change in scavenging activity
BT	-	-	-	-	-	-
OG	12.03±0.76^{a} **(-)**	16.03±1.00^{a} **(-)**	19.00±5.78^{a} **(-)**	7.46±1.60^{a} **(-)**	8.33±1.52^{a} **(-)**	12.57±4.94 **(-)**
OS	46.30±0.65^{b} **(-)**	72.66±0.75^{b} **(-)**	73.66±1.92^{b} **(-)**	61.13±0.25^{b} **(-)**	14.00±3.00^{b} **(+)**	47.60±24.72 **(-)**
OC	61.00±0.91^{c} (-)	80.33±0.05^{c} **(-)**	79.00±0.36^{c} **(-)**	69.50±0.85^{c} **(-)**	44.33±1.52^{c} **(-)**	69.95±14.93 **(-)**
BTOG	68.50±4.67^{d} **(+)**	61.56±5.60^{d} **(+)**	68.50±17.17^{d} **(+)**	88.36±16.69^{d} **(+)**	68.33±6.02^{d} **(+)**	71.05±10.12 **(+)**
BTOS	14.66±1.10^{e} **(+)**	41.73±1.35^{e} **(-)**	28.20±0.70^{e} **(-)**	3.33±0.76^{e} **(+)**	2.66±1.52^{e} **(+)**	10.00±16.79 **(-)**
BTOC	24.33±2.02^{f} **(-)**	46.20±0.75^{f} **(-)**	42.56±2.85^{f} **(-)**	37.16±44.40^{f} **(-)**	40.33±0.57^{f} **(-)**	38.11±8.38 **(-)**

Values are represented as MEAN ± S.D, n = 3replicates. Different alphabetical letters on the column showed significant differences ($P \leq 0.05$) between different infusions BT – Black Tea, OG – *O. gratissimum*, OS – *O. sanctum*, OCS – *O. canum*. BTOG – Black Tea + *O. gratissimum*, BTOS – Black Tea + *O. sanctum*, BTOC– Black Tea + *O. canum* for the determination of antioxidant potential via different parameters of various infusion types in alone and in combination such as (DPPH – 2,2 – diphenyl 1-1, picryl hydrazyl, ABTS - 2,2'-azino-bis(ethylbenzthiazoline-6-sulfonic acid, NO – Nitric Oxide scavenging activity, LPO – Lipid Peroxidation scavenging activity). Values in parenthesis are arcsine transformed values; a-f represents the levels of treatments: 'a' = highest effect and 'f ' = less effect.

Interestingly, the addition of *O. gratissimum* to black tea substantially enhanced the antioxidant capability (71.05%) but supplementation of *O. sanctum* demonstrated a decline in overall scavenging activity (10.00%). However, tea (38.11%). While, BTOG combination displayed nearly additive interaction in various antioxidant assays whereas BTOS exhibited moderate antagonism to antagonism, and BTOC showed antagonism in all studied antioxidant parameters as illustrated in **Fig's. 33, 34, 35, 36, 37, 38** and **Table 15**.

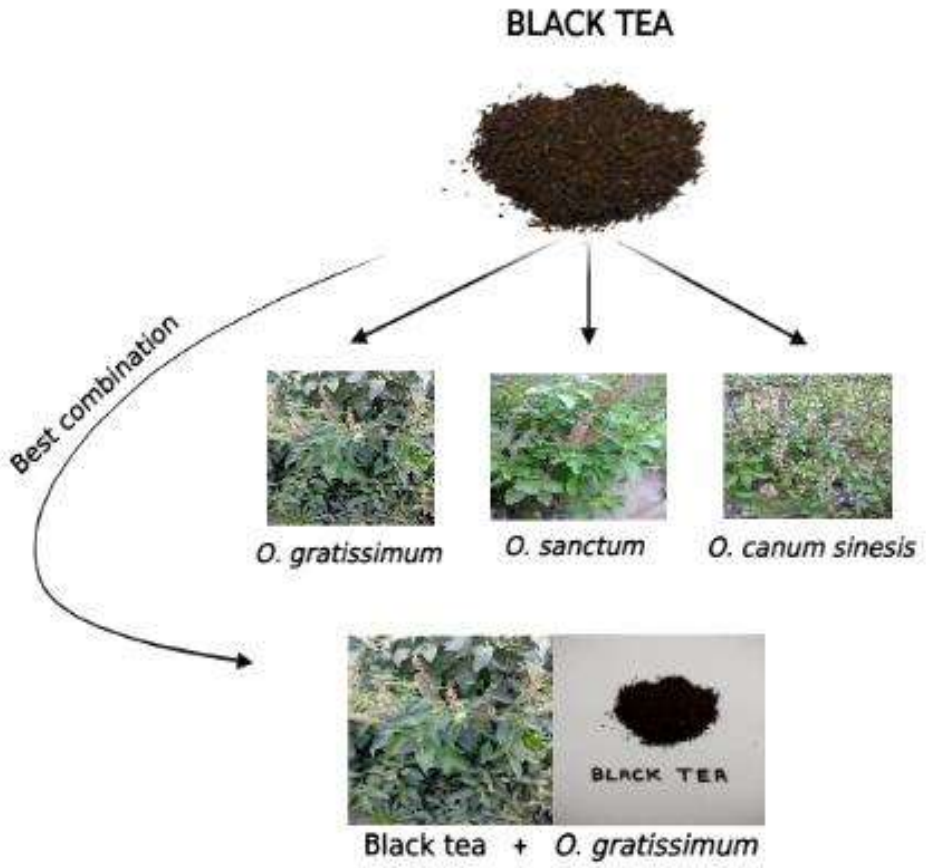


Fig. 43: Systematic diagram of preliminary screening of Black Tea and its best combination

The supplementation of *O. gratissimum* to Black Tea enhanced the radical quenching ability from 68% to 88% in various antioxidant assays performed. The binary combination of Black Tea and *O. gratissimum* was found to be the best among the studied pairs in terms of antioxidant potential as shown in **Fig. 43**.

6.3 Determining the effect of their proportion on antioxidant and antigenotoxic potential of most active black tea and *O. gratissimum* combination.

6.3.1 DPPH assay

It was observed that black tea contained significantly higher antioxidant potency with less EC_{50}: 78.23 µg/ml than *O. gratissimum* (EC_{50}: 93.35 µg/ml), respectively.

The effect of antioxidant potency between BT and OG combination in different ratios

The maximum antioxidant potential was noted in 3:1- 89.13 and 1:2 - 91.43 µg/ml, followed by 1:1 - 93.24 µg/ml, 1:3- 106.96 µg/ml) and 2:1 - 91.72 µg/ml, respectively shown in **Fig. 44** and **Table 21**.

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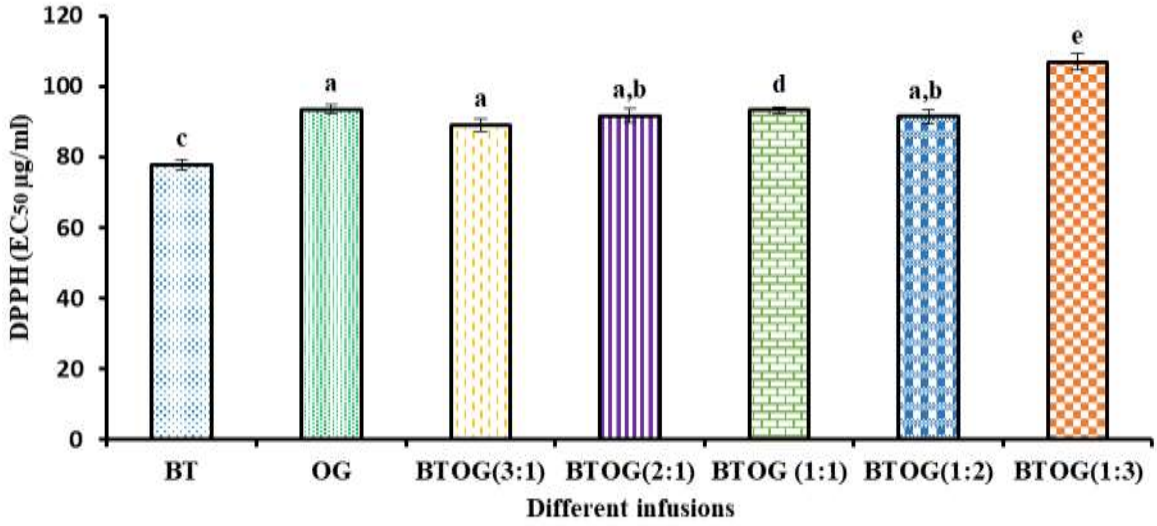


Fig. 44: DPPH scavenging activity of Black Tea (BT) and *O. gratissimum* (OG) infusions in a different ratio. EC_{50} (Effective concentration showing 50% scavenging activity); Values are represented as MEAN± S.D of 3 replicates). Different alphabets on the histogram columns depicts significant difference at $p \leq 0.05$ among the different infusions of BT and OG with respect to DPPH activity. Values in parenthesis are arcsine transformed values; a-e represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

Table 21: EC_{50} values of DPPH scavenging activity of black tea (BT) and *O. gratissimum* (OG) infusions in different ratios.

Sample Extract	EC_{50} (µg/ml)
BT	78.23[c]
OG	93.35[a]
BTOG (1:1)	93.24[a]
BTOG (1:2)	91.43[a,b]
BTOG (1:3)	106.96[d]
BTOG (2:1)	91.72[a,b]
BTOG (3:1)	89.13[a]

Values are represented as MEAN ± S.D, n = 3 replicates. EC_{50} indicates (Effective concentration causing 50% scavenging activity. Same alphabets showed insignificant differences ($P \leq 0.05$) between different infusions of BT – Black Tea, OG – *O. gratissimum*, BTOG – Black Tea + *O. gratissimum*. Different alphabets showed significant differences ($P \leq 0.05$) between different infusions BT – Black Tea, OG – *O. gratissimum*, BTOG – Black Tea + *O. gratissimum* for an antioxidant parameter (DPPH – 2,2 diphenyl 1,1 picryl hydrazyl) in different ratios. Values in parenthesis are arcsine transformed values; a-d represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

6.3.2 ABTS assay

It was noted that black tea contained significantly higher antioxidant potency with less EC_{50}: 44.37 µg/ml than *O. gratissimum* (EC_{50}: 56.70 µg/ml), respectively.

The effect of antioxidant potency between BT and OG combination in different ratios

The maximum antioxidant potential was noted in 3:1 - 51.13 and 1:2 - 55.11 µg/ml, followed by 1:1 - 59.58 µg/ml, 1:3 - 60.51 µg/ml, and 2:1- 57.59 µg/ml, respectively as shown in **Fig. 45** and **Table 22**.

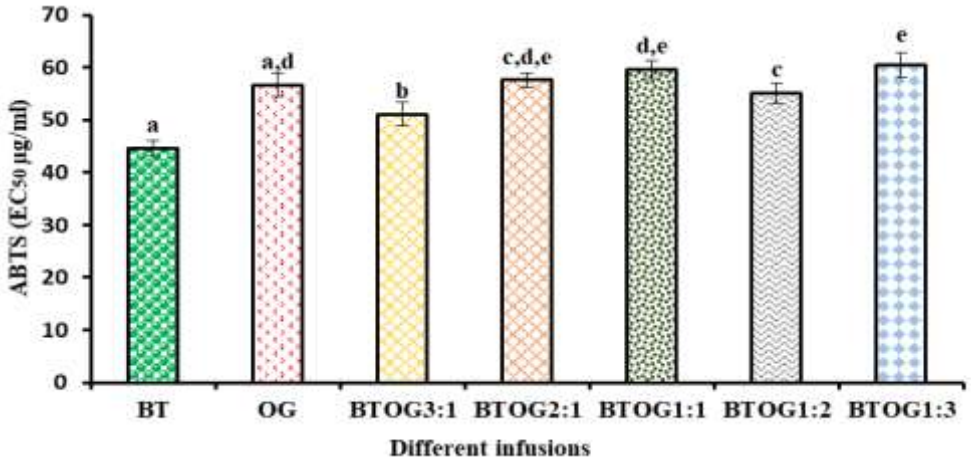


Fig. 45: ABTS scavenging activity of Black Tea (BT) and *O. gratissimum* (OG) infusions in a different ratio. EC_{50} (Effective concentration showing 50% scavenging activity); Values are represented as MEAN± S.D of 3 replicates). Different alphabets on the histogram columns depicts significant difference at $p \leq 0.05$ among the different infusions of BT and OG with respect to DPPH activity. Values in parenthesis are arcsine transformed values; a-e represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

Table 22: EC_{50} values of ABTS scavenging activity of black tea (BT) and *O. gratissimum* (OG) infusions in different ratios.

Sample Extract	EC_{50} (µg/ml)
BT	44.37^{a}
OG	$56.70^{a,d}$
BTOG (3:1)	51.13^{b}
BTOG (2:1)	$57.59^{c,d,e}$
BTOG (1:1)	$59.58^{d,e}$
BTOG (1:2)	55.11^{c}
BTOG (1:3)	60.51^{e}

Values are represented as MEAN ± S.D, n = 3 replicates. EC_{50} indicates (Effective concentration causing 50% scavenging activity. Same alphabets showed insignificant differences ($P \leq 0.05$) between different infusions of BT – Black Tea, OG – *O. gratissimum*, BTOG – Black Tea + *O. gratissimum*. Different alphabets showed significant differences ($P \leq 0.05$) between different infusions BT – Black Tea, OG – *O. gratissimum*, BTOG – Black Tea + *O. gratissimum* for an antioxidant parameter (ABTS- 2,2'-azino-bis (ethylbenzthiazoline-6-sulfonic acid) in different ratios. Values in parenthesis are arcsine transformed values; a-e represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

6.3.3 NO assay

Black tea contained significantly higher antioxidant activity with lower effective concentration (EC_{50}: 48.35 µg/ml) than *O. gratissimum* (EC_{50}: 50.61 µg/ml), respectively.

The effect of antioxidant potency between BT and OG combination in different ratios

The maximum antioxidant capability was reported in 3:1- 47.99 µg/ml and 1:2 – 46.36 µg/ml then 1:1- 55.77 µg/ml, 1:3- 84.35 µg/ml and 2:1- 49.96 µg/ml, respectively as shown in **Fig. 46** and **Table 23**.

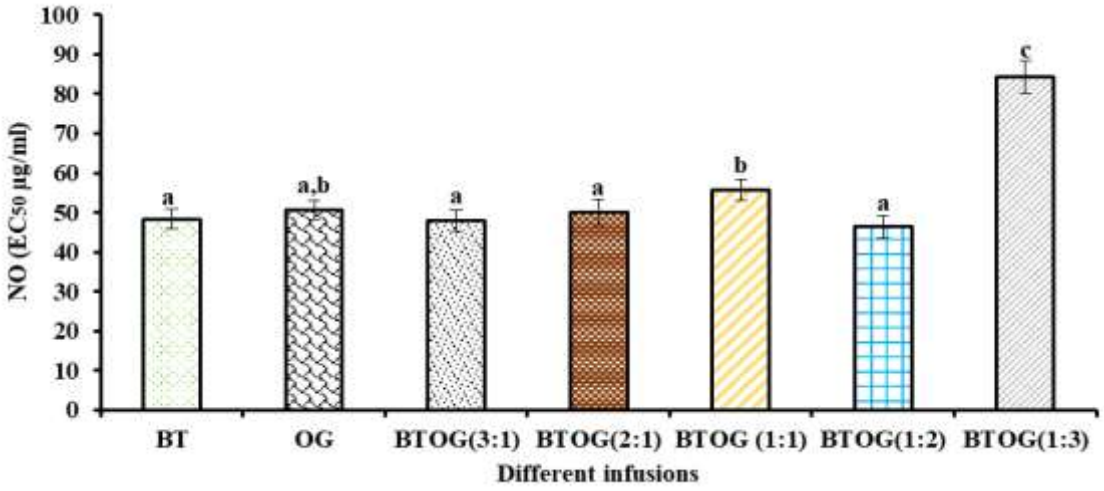


Fig. 46: NO scavenging activity of Black Tea (BT) and *O. gratissimum* (OG) infusions in a different ratio. EC_{50} (Effective concentration showing 50% scavenging activity); Values are represented as MEAN± S.D of 3 replicates). Different alphabets on the histogram columns depicts significant difference at $p \leq 0.05$ among the different infusions of BT and OG with respect to DPPH activity. Values in parenthesis are arcsine transformed values; a-b represents the levels of treatments: 'a' = highest effect and 'b' = less effect.

Table 23: EC_{50} values of NO scavenging activity of black tea (BT) and *O. gratissimum* (OG) infusions in different ratios.

Sample Extract	EC_{50} (µg/ml)
BT	48.95[a]
OG	50.61[a,b]
BTOG (3:1)	47.99[a]
BTOG (2:1)	49.96[a]
BTOG (1:1)	55.77[b]
BTOG (1:2)	49.96[a]
BTOG (1:3)	47.99[a]

Values are represented as MEAN ± S.D, n = 3 replicates. EC_{50} indicates (Effective concentration causing 50% scavenging activity. Same alphabets showed insignificant differences ($P \leq 0.05$) between different infusions of BT – Black Tea, OG – *O. gratissimum*, BTOG – Black Tea + *O. gratissimum*. Different alphabets showed significant differences ($P \leq 0.05$) between different infusions BT – Black Tea, OG – *O. gratissimum*, BTOG – Black Tea + *O. gratissimum* for an antioxidant parameter (NO – Nitric Oxide scavenging activity) in different ratios. Values in parenthesis are arcsine transformed values; a-b represents the levels of treatments: 'a' = highest effect and 'b' = less effect.

6.3.4 LPO assay

Black tea contained significantly higher antioxidant activity with increased concentration (EC_{50}: 43.81 µg/ml) than *O. gratissimum* (EC_{50}: 49.28 µg/ml) respectively.

The effect of antioxidant potency between BT and OG combination in different ratios:

The maximum antioxidant capability was reported in 3:1 (43.62 µg/ml) and 1:2 – (50.13 µg/ml), followed by 1:1- (55.35 µg/ml), 1:3- (43.62 µg/ml) and 2:1- (46.72 µg/ml), respectively as shown in **Fig. 47** and **Table 24**.

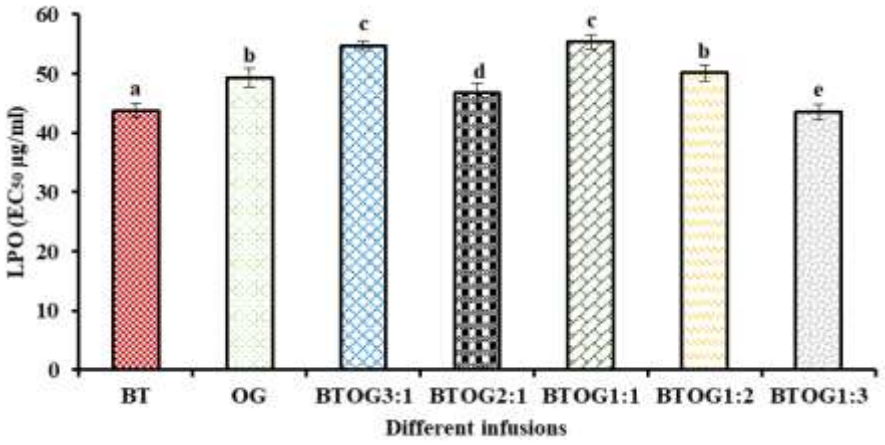


Fig. 47: LPO scavenging activity of Black Tea (BT) and *O. gratissimum* (OG) infusions in a different ratio. EC_{50} (Effective concentration showing 50% scavenging activity); Values are represented as MEAN± S.D of 3 replicates. Different alphabets on the histogram columns depicts significant difference at $p \leq 0.05$ among the different infusions of BT and OG with respect to DPPH activity. Values in parenthesis are arcsine transformed values; a-d represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

Table 24: EC_{50} values of lipid peroxidation scavenging activity of black tea (BT) and *O. gratissimum* (OG) infusions in different ratios.

Sample Extract	EC_{50} (µg/ml)
BT	43.81^{a}
OG	49.28^{b}
BTOG (3:1)	43.62^{c}
BTOG (2:1)	46.72^{d}
BTOG (1:1)	55.35^{c}
BTOG (1:2)	50.13^{b}
BTOG (1:3)	43.62^{a}

Values are represented as MEAN ± S.D, n = 3 replicates. EC_{50} indicates (Effective concentration causing 50% scavenging activity. Same alphabets showed insignificant differences ($P \leq 0.05$) between different infusions of BT – Black Tea, OG – *O. gratissimum*, BTOG – Black Tea + *O. gratissimum*. Different alphabets showed significant differences ($P \leq 0.05$) between different infusions BT – Black Tea, OG – *O. gratissimum*, BTOG – Black Tea + *O. gratissimum* for an antioxidant parameter (LPO – lipid peroxidation scavenging activity) in different ratios. Values in parenthesis are arcsine transformed values; a-d represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

6.3.5 Hemolysis

Black tea confined more antioxidant activity with increased concentration (EC_{50}: 36.03 µg/ml) than *O. gratissimum* (EC_{50}: 39.10 µg/ml), respectively.

The effect of antioxidant potency between BT and OG combination in different ratios

The maximum antioxidant capability was reported in 3:1 (36.72 µg/ml) and 1:2 – (37.67 µg/ml), followed by 1:1- (44.30 µg/ml), 1:3- (45.06 µg/ml) and 2:1- (39.55 µg/ml), respectively as depicted in **Fig. 48** and **Table 25.**

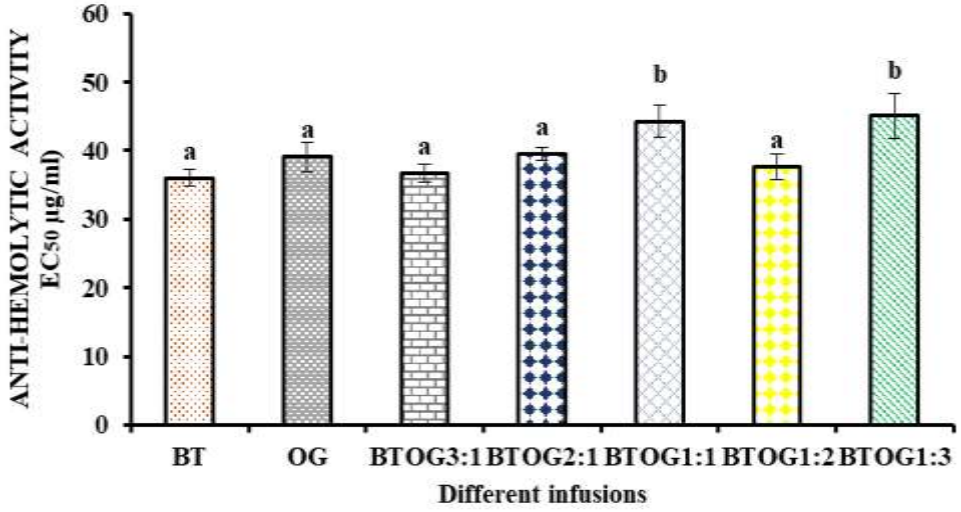


Fig. 48: Antihemolytic scavenging activity of Black Tea (BT) and *O. gratissimum* (OG) infusions in a different ratio. EC_{50} (Effective concentration showing 50% scavenging activity); Values are represented as MEAN± S.D of 3 replicates. Different alphabets on the histogram columns depicts significant difference at $p \leq 0.05$ among the different infusions of BT and OG with respect to DPPH activity. Values in parenthesis are arcsine transformed values; a-b represents the levels of treatments: 'a' = highest effect and 'b' = less effect.

Table 25: EC_{50} values of anti-hemolytic scavenging activity of black tea (BT) and *O. gratissimum* (OG) infusions in different ratios.

Sample Extract	EC_{50} (µg/ml)
BT	36.03[a]
OG	39.10[a]
BTOG (3:1)	36.72[a]
BTOG (2:1)	39.55[a]
BTOG (1:1)	44.30[b]
BTOG (1:2)	37.67[a]
BTOG (1:3)	45.06[b]
BTOG (1:3)	45.06[b]

Values are represented as MEAN ± S.D, n = 3 replicates. EC_{50} indicates (Effective concentration causing 50% scavenging activity. Same alphabets showed insignificant differences ($P \leq 0.05$) between different infusions of BT – Black Tea, OG – *O. gratissimum*, BTOG – Black Tea + *O. gratissimum*. Different alphabets showed significant differences ($P \leq 0.05$) between different infusions BT – Black Tea, OG – *O. gratissimum*, BTOG – Black Tea + *O. gratissimum* for an antioxidant parameter (Anti- hemolytic scavenging activity) in different ratios. Values in parenthesis are arcsine transformed values; a-d represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

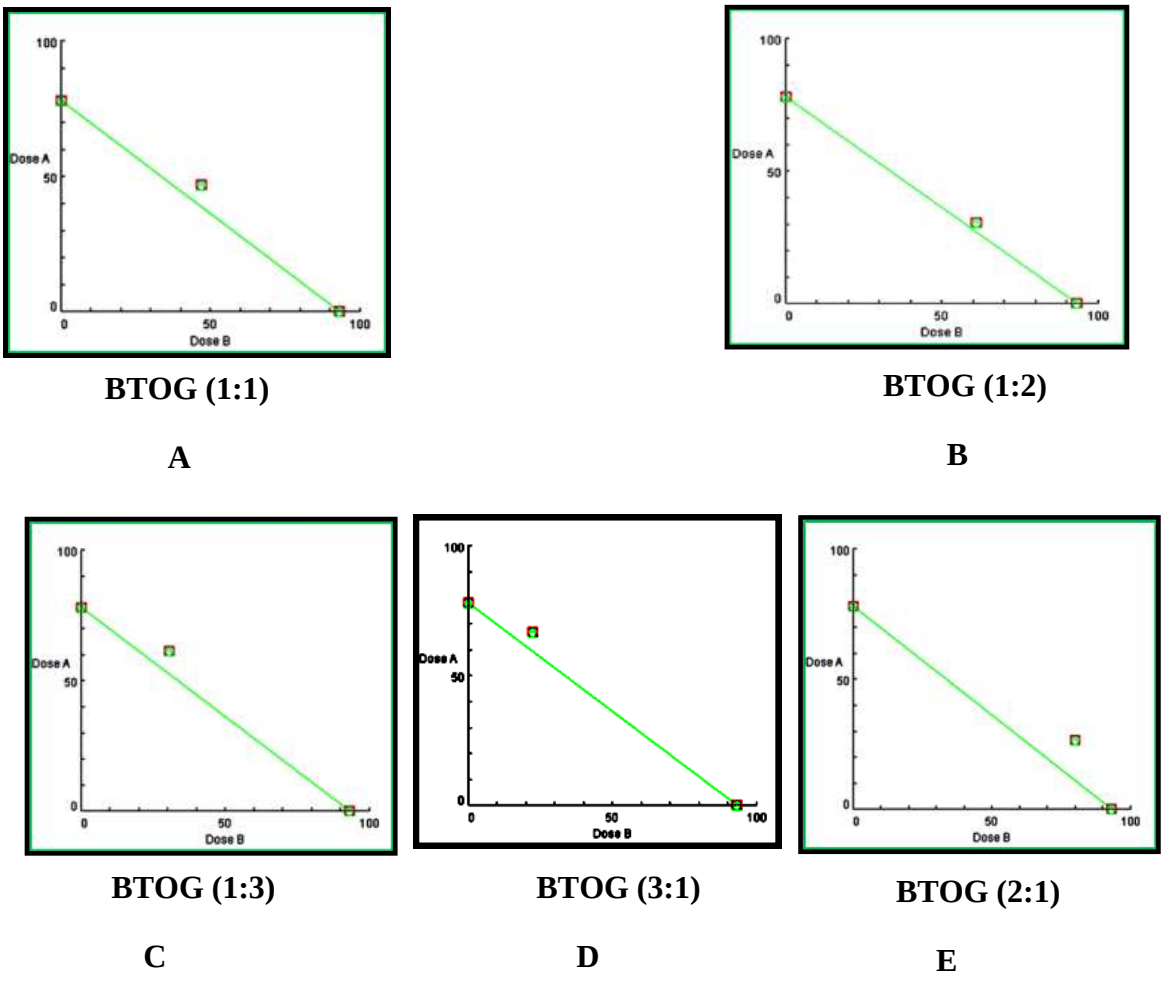
Table 26: The radical quenching capability of individual and combined infusion (BTOG) in different ratios (3:1, 2:1, 1:1, 1:2, and 1:3) in comparison of Black Tea.

Infusion type	% Change in DPPH activity	% Change in ABTS activity	% Change in NO activity	% Change in LPO activity	% Change in anti-hemolytic activity	% Average change in scavenging activity
BT	-	-	-	-	-	-
OG	17.34±1.41[b](-)	23.29±3.96[b](-)	7.01±3.99[a](-)	11.09±0.68[a](-)	10.30±2.16[a](-)	13.80±2.44(-)
BTOG (1:1)	66.64±1.41[a](+)	50.05±5.30[a](+)	69.09±8.49[b](+)	59.55±3.96[b](+)	62.74±9.31[b](+)	61.61±5.69(+)
BTOG (1:2)	69.18±2.37[a](+)	62.31±6.39[c](+)	104.76±8.48[c](+)	86.90±1.95[c](+)	87.67±2.20[c](+)	82.16±4.27(+)
BTOG (1:3)	45.04±1.80[c](+)	48.96±7.86[d](+)	13.39±1.59[d](+)	56.94±2.62[b](+)	64.31±16.21[b](+)	45.72±6.01(+)
BTOG (2:1)	68.64±1.23[a](+)	51.94±3.53[a](+)	92.52±1.41[e](+)	75.06±0.53[d](+)	79.88±4.73[d](+)	73.60±2.28(+)
BTOG (3:1)	74.10±2.07[d](+)	71.83±3.05[e](+)	103.28±3.25[c](+)	96.63±7.71[e](+)	93.81±5.74[e](+)	87.93±4.36(+)

Values are represented as MEAN ± S.D; n = 3 replicates. Different alphabetical letters on the column showed significant differences ($P \leq 0.05$) between different infusions BT – Black Tea, OG – *O. gratissimum*. BTOG – Black Tea + *O. gratissimum* in different ratios (1:1, 1:2, 1:3, 2:1, and 3:1) for the determination of antioxidant potential via different parameters of various infusion types in alone and in combination such as (DPPH – 2,2 – diphenyl 1-1, picryl hydrazyl, ABTS - 2,2'-azino-bis(ethylbenzthiazoline-6-sulfonic acid, NO – Nitric Oxide scavenging activity, LPO – Lipid Peroxidation scavenging activity). Values in parenthesis are arcsine transformed values; a-e represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

6.4 Antioxidant interactions between Black Tea (BT) and *O. grattissimum* (OG) were done by the Combination index (CI), Isobologram, and Polygonogram

6.4.1 Isobologram of Black Tea with *O. gratissimum* in five different ratios



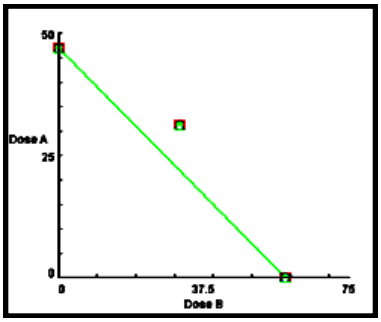
BTOG (1:1) **BTOG (1:2)**

A **B**

BTOG (1:3) **BTOG (3:1)** **BTOG (2:1)**

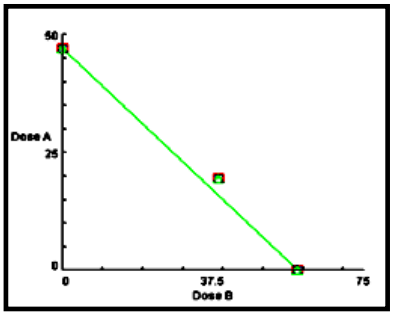
C **D** **E**

Fig. 49: Isobologram plots showing interaction between Black tea (BT) and *O. gratissimum* (OG); having different combination in ratio (1:1, 1:2, 1:3, 2:1, and 3:1) for BT and OG respectively for DPPH scavenging activity (A-E). Dose A represents the EC_{50} (µg/ml) of BT in the graph and Dose B stands for the EC_{50} of OG. The straight line (additive line) is made by dose A and B (EC_{50} value). If the square symbol lies below the line of additivity it indicates synergistic interaction. When it is plotted on this line, it shows an additive effect. If drawn above the line, it is directed toward antagonistic interaction. The data are shown as MEAN ± S.D for 3 independent experiments (each with three replicates of each test point).



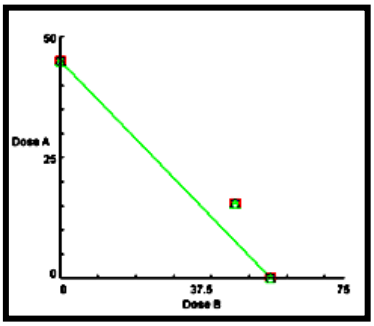
BTOG (1:1)

F



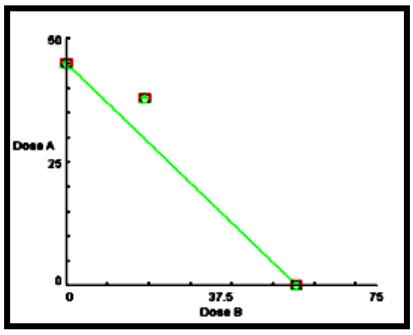
BTOG (1:2)

G



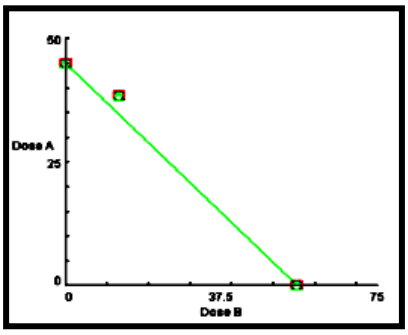
BTOG (1:3)

H



BTOG (2:1)

I



BTOG (3:1)

J

Fig. 50: Isobologram plots showing interaction between Black tea (BT) and *O. gratissimum* (OG); having different combination in ratio (1:1, 1:2, 1:3, 2:1, and 3:1) for BT and OG respectively for ABTS scavenging activity (F-J). Dose A represents the EC_{50} (µg/ml) of BT in the graph and Dose B stands for the EC_{50} of OG. The straight line (additive line) is made by dose A and B (EC_{50} value). If the square symbol lies below the line of additivity it indicates synergistic interaction. When it is plotted on this line, it shows an additive effect. If drawn above the line, it is directed toward antagonistic interaction. The data are shown as MEAN ± S.D for 3 independent experiments (each with three replicates of each test point).

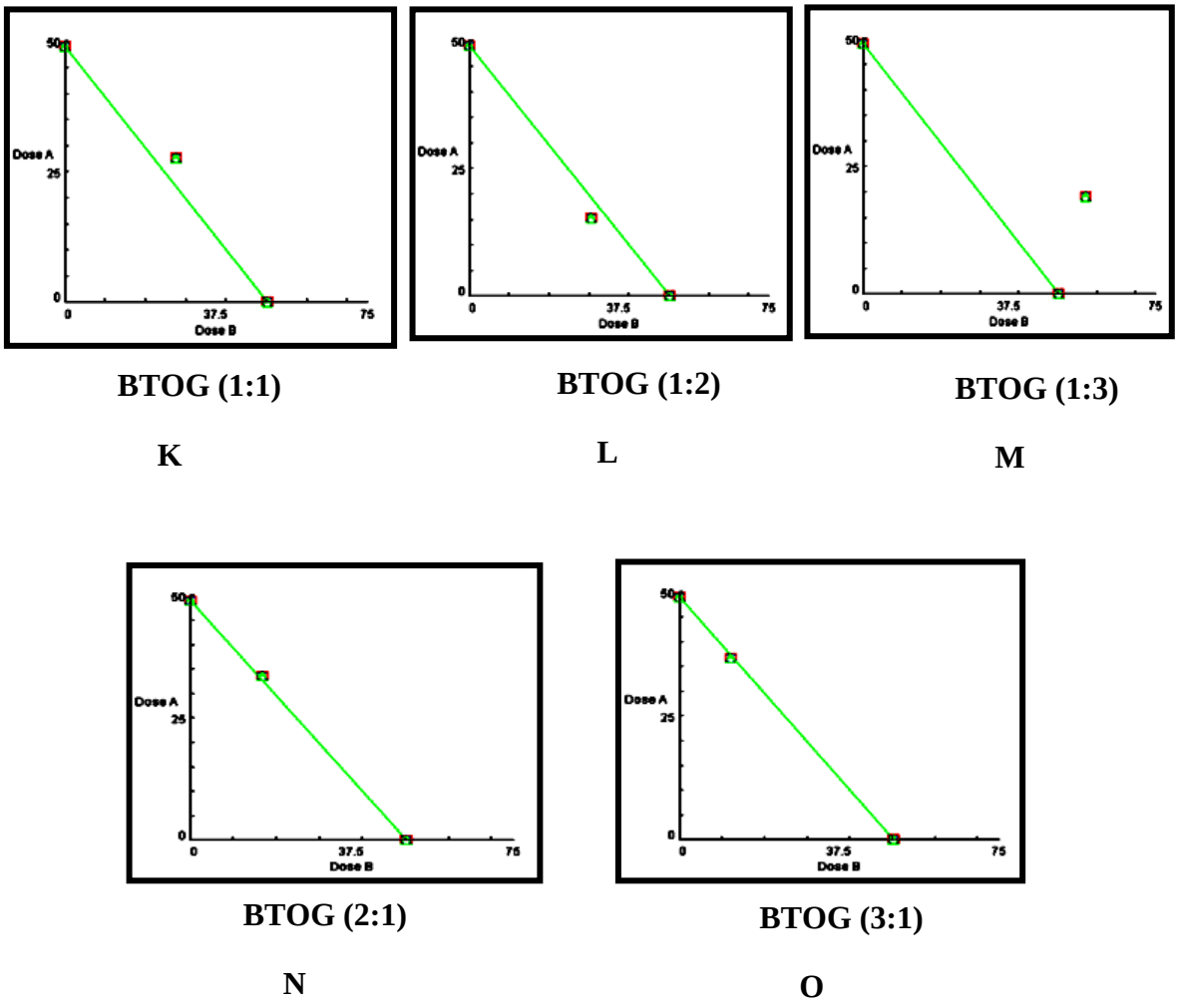


Fig. 51: Isobologram plots showing interaction between Black tea (BT) and *O. gratissimum* (OG); having different combination in ratio (1:1, 1:2, 1:3, 2:1, and 3:1) for BT and OG respectively for NO scavenging activity (K-O). Dose A represents the EC_{50} (µg/ml) of BT in the graph and Dose B stands for the EC_{50} of OG. The straight line (additive line) is made by dose A and B (EC_{50} value). If the square symbol lies below the line of additivity it indicates synergistic interaction. When it is plotted on this line, it shows an additive effect. If drawn above the line, it is directed toward antagonistic interaction. The data are shown as MEAN ± S.D for 3 independent experiments (each with three replicates of each test point).

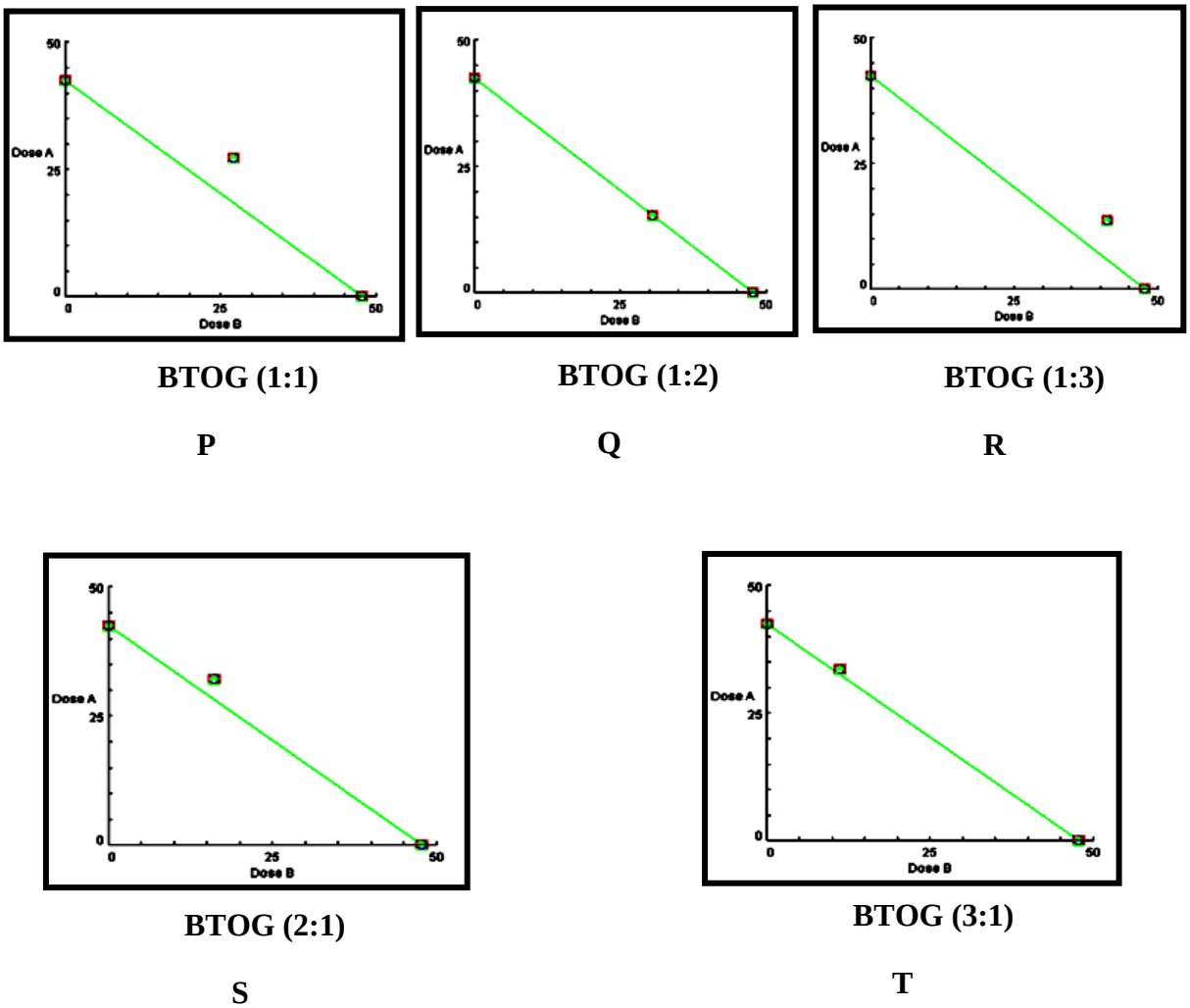
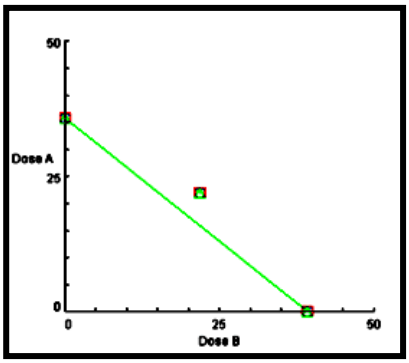
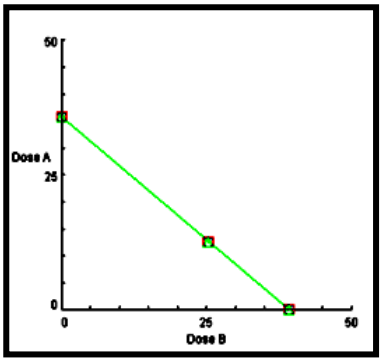


Fig. 52: Isobologram plots showing interaction between Black tea (BT) and *O. gratissimum* (OG); having different combination in ratio (1:1, 1:2, 1:3, 2:1, and 3:1) for BT and OG respectively for LPO scavenging activity (P-T). Dose A represents the EC_{50} (µg/ml) of BT in the graph and Dose B stands for the EC_{50} of OG. The straight line (additive line) is made by dose A and B (EC_{50} value). If the square symbol lies below the line of additivity it indicates synergistic interaction. When it is plotted on this line, it shows an additive effect. If drawn above the line, it is directed toward antagonistic interaction. The data are shown as MEAN ± S.D for 3 independent experiments (each with three replicates of each test point).



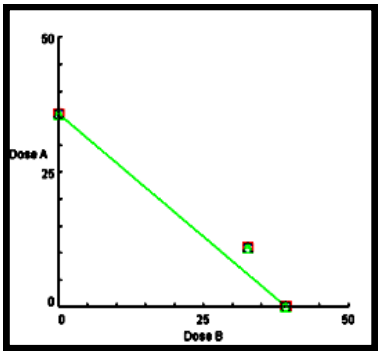
BTOG (1:1)

U



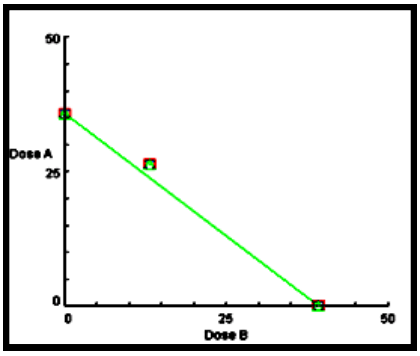
BTOG (1:2)

V



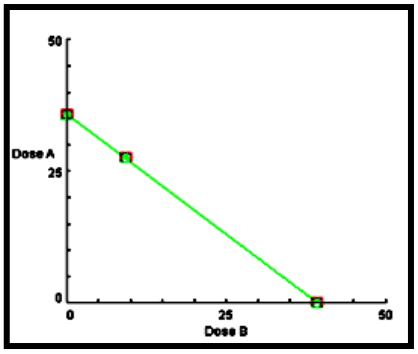
BTOG (1:3)

W



BTOG (2:1)

X



BTOG (3:1)

Y

Fig. 53: Isobologram plots showing interaction between Black tea (BT) and *O. gratissimum* (OG); having different combination in ratio (1:1, 1:2, 1:3, 2:1, and 3:1) for BT and OG respectively for Antihemolytic activity (U-Y). Dose A represents the EC_{50} (µg/ml) of BT in the graph and Dose B stands for the EC_{50} of OG. The straight line (additive line) is made by dose A and B (EC_{50} value). If the square symbol lies below the line of additivity it indicates synergistic interaction. When it is plotted on this line, it shows an additive effect. If drawn above the line, it is directed toward antagonistic interaction. The data are shown as MEAN ± S.D for 3 independent experiments (each with three replicates of each test point).

6.4.2 **Polygonogram plot of combined Black Tea and *O. gratissimum* sample tea extract in five different proportions (3:1, 2:1, 1:1, 1:2, and 1:3) at various antioxidant assays.**

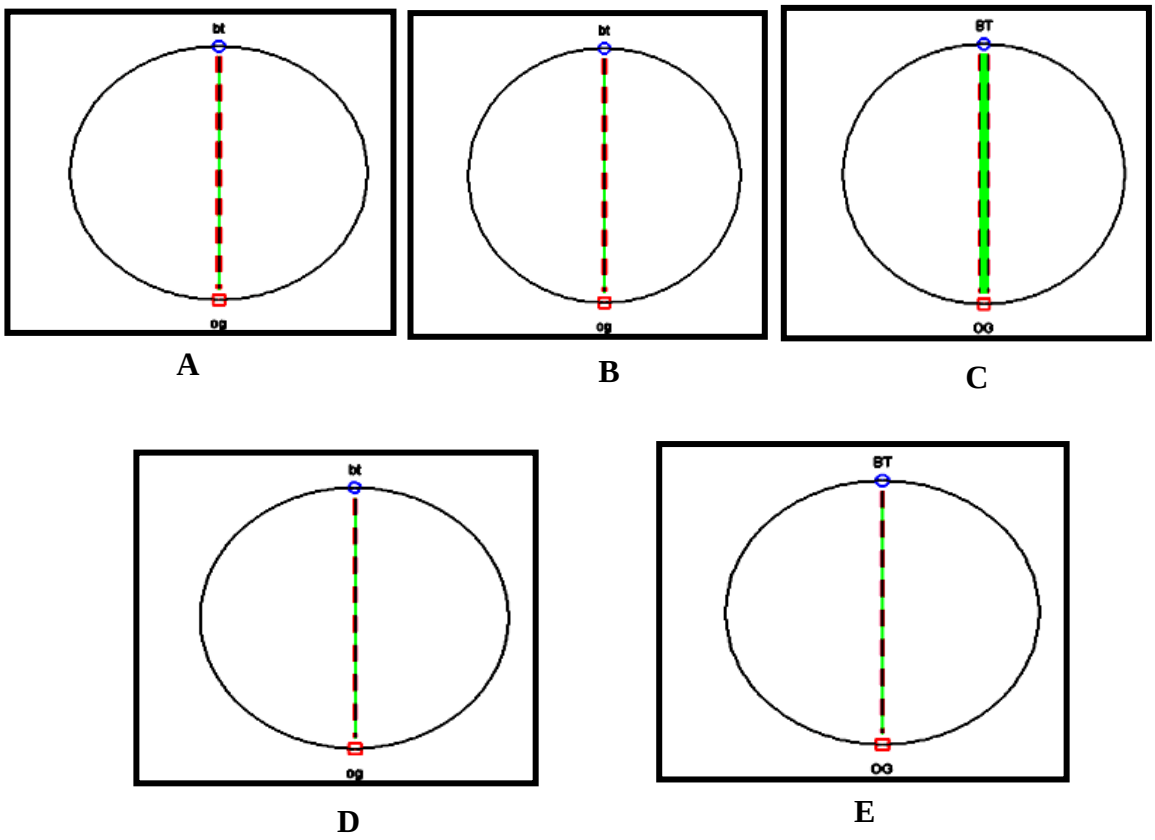


Fig. 54: Polygonogram demonstrating the antioxidant interactions (Additive, Antagonistic and Synergistic) between the black tea and *O. gratissimum* respectively, while combining them in different ratios (3:1, 2:1, 1:1, 1:2, and 1:3) for (A) DPPH (B) ABTS (C) NO (D) LPO and (E) Haemolysis. The data are shown as MEAN ± S.D for 3 independent experiments (each with three replicates of each test point). The 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), lipid peroxidation (LPO) and nitric oxide (NO).

In the DPPH test, BTOG (1:1) (CI = 1.10) showed slight antagonism (red line, less thickness), BTOG (1:2) (CI = 1.04) – nearly additive (green line, less thickness), BTOG (1:3) (CI = 1.20) – slight antagonism (red line, less thickness), BTOG (2:1) (CI = 1.11) – slight antagonism (red line, less thickness), and BTOG (3:1) (CI = 1.09) – nearly additive (green line, less thickness) at EC_{50} as shown in **Fig. 54.**

BTOG (1:1) (CI = 1.20) displayed slight antagonism (red line, less thickness), BTOG (1:2) (CI = 1.07) – nearly additive (green line, less thickness), BTOG (1:3) (CI = 1.17) – slight antagonism (red line, less thickness), BTOG (2:1) (CI = 1.18) – slight

antagonism (red line, less thickness), & BTOG (3:1) (CI = 1.08) – nearly additive interaction (green line, less thickness) in the ABTS test at EC_{50}.

In the NO assay, BTOG (1:1) (CI = 2.14) expressed antagonism (red line, more thickness), BTOG (1:2) (CI = 3.37) – strong antagonism (red line, more thickness), BTOG (1:3) (CI = 2.40) – strong antagonism (red line, more thickness), BTOG (2:1) (CI = 2.57) – strong antagonism (red line, more thickness), & BTOG (3:1) (CI = 0.66) – synergism (green line, more thickness) at EC_{50}. For LPO test. BTOG (1:1) (CI = 1.16) – slight antagonism (red line, less thickness), BTOG (1:2) (CI = 0.98) – nearly additive (green line, less thickness), BTOG (1:3) (CI = 1.15) – slight antagonism (red line, less thickness) BTOG (2:1) (CI = 1.11) – slight antagonism (red line, less thickness), & BTOG (3:1) (CI = 0.95) – nearly additive (green line, less thickness). Whereas, in hemolysis test, BTOG (1:1) (C = 1.12) – slight antagonism (red line, less thickness), BTOG (1:2) (CI = 0.95) – nearly additive (green line, less thickness), BTOG (1:3) (CI = 1.12) – slight antagonism (red line, less thickness), BTOG (2:1) (CI = 1.14) – slight antagonism (red line, less thickness), and BTOG (3:1) (CI = 0.94) – nearly additive interaction (green line, less thickness) respectively as illustrated in **Fig. 54** and **Table 27.**

Table 27: The EC_{50}, combination index, and type of antioxidant interactions of Black Tea and *O. gratissimum* combination for antioxidant parameters.

Assay	Binary combination	EC_{50} (µg/ml)	CI at EC_{50}	Type of interaction
DPPH	1:0	77.86 ± 1.49^{a}	-	
	3:1	89.13 ± 1.80^{b}	1.09	Nearly additive
	2:1	$91.72 \pm 2.05^{b,c}$	1.08	Nearly additive
	1:1	93.24 ± 0.83^{c}	1.10	Slight antagonism
	1:2	$91.43 \pm 1.89^{b,c}$	1.04	Nearly additive
	1:3	106.96 ± 2.30^{d}	1.20	Antagonism
	0:1	93.47 ± 1.33^{c}	-	-
ABTS	1:0	44.57 ± 1.46^{a}	-	-
	3:1	51.13 ± 2.16^{b}	1.08	Nearly additive
	2:1	57.59 ± 1.46^{c}	1.18	Antagonism

	1:1	59.58 ± 1.74^{c,d}	1.20	Antagonism
	1:2	55.11 ± 2.01^{c}	1.07	Nearly additive
	1:3	60.51 ± 2.32^{e}	1.04	Nearly additive
	0:1	56.70 ± 2.34^{c,d}	-	
NO	1:0	48.53 ± 2.57^{a}	-	
	3:1	47.99 ± 2.60^{a}	0.94	Nearly additive
	2:1	49.96 ± 3.35^{a}	1.00	Slight antagonism
	1:1	55.77 ± 2.74^{b}	1.17	Antagonism
	1:2	46.36 ± 2.87^{a}	0.94	Nearly additive
	1:3	84.35 ± 4.10^{c}	1.69	Antagonism
	0:1	50.61 ± 2.30^{a}	-	
LPO	1:0	43.62 ± 0.71^{a}	-	
	3:1	43.62 ± 0.71^{a}	0.98	Nearly additive
	2:1	50.13 ± 1.65^{b}	1.10	Slight antagonism
	1:1	54.64 ± 1.20^{c}	1.18	Antagonism
	1:2	46.72 ± 1.41^{d}	0.99	Nearly additive
	1:3	55.35 ± 1.25^{c}	1.16	Antagonism
	0:1	49.28 ± 1.55^{d}	-	-
Hemolysis	1:0	36.03 ± 1.21^{a}	-	
	3:1	36.72 ± 1.40^{a}	1.00	Nearly additive
	2:1	39.55 ± 0.93^{b}	1.07	Nearly additive
	1:1	44.30 ± 2.26^{c}	1.20	Antagonism
	1:2	37.67 ± 1.95^{a}	0.99	Nearly additive
	1:3	45.06 ± 3.33^{c}	1.21	Antagonism
	0:1	39.10 ± 2.18^{a}	-	-

EC_{50} shows (effective concentration causing 50% scavenging activity), CI (combination index). Data is shown as MEAN ± S.D (n = 3 replicates). The range of CI values and type of interaction as given by Chou (2010) are as follows: < 0.1: very strong synergism, 0.1 – 0.3: strong synergism, 0.3 – 0.7: synergism, 0.7 - 0.85: moderate synergism, 0.85 – 0.90: slight synergism, 0.90

– 1.10: nearly additive, 1.20 – 1.45: moderate antagonism, and 1.45 – 3.3: antagonism. Different alphabets on the histogram columns in the same assay depict significant difference at $p \leq 0.05$ for a assay type. Values in parenthesis are arcsine transformed values; a-e represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

6.4.3 Phytochemical analysis of Black Tea and *O. gratissimum* in different proportions (3:1, 2:1, 1:1, 1:2, and 1:3)

6.4.3.1 Estimation of phenolic compounds

Black tea contained a high amount of phenolic content (134.88 µg/100ml) as compared to *O. gratissimum* (66.88 µg/100ml), respectively.

Black tea and its combination with *O. gratissimum* in five different ratios (1:1, 1:2, 1:3, 2:1, and 3:1)

BTOG in combination with confined maximum phenolic content in all ratios are in the following order as 3:1 (104.27 µg/100ml) > 2:1 (102.55 µg/ml) > 1:2 (98.38 µg/ml) > 1:3 (95.60 µg/ml) > 1:1 (93.60 µg/ml), respectively as illustrated in the **Fig's. 55(a) & (b)** and **Table 28**.

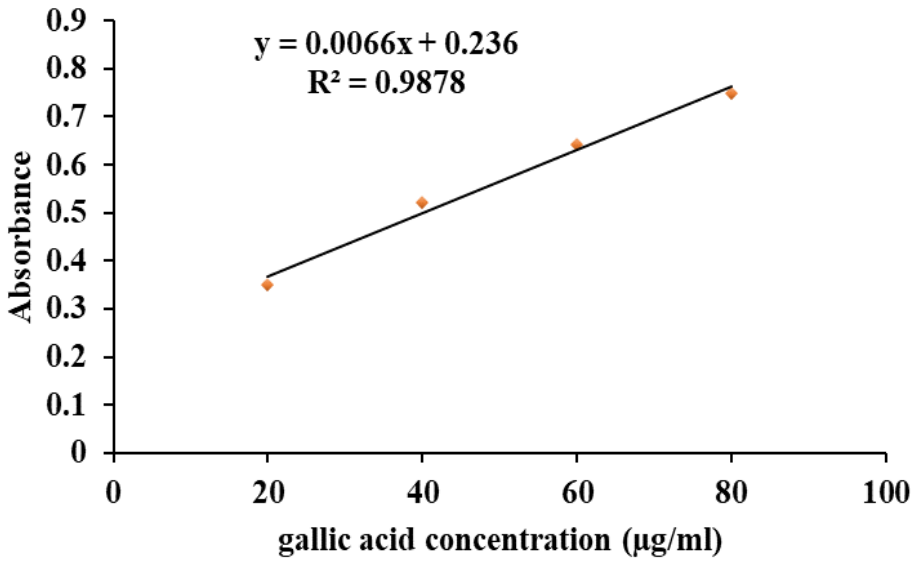


Fig. 55: (a) Standard calibration curve of gallic acid measured by GAE (gallic acid equivalent)

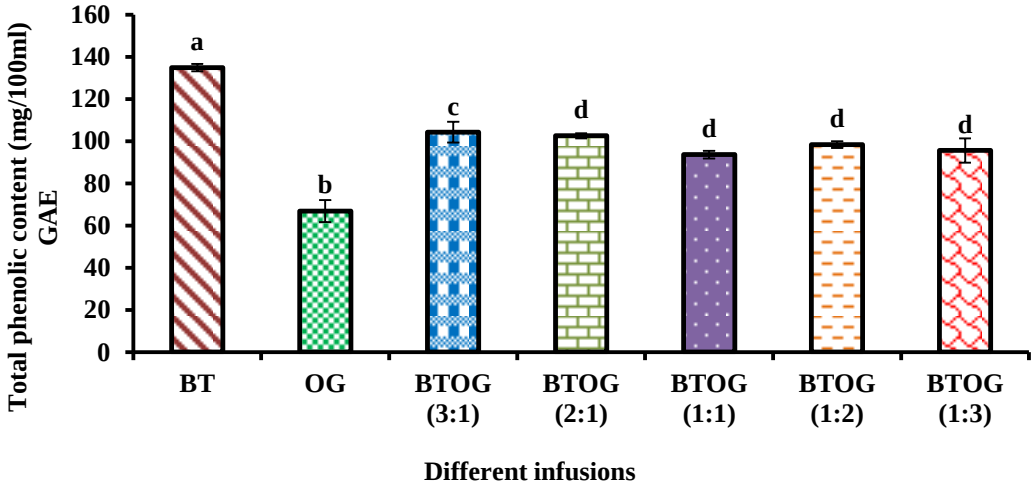


Fig. 55: (b) Total phenolic content of Black tea (BT) and *O. gratissimum* (OG); in different infusions alone and in combination. The data are shown as MEAN ± S.D for 3 independent tests (each with three replicates of each test point). Different alphabets depicted significant difference at $p \leq 0.05$ among the TPC of different infusions. Values in parenthesis are arcsine transformed values; a-d represents the levels of treatments: 'a' = highest effect and 'd' = less effect.

Table 28: Total phenolic content of Black tea (BT) and *O. gratissimum* (OG) sample extracts taken alone and in binary combination having different ratios.

Sample Extract	Total Phenolic Content (mg GAE/g) per 100ml
BT	134.88 ± 1.73^{a}
OG	66.88 ± 5.18^{b}
BTOG (3:1)	104.27 ± 4.94^{c}
BTOG (2:1)	102.55 ± 1.25^{d}
BTOG (1:1)	93.60 ± 1.80^{d}
BTOG (1:2)	98.38 ± 1.64^{d}
BTOG (1:3)	95.60 ± 5.73^{d}

Values are represented as MEAN ± S.D; n = 3 replicates. Different alphabets on the calculated values depicted significant difference at $p \leq 0.05$ among the different infusion's types BT – Black Tea, OG – *O. gratissimum*, BTOG – Black Tea + *O. gratissimum* in different ratios with respect to total phenolic content. Values in parenthesis are arcsine transformed values; a-d represents the levels of treatments: 'a' = highest effect and 'd' = less effect.

6.4.3.2 Determination of flavonoid compounds:

O. gratissimum (83.99 µg/100ml) displayed the highest flavonoid content, followed by black tea (31.61 µg/100ml).

Black tea and its combination with *O. gratissimum* in five different ratios (1:1, 1:2, 1:3, 2:1 and 3:1)

BTOG in combination contained more flavonoid content in five different ratios in the following order: 3:1 (87.32 µg/ml) > 1:1 (86.66 µg/ml) > 1:3 (83.61 µg/ml) > 2:1 (83.33 µg/ml) > 1:2 (83.04 µg/ml), respectively as shown in **Fig's. 56(a) & (b)** and **Table 29**.

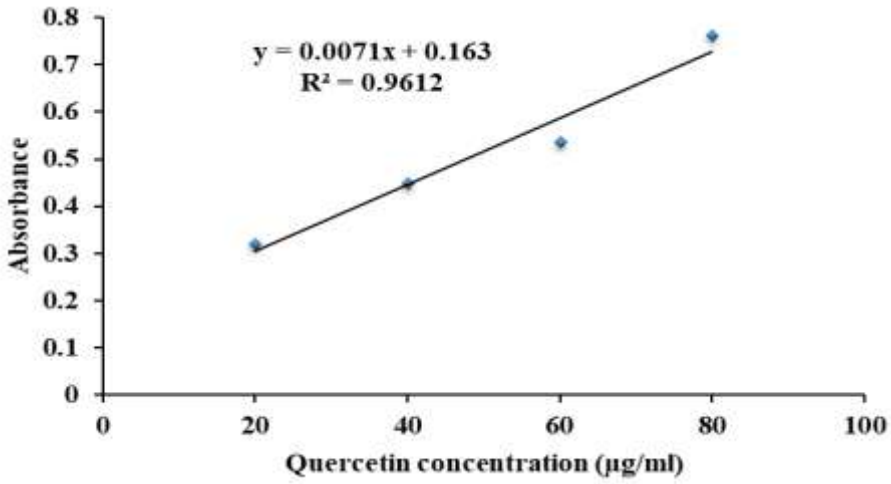


Fig. 56: (a) Standard calibration curve of quercetin measured by QE (quercetin equivalent)

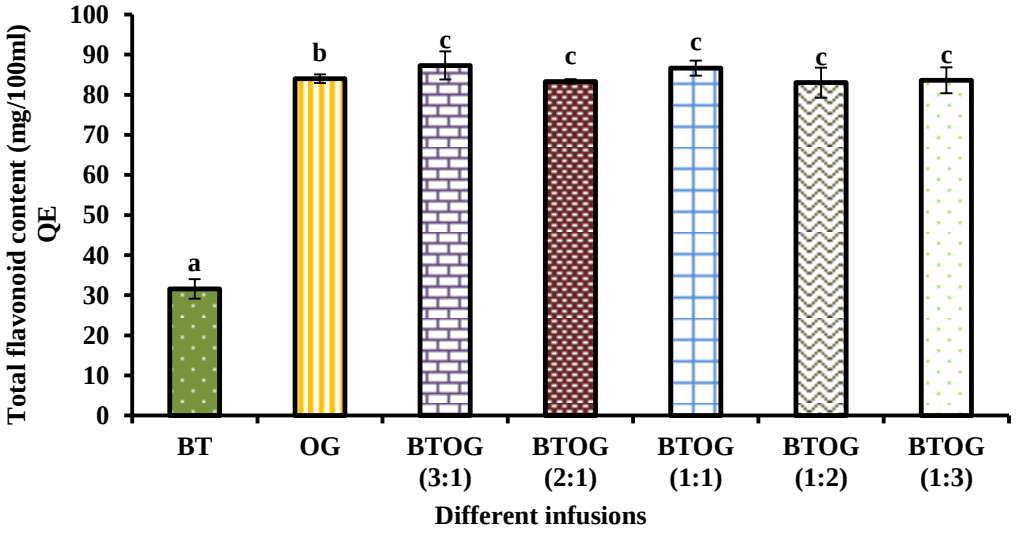


Fig. 56: (b) Total flavonoids content of Black tea (BT) and *O. gratissimum* (OG); in different infusions alone and in combination. The data are shown as MEAN ± S.D for 3 independent tests (each with three replicates of each test point). Different alphabets depicted significant difference at $p \leq 0.05$ among the TFC of different infusions. Values in parenthesis are arcsine transformed values; a-c represents the levels of treatments: 'a' = highest effect and 'c' = less effect.

Table 29: Total flavonoid content of Black tea (BT) and *O. gratissimum* (OG) sample extracts taken alone and in binary combination having different ratios.

Sample Extract	Total Flavonoid Content (mg QE/g) per 100ml
BT	31.61 ± 2.42^{a}
OG	83.99 ± 1.08^{b}
BTOG (3:1)	87.32 ± 3.50^{c}
BTOG (2:1)	83.33 ± 0.53^{c}
BTOG (1:1)	86.66 ± 1.88^{c}
BTOG (1:2)	83.04 ± 3.76^{c}
BTOG (1:3)	83.61 ± 3.23^{c}

Values are represented as MEAN ± S.D; n = 3 replicates. Different alphabets on the calculated values depicted significant difference at $p \leq 0.05$ among the different infusions types BT – Black Tea, OG – *O. gratissimum*, BTOG – Black Tea + *O. gratissimum* in different ratios with respect to total flavonoid content. Values in parenthesis are arcsine transformed values; a-c represents the levels of treatments: 'a' = highest effect and 'c' = less effect.

Table 30: Correlation between phenolic/flavonoid concentration and EC_{50} values of aqueous infusions of BT and OG alone and in combination at five different ratios (3:1, 2:1, 1:1, 1:2 and 1:3).

SAMPLE EXTRACT	TPC	DPPH	correlation	ABTS	correlation	NO	correlation	LPO	correlation	Anti - haemolysis	correlation
BT	134.88	77.86		44.57		48.353		43.81		36.03	
OG	66.88	93.47	-1	56.7	-1	50.615	-1	49.28	-1	39.1	-1
BTOG (1:1)	93.6	93.24		51.13		55.778		43.62		36.72	
BTOG (1:2)	98.38	91.43		57.59		46.363		50.13		39.55	
BTOG (1:3)	95.6	106.96	-0.558978	59.58	0.501807	84.353	-0.535627	54.64	0.3870783	44.3	0.3216738
BTOG (2:1)	102.55	91.72		55.11		49.966		46.72		37.67	
BTOG (3:1)	104.27	89.13		60.51		47.991		55.35		45.06	

Values are represented as mean ± s.d, n = 3 of three independent tests (each with triplicates for each point). BT- Black Tea, OG- *O. gratissimum*, BTOG- Black Tea+ *O. gratissimum* for an antioxidant parameter (DPPH- 2, 2-diphenyl-1-picryl hydrazyl, ABTS- 2,2'-azino-bis(ethylbenzthiazoline-6-sulfonic acid, NO- Nitric oxide scavenging activity, LPO- Lipid Peroxidation) (+) & (-) symbols showed an increase and decrease in activity as compared to Black Tea.

A correlation between phenolic and flavonoid with EC_{50} of antioxidant assays (DPPH, ABTS, NO, and LPO) was expressed with Pearson's correlation coefficient (r). A high negative correlation was found between EC_{50} and total phenolic content [DPPH (r = -1), ABTS (r = -1), LPO (r = -1), NO (r = -1), hemolysis (r = -1),] or strong positive correlation was observed between EC_{50} and flavonoid content [DPPH (r = 1), ABTS (r = 1), NO (r = 1), LPO (r = 1) and hemolysis (r = 1)] for individual infusions. Whereas, various types of correlation were found (BTOG 1:1, BTOG 1:2, BTOG 1:3, BTOG 2:1, and BTOG 3:1) between EC_{50} and total phenolic content [DPPH (r = -0.558, moderate positive), NO (r = -0.535, moderate negative), ABTS (r = -0.501, moderate negative), LPO (r = 0.387, weak positive), and hemolysis (r= 0.321, weak positive)] or total flavonoid content [NO (r = -0.676, strongly positive), DPPH (r = 0.619, strongly positive), ABTS (r = -0.201, weak negative), & hemolysis (r = -0.048, very weak)] in binary combinations, respectively as depicted in **Table 30.**

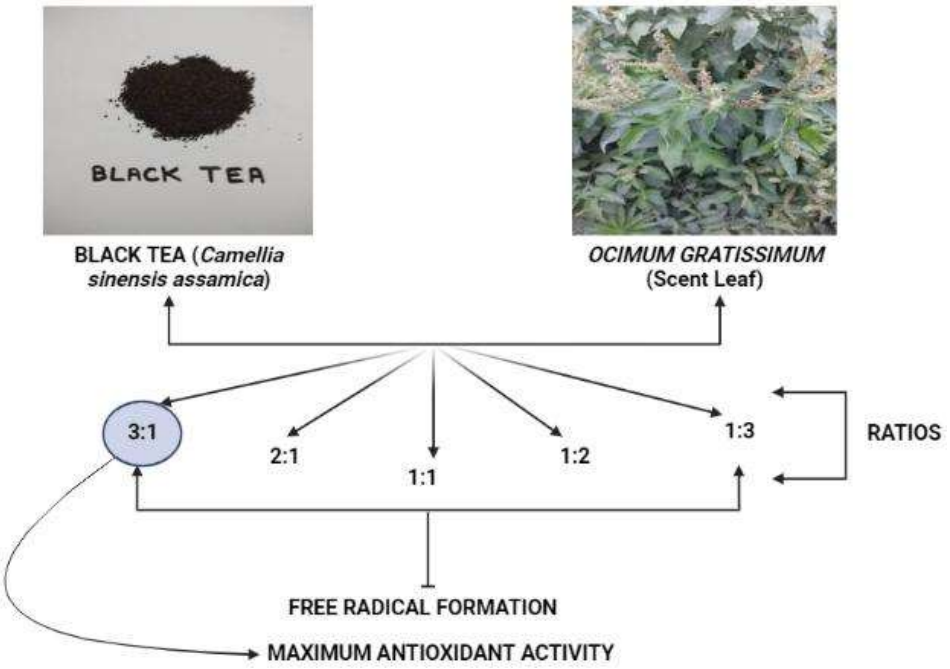


Fig. 57: The maximum antioxidant effect of a 3:1 ratio of Black tea and *O. gratissimum*

O. gratissimum (OG) at the different ratios with Black Tea (BT) alters the radical quenching ability of the binary mixture. Among the ratios, 3:1 showed maximum radical quenching ability in both *in vitro* and *ex vivo* assays, and the interaction observed was additive, as illustrated in **Fig. 47, 48, 49, 50, 51 and 55**.

6.5 Assessment of *S. rebaudiana* (leaves) supplementation to Black Tea and *O. gratissimum* (3:1) on radical quenching ability and H_2O_2 –induced DNA damage

6.5.1 DPPH assay

The activities were measured in terms of EC_{50}; the lower the EC_{50} higher is the antioxidant potential. Stevia (St) displayed the highest ability to reduce DPPH, with an EC_{50} (µg/ml) value of about 26.18 µg/ml followed by BT (38.26 µg/ml) and OG (47.13 µg/ml), respectively. Whereas in the combination black tea (BT) with *O. gratissimum* (OG) expressed maximum quenching ability than other combinations in the following order as BTOG (29.66 µg/ml) > OGSt (34.78 µg/ml) > BTSt (39.66 µg/ml) > BTOGSt (41.75 µg/ml), respectively as shown in **Fig. 58** and **Table 31**.

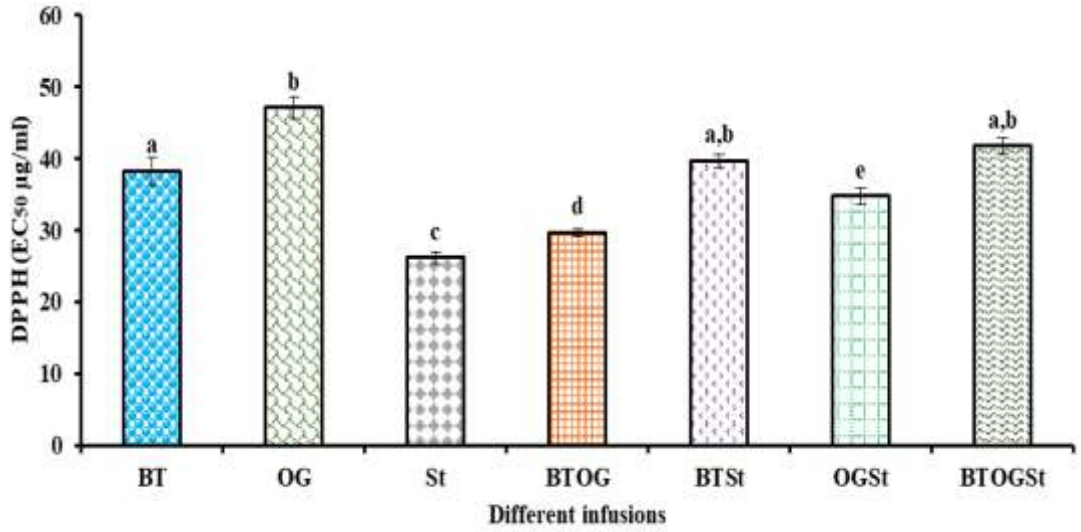


Fig. 58: The DPPH antioxidant power of aqueous extracts including- Black Tea (BT), *O. gratissimum* (OG), and *S. rebaudiana* (St) infusions alone and in combination. EC_{50} undergoes as (Effective concentration causing 50% scavenging activity). Values are represented as MEAN ± S.D n = 3). Different alphabets depicted significant difference at $p \leq 0.05$ among different infusions in alone (BT, OG and St) as well as in combination (BT+OG – Black Tea + *O. gratissimum*), (BT+ St – Black Tea + *Stevia rebaudiana*), (OG + St- *O. gratissimum* + *Stevia rebaudiana*), and (BT+ OG+ St – Black Tea + *O. gratissimum* + *Stevia rebaudiana*). Values in parenthesis are arcsine transformed values; a-e represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

Table 31: EC_{50} values of DPPH scavenging activity of individual and combined infusions of BT, OG and St.

Sample Extract	EC_{50} (µg/ml)
BT	38.26^{a}
OG	47.13^{b}
St	26.18^{c}
BTOG	29.66^{d}
BTSt	$39.66^{a,b}$
OGSt	34.78^{e}
BTOGSt	$41.75^{a,b}$

Values are represented as MEAN ± S.D; n = 3 replicates). EC_{50} shows (Effective concentration causing 50% scavenging activity). Same alphabets on the EC_{50} values indicates non-significant differences ($P \leq 0.05$) between different infusions and different alphabets on the EC_{50} values showed significant differences ($P \leq 0.05$) between different infusions BT – Black Tea, OG – *O. gratissimum*, St – *S. rebaudiana*. BTOG – Black Tea + *O. gratissimum*, BTSt – Black Tea + *S. rebaudiana*, OGSt- *O. gratissimum S. rebaudiana*, BTOGSt – Black Tea + *O. gratissimum* + *S. rebaudiana* for the antioxidant parameter (DPPH – 2,2 diphenyl hydrazyl). Values in parenthesis are arcsine transformed values; a-e represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

6.5.2 ABTS assay

The activities were measured in terms of EC_{50}; the lower the EC_{50} (µg/ml) higher is the antioxidant potential. Black tea (29.92 µg/ml) was found to have a more scavenging effect as compared to stevia (32.05 µg/ml) and *O. gratissimum* (45.08 µg/ml), respectively. While black tea in the combination with *O. gratissimum* (OG) confined maximum quenching ability than other combinations were as follows: BTOG (30.42 µg/ml) > BTOGSt (36.48 µg/ml) > BTSt (44.88 µg/ml) > OGSt (56.46 µg/ml) as shown in **Fig. 59** and **Table 32**.

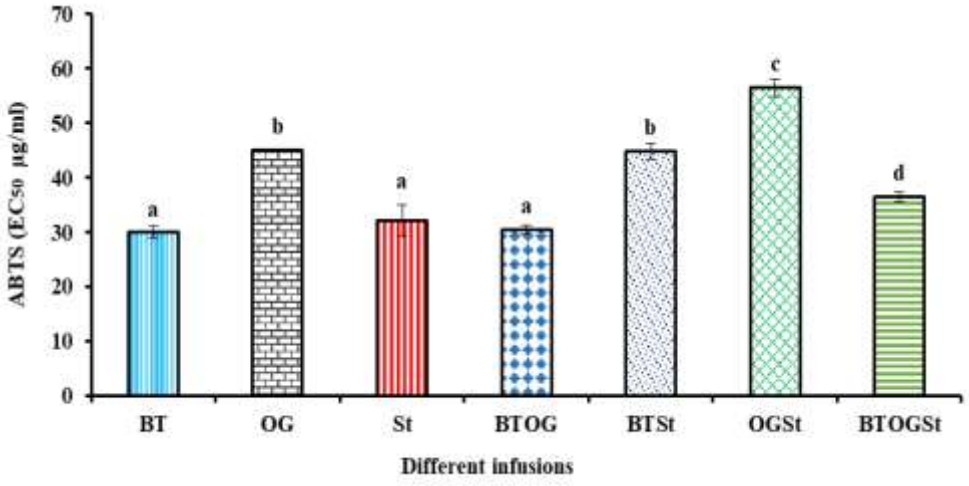


Fig. 59: ABTS scavenging activity of aqueous extracts including- Black Tea (BT), *O. gratissimum* (OG), and *S. rebaudiana* (St) infusions alone and in combination. EC_{50} undergoes as (Effective concentration causing 50% scavenging activity). Values are represented as MEAN ± S.D n = 3). Different alphabets depicted significant difference at $p \leq 0.05$ among different infusions in alone (BT and OG) as well as in combination (BT+OG – Black Tea + *O. gratissimum*), (BT+ St – Black Tea + *Stevia rebaudiana*), (OG + St- *O. gratissimum* + *Stevia rebaudiana*), and (BT+ OG+ St – Black Tea + *O. gratissimum* + *Stevia rebaudiana*). Values in parenthesis are arcsine transformed values; a-d represents the levels of treatments: 'a' = highest effect and 'd' = less effect.

Table 32: EC_{50} values of ABTS scavenging activity of individual and combined infusions BT, OG and St.

Sample Extract	EC_{50} (µg/ml)
BT	29.92^{a}
OG	45.08^{b}
St	32.05^{a}
BTOG	30.42^{a}
BTSt	44.88^{b}
OGSt	56.46^{c}
BTOGSt	36.48^{d}

Values are represented as MEAN ± S.D; n = 3 replicates). EC_{50} shows (Effective concentration causing 50% scavenging activity). Same alphabets on the EC_{50} values indicates non-significant differences ($P \leq 0.05$) between different infusions and different alphabets on the EC_{50} values showed significant differences ($P \leq 0.05$) between different infusions BT – Black Tea, OG – *O. gratissimum*, St – *S. rebaudiana*. BTOG – Black Tea + *O. gratissimum*, BTSt – Black Tea + *S. rebaudiana*, OGSt- *O. gratissimum S. rebaudiana*, BTOGSt – Black Tea + *O. gratissimum* + *S. rebaudiana* for the antioxidant parameter (ABTS). Values in parenthesis are arcsine transformed values; a-d represents the levels of treatments: 'a' = highest effect and 'd' = less effect.

6.5.3 NO assay

The activities were measured in terms of EC_{50}; the lower the EC_{50} (µg/ml) higher is the antioxidant potential. Stevia (St) showed a more quenching effect than black tea (BT) and *O. gratissimum* (OG). The scavenging potential was as follows: St (16.37µg/ml) > BT (27.53 µg/ml) > OG (49.51 µg/ml), respectively. While in combination BTOG expressed maximum antioxidant potential than other combinations in the following order: BTOG (29.74 µg/ml) > BTOGSt (39.37 µg/ml) > BTSt (44.72 µg/ml) > OGSt (54.83 µg/ml) as depicted in **Fig. 60** and **Table 33**.

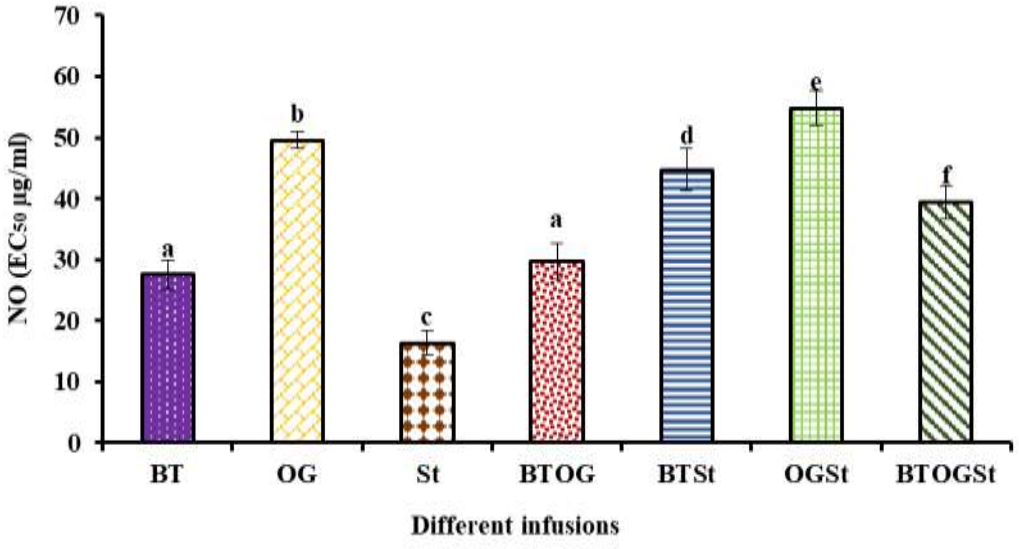


Fig. 60: NO quenching ability of aqueous extracts including- Black Tea (BT), *O. gratissimum* (OG), and *S. rebaudiana* (St) infusions alone and in combination. EC_{50} undergoes as (Effective concentration causing 50% scavenging activity). Values are represented as MEAN ± S.D n = 3). Different alphabets depicted significant difference at $p \leq 0.05$ among different infusions in alone (BT and OG) as well as in combination (BT+OG – Black Tea + *O. gratissimum*), (BT+ St – Black Tea + *Stevia rebaudiana*), (OG + St- *O. gratissimum* + *Stevia rebaudiana*), and (BT+ OG+ St – Black Tea + *O. gratissimum* + *Stevia rebaudiana*). Values in parenthesis are arcsine transformed values; a-f represents the levels of treatments: 'a' = highest effect and 'f' = less effect.

Table 33: EC_{50} values of NO scavenging activity of individual and combined infusions BT, OG and St.

Sample Extract	EC_{50} (µg/ml)
BT	27.53^{a}
OG	49.51^{b}
St	16.37^{c}
BTOG	29.74^{a}
BTSt	44.72^{d}
OGSt	54.83^{e}
BTOGSt	39.37^{f}

Values are represented as MEAN ± S.D; n = 3 replicates). EC_{50} shows (Effective concentration causing 50% scavenging activity). Same alphabets on the EC_{50} values indicates non-significant differences ($P \leq 0.05$) between different infusions and different alphabets on the EC_{50} values showed significant differences ($P \leq 0.05$) between different infusions BT – Black Tea, OG – *O. gratissimum*, St – *S. rebaudiana*. BTOG – Black Tea + *O. gratissimum*, BTSt – Black Tea + *S. rebaudiana*, OGSt- *O. gratissimum S. rebaudiana*, BTOGSt – Black Tea + *O. gratissimum* + *S. rebaudiana* for the antioxidant parameter (NO scavenging activity). Values in parenthesis are arcsine transformed values; a-f represents the levels of treatments: 'a' = highest effect and 'f' = less effect.

6.5.4 Hemolysis

The activities were measured in terms of EC_{50}, the lower the EC_{50} (µg/ml), higher is the antioxidant potential. The aqueous extract of stevia (EC_{50} – 30.75 µg/ml) exhibited the maximum hemolytic activity towards human erythrocytes as compared to black tea (EC_{50} – 68.90 µg/ml) and *O. gratissimum* (EC_{50} – 76.16 µg/ml), respectively. Whereas, the hemolytic activity of black tea in the combination (BTOG, BTSt, OGSt & BTOGSt) was found in the following order: BTOG (57.43 µg/ml) > BTOGSt (84.86 µg/ml) > BTSt (100.16 µg/ml) > OGSt (110.53 µg/ml), respectively as shown in **Fig. 61 and Table 34**.

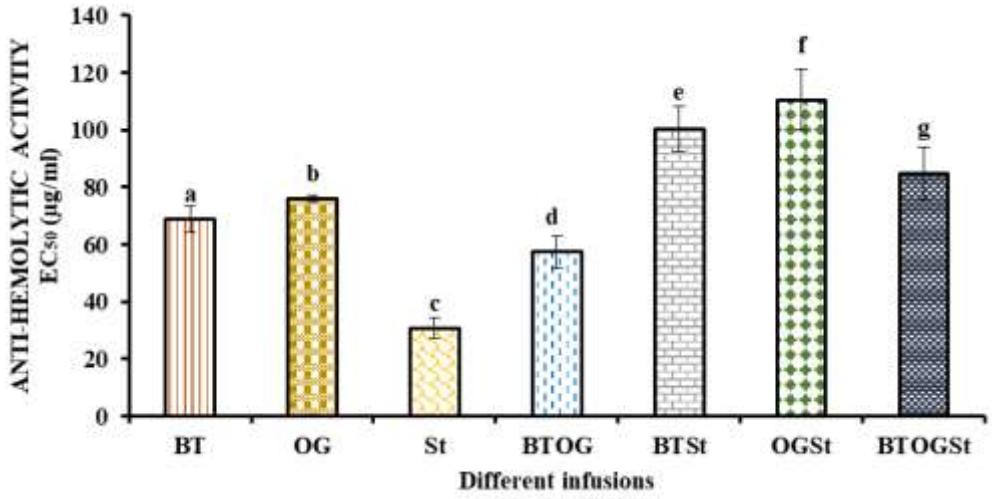


Fig. 61: Antihemolytic activity of aqueous extracts including- Black Tea (BT), *O. gratissimum* (OG), and *S. rebaudiana* (St) infusions alone and in combination. EC_{50} undergoes as (Effective concentration causing 50% scavenging activity). Values are represented as MEAN ± S.D n = 3). Different alphabets depicted significant difference at $p \leq 0.05$ among different infusions in alone (BT and OG) as well as in combination (BT+OG – Black Tea + *O. gratissimum*), (BT+ St – Black Tea + *Stevia rebaudiana*), (OG + St- *O. gratissimum* + *Stevia rebaudiana*), and (BT+ OG+ St – Black Tea + *O. gratissimum* + *Stevia rebaudiana*). Values in parenthesis are arcsine transformed values; a-g represents the levels of treatments: 'a' = highest effect and 'g' = less effect.

Table 34: EC_{50} values of Anti-hemolytic activity of individual and combined infusions of BT, OG and St.

Sample Extract	EC_{50} (µg/ml)
BT	68.90^{a}
OG	76.16^{b}
St	30.75^{c}
BTOG	57.43^{d}
BTSt	100.16^{e}
OGSt	110.53^{f}
BTOGSt	84.86^{g}

Values are represented as MEAN ± S.D; n = 3 replicates). EC_{50} shows (Effective concentration causing 50% scavenging activity). Same alphabets on the EC_{50} values indicates non-significant differences ($P \leq 0.05$) between different infusions and different alphabets on the EC_{50} values showed significant differences ($P \leq 0.05$) between different infusions BT – Black Tea, OG – *O. gratissimum*, St – *S. rebaudiana*. BTOG – Black Tea + *O. gratissimum*, BTSt – Black Tea + *S. rebaudiana*, OGSt- *O. gratissimum S. rebaudiana*, BTOGSt – Black Tea + *O. gratissimum* + *S. rebaudiana* for the antioxidant parameter (Antihemolytic activity). Values in parenthesis are arcsine transformed values; a-g represents the levels of treatments: 'a' = highest effect and 'g' = less effect.

6.5.5 Lipid peroxidation (LPO) assay

The activities were measured in terms of EC_{50}; the lower the EC_{50} (µg/ml), higher is the antioxidant potential. Stevia showed the highest lipid peroxidation inhibitory capacity with the lowest EC_{50} value – 21.07 µg/ml followed by black tea (43.56 µ/ml) and *O. gratissimum* (50.11 µg/ml), respectively. While, in combination, black tea with *O. gratissimum* (EC_{50} – 53.97 µg/ml) expressed a maximum inhibitory effect as compared to other combinations BTSt (93.06 µg/ml), OGSt (96.03 µg/ml) & BTOGSt (79.36 µg/ml) respectively as illustrated in **Fig. 62** and **Table 35.**

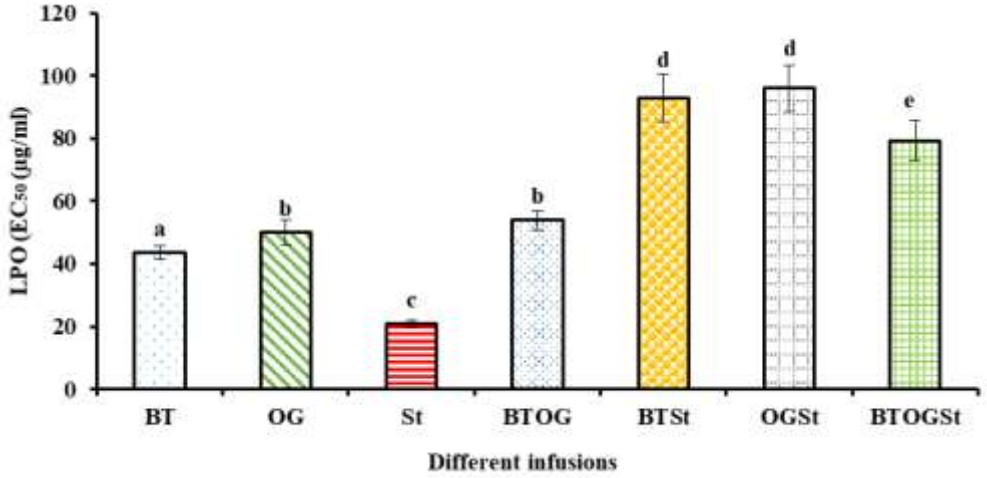


Fig. 62: Lipid peroxidation inhibitory capacity of aqueous extracts including- Black Tea (BT), *O. gratissimum* (OG), and *S. rebaudiana* (St) infusions alone and in combination. EC_{50} undergoes as (Effective concentration causing 50% scavenging activity). Values are represented as MEAN ± S.D n = 3). Different alphabets depicted significant difference at $p \leq 0.05$ among different infusions in alone (BT and OG) as well as in combination (BT+OG – Black Tea + *O. gratissimum*), (BT+ St – Black Tea + *Stevia rebaudiana*), (OG + St- *O. gratissimum* + *Stevia rebaudiana*), and (BT+ OG+ St – Black Tea + *O. gratissimum* + *Stevia rebaudiana*). Values in parenthesis are arcsine transformed values; a-e represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

Table 35: EC_{50} values of LPO scavenging activity of individual and combined infusions of BT, OG and St.

Sample Extract	EC_{50} (µg/ml)
BT	43.56^{a}
OG	50.11^{b}
St	21.07^{c}
BTOG	53.97^{b}
BTSt	93.06^{d}
OGSt	96.03^{d}
BTOGSt	79.36^{e}

Values are represented as MEAN ± S.D; n = 3 replicates). EC_{50} shows (Effective concentration causing 50% scavenging activity). Same alphabets on the EC_{50} values indicates non-significant differences ($P \leq 0.05$) between different infusions and different alphabets on the EC_{50} values showed significant differences ($P \leq 0.05$) between different infusions BT – Black Tea, OG – *O. gratissimum*, St – *S. rebaudiana*. BTOG – Black Tea + *O. gratissimum*, BTSt – Black Tea + *S. rebaudiana*, OGSt- *O. gratissimum S. rebaudiana*, BTOGSt – Black Tea + *O. gratissimum* + *S. rebaudiana* for the antioxidant parameter (LPO – Lipid Peroxidation Inhibitory Capacity). Values in parenthesis are arcsine transformed values; a-d represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

Table 36: EC_{50}, combination index and type of antioxidant interactions between black Tea, *O. gratissimum and S. rebaudiana* sample extracts in alone, binary and ternary combination for different antioxidant assays.

Assay	Binary combinations	EC_{50} (µg/ml)	CI at EC_{50}	Type of interactions
DPPH	BT	38.26 ± 1.95^{a}	-	-
	OG	47.13 ± 1.51^{b}	-	-
	St	26.18 ± 0.87^{c}	-	-
	BTOG (3:1)	29.66 ± 0.63^{d}	0.75	Nearly additive
	BTSt	$39.66 \pm 0.87^{a,b}$	1.23	Antagonism
	OGSt	34.78 ± 1.13^{e}	0.94	Nearly additive
	BTOGSt	$41.75 \pm 1.12^{a,b}$	1.25	Antagonism
ABTS	BT	29.22 ± 1.04^{a}	-	-
	OG	45.08 ± 0.11^{b}	-	-
	St	32.05 ± 2.90^{a}	-	-

	BTOG (3:1)	30.42 ± 0.88[a]	0.89	Nearly additive
	BTSt	44.88 ± 1.46[b]	1.42	Antagonism
	OGSt	56.46 ± 1.56[c]	1.44	Antagonism
	BTOGSt	36.48 ± 0.82[d]	1.10	Slight antagonism
NO	BT	27.53 ± 2.50[a]	-	
	OG	49.51 ± 1.38[b]	-	-
	St	16.37 ± 1.90[c]	-	-
	BTOG (3:1)	29.74 ± 2.87[a]	0.96	Nearly additive
	BTSt	44.72 ± 3.47[d]	1.98	Antagonism
	OGSt	54.83 ± 2.80[e]	1.87	Antagonism
	BTOGSt	39.37 ± 2.66[f]	1.62	Antagonism
LPO	BT	43.56 ± 2.09[a]	-	-
	OG	50.11 ± 4.11[b]	-	-
	St	21.07 ± 1.05[c]	-	-
	BTOG (3:1)	53.97 ± 3.07[b]	1.22	Antagonism
	BTSt	93.06 ± 7.52[d]	2.93	Strong antagonism
	OGSt	96.03 ± 7.48[d]	2.81	Strong antagonism
	BTOGSt	79.36 ± 6.34[e]	2.43	Strong antagonism
Hemolysis	BT	68.90 ± 4.52[a]	-	-
	OG	76.16 ± 0.90[b]	-	-
	St	30.75 ± 3.58[c]	-	-
	BTOG (3:1)	57.43 ± 5.56[d]	0.80	Nearly additive
	BTSt	100.16 ± 7.98[e]	2.06	Slight antagonism
	OGSt	110.53 ± 10.62[f]	2.16	Strong antagonism

Different alphabets showed significant differences ($P \leq 0.05$) between different infusions BT – Black Tea, OG – *O. gratissimum*, St – *S. rebaudiana*. BTOG – Black Tea + *O. gratissimum*, BTSt – Black Tea + *S. rebaudiana*, BTOGSt – Black Tea + *O. gratissimum* + *S. rebaudiana*. EC_{50} represents (effective concentration causing 50% scavenging activity), CI (combination index). Data are shown as the MEAN ± S.D; n = 3 replicates). The range of CI values and type of interaction as given by Chou (2010)

are as follows: < 0.1: very strong synergism, 0.1 – 0.3: strong synergism, 0.3 – 0.7: synergism, 0.7 - 0.85: moderate synergism, 0.85 – 0.90: slight synergism, 0.90 – 1.10: nearly additive, 1.20 – 1.45: moderate antagonism, and 1.45 – 3.3: antagonism.Values in parenthesis are arcsine transformed values; a-f represents the levels of treatments: 'a' = highest effect and 'f ' = less effect.

In the DPPH test, BTOG (CI = 0.75) – nearly additive (green line, less thickness), BTSt (CI = 1.23) – antagonism (red line, more thickness), OGSt (CI = 0.94) – nearly additive (green line, less thickness) & BTOGSt (CI = 1.25) – antagonism (red line, more thickness) at EC_{50} as shown in **Fig. 65** and **Table 36.**

BTOG (CI = 0.89) – nearly additive (green line, less thickness), BTSt (CI = 1.42) – antagonism (red line, more thickness), OGSt (CI = 1.44) – antagonism (red line, more thickness) & BTOGSt (CI = 1.10) – slight antagonism in the ABTS assay. In the NO test, BTOG (CI = 0.96) – nearly additive, BTSt (CI = 1.98) – antagonism (red line, more thickness), OGSt (CI = 1.87) – antagonism (red line, more thickness) & BTOGSt (CI = 1.62) – antagonism (red line, more thickness). For LPO assay, BTOG (CI = 1.22) – antagonism (red line, more thickness), BTSt (CI = 2.93) – strong antagonism (red line, more thickness), OGSt (CI = 2.81) – strong antagonism (red line, more thickness) and BTOGSt (CI = 2.43) – strong antagonism (red line, more thickness). Whereas, in hemolysis test, BTOG (CI = 0.80) – nearly additive (green line, less thickness), BTSt (CI = 2.06) – slight antagonism (red line, less thickness), OGSt (CI = 2.16) – strong antagonism (red line, more thickness) & BTOGSt (CI = 1.74) – strong antagonism interaction (red line, more thickness) respectively as depicted in **Fig. 65** and **Table 36**.

6.5.6 Total Phenolic Content

Among the tea infusions, the highest phenolic content was found in Black Tea (104.16 mg/100ml) followed by *O. gratissimum* (68.69 mg/100ml) and stevia (62.71 mg/100ml). Whereas, in combination BTOGSt (Black Tea + *O. gratissimum* + stevia) (117.33 mg/100ml) contained a maximum amount of phenolic content than other combinations BTSt (100.15 mg/100ml), BTOG (92.10 mg/100ml) & OGSt (90.02 mg/100ml) as depicted in **Fig's. 63(a), (b)** and **Table 37**.

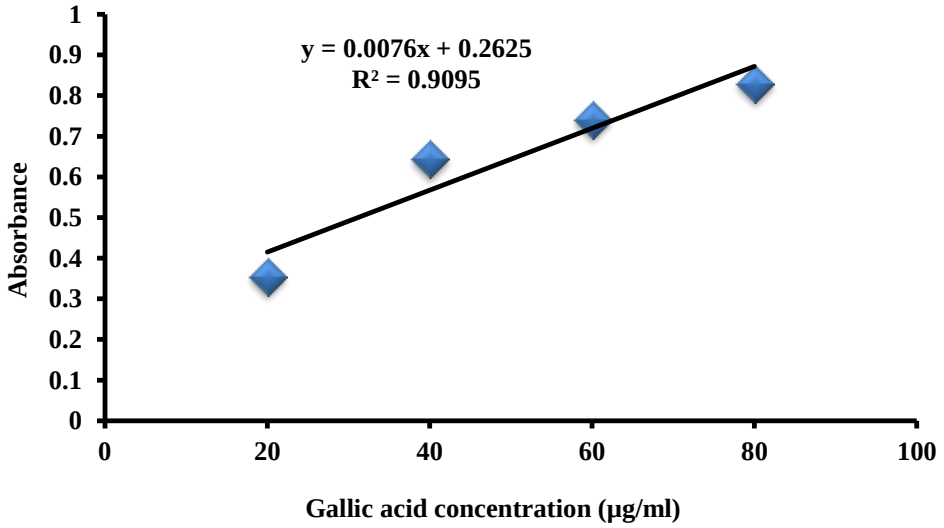


Fig. 63: (a) Standard calibration curve of gallic acid measured by GAE (gallic acid equivalent)

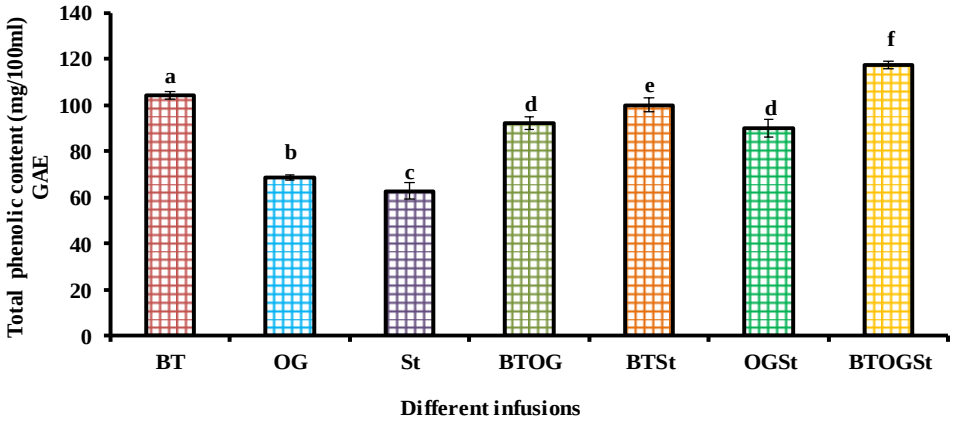


Fig. 63: (b) The Total Phenolic Content of Black Tea (BT), *O. gratissimum* (OG), and *Stevia rebaudiana* (St) in alone and in binary and ternary combination [Black Tea + *O. gratissimum* (BTOG), Black Tea + Stevia (BTSt), *O. gratissimum* + Stevia (OGSt) and Black Tea + *O. gratissimum* + Stevia (BTOGSt)]. Values are represented as MEAN± S.D; n = 3 replicates. The dissimilar alphabets illustrated significant difference ($p \leq 0.05$) with respect to the total phenolic content. Values in parenthesis are arcsine transformed values; a-f represents the levels of treatments: 'a' = highest effect and 'f' = less effect.

Table 37: Total phenolic content of sample extracts when taken alone, binary and ternary combination.

Sample	TPC (mg/100ml) GAE
BT	104.16± 1.47[a]
OG	68.69±1.29[b]
St	62.71±3.47[c]
BTOG	92.10±2.91[d]
BTSt	100.15±2.89[e]
OGSt	90.02±3.86[d]
BTOGSt	117.33±1.59[f]

Values are represented as MEAN ± S.D, n = 3 replicates. EC_{50} shows (Effective concentration causing 50% scavenging activity).. Different alphabets on the calculated values showed significant differences ($P \leq 0.05$) between different infusions -BT – Black Tea, OG – *O. gratissimum*, St – *S. rebaudiana*. BTOG – Black Tea + *O. gratissimum*, BTSt – Black Tea + *S. rebaudiana*, BTOGSt – Black Tea + *O. gratissimum* + *S. rebaudiana* for the (TPC – Total Phenolic Content). Values in parenthesis are arcsine transformed values; a-f represents the levels of treatments: 'a' = highest effect and 'f ' = less effect.

6.5.7 Total Flavonoid Content

The highest amount of flavonoid content was detected in the aqueous extract of *O. gratissimum* (82.20 mg/100ml) than St (55.53 mg/100ml) & BT (39.96 mg/100ml), respectively. While, Black Tea, *O. gratissimum*, and stevia in combined form (BTOGSt) confined maximum flavonoid content (88.63 mg/100ml) as compared to other combinations (OGSt- 86.92 mg/100ml, BTOG- 84.43 mg/100ml & BTSt- 81.60 mg/100ml), respectively as shown in **Fig's. 64(a), (b)** and **Table 38**.

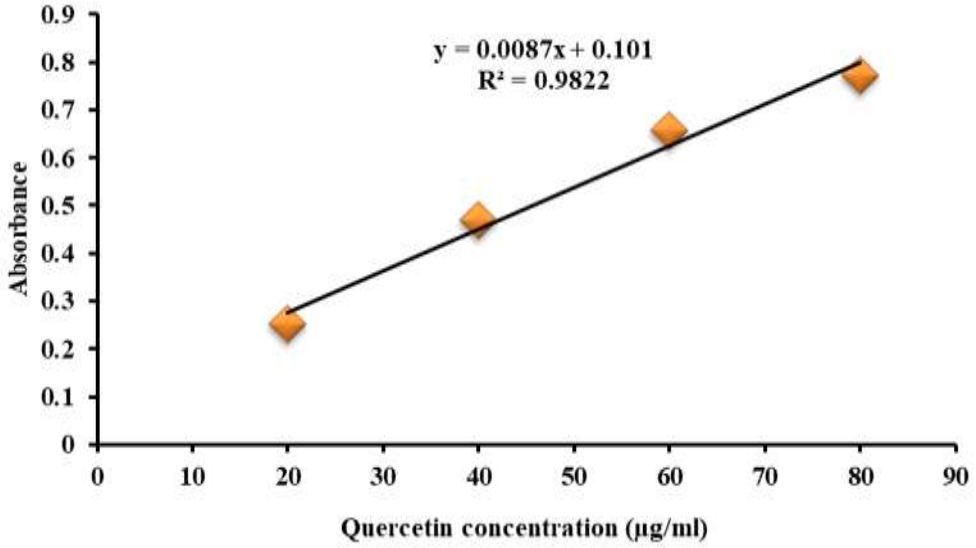


Fig.64: (a) Standard calibration curve of quercetin measured by quercetin equicalent (QE)

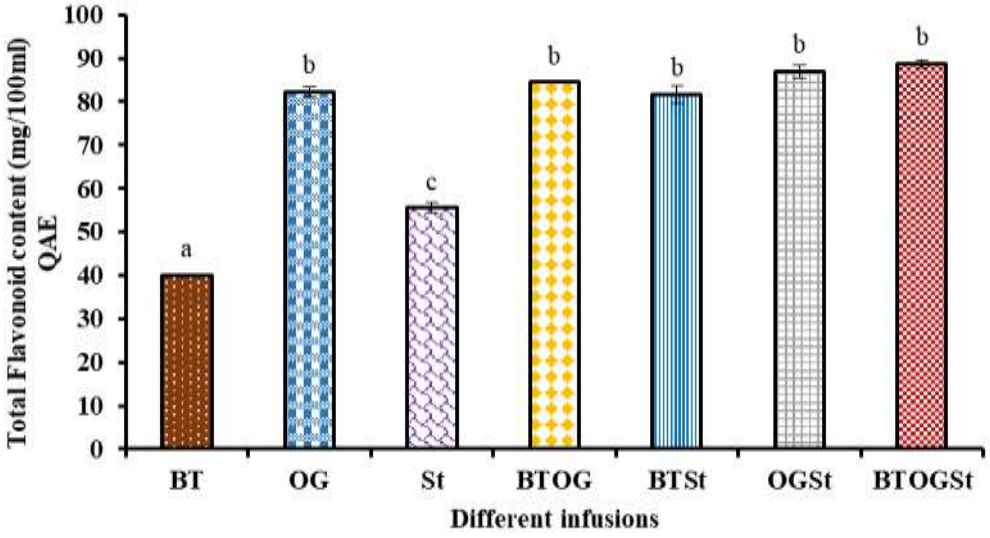


Fig. 64: (b) The Total flavonoid Content of aqueous infusions of Black Tea (BT), *O. gratissimum* (OG), and *Stevia rebaudiana* (St) in alone, binary and ternary combination - [Black Tea + *O. gratissimum* (BTOG), Black Tea + Stevia (BTSt), *O. gratissimum* + Stevia (OGSt) and Black Tea + *O. gratissimum* + Stevia (BTOGSt)]. Values are represented as MEAN± S.D; n = 3 replicates. The dissimilar alphabets illustrated significant difference ($p \leq 0.05$) with respect to the total flavonoid content. Values in parenthesis are arcsine transformed values; a-c represents the levels of treatments: 'a' = highest effect and 'c' = less effect.

Table 38: Total flavonoid content of sample extracts when taken alone, binary and ternary combination.

Sample	TFC (mg/100ml) QE
BT	39.96±0.05[a]
OG	82.20±1.09[b]
St	55.53±1.16[c]
BTOG	84.43±0.26[b]
BTSt	81.60±2.17[b]
OGSt	86.92±1.34[b]
BTOGSt	88.63±0.95[b]

Values are represented as MEAN ± S.D, n = 3 replicates. EC_{50} shows (Effective concentration causing 50% scavenging activity).. Different alphabets on the calculated values showed significant differences ($P \leq 0.05$) between different infusions -BT – Black Tea, OG – *O. gratissimum*, St – *S. rebaudiana*. BTOG – Black Tea + *O. gratissimum*, BTSt – Black Tea + *S. rebaudiana*, BTOGSt – Black Tea + *O. gratissimum* + *S. rebaudiana* for the (TPC – Total Flavonoid Content). Values in parenthesis are arcsine transformed values; a-c represents the levels of treatments: 'a' = highest effect and 'c' = less effect.

Table 39: Correlation between phenolic level & EC_{50} values of aqueous infusions of BT, OG and St alone and in binary/ternary combination BTOG, BTSt, OGSt and BTOGSt.

SAMPLE EXTRACT	TPC	DPPH	correlation	ABTS	correlation	NO	correlation	LPO	correlation	Anti - hemolysis	correlation
BT	78.51	38.26		29.92		27.53		43.56		68.90	
OG	44.28	47.13	-1	45.08	-1	49.51	-1	50.11	-1	76.16	-1
St	62.71	26.18		32.05		16.37		21.07		30.75	
BTOG	92.10	29.66		30.42		29.74		53.97		57.43	
BTSt	100.15	39.66		44.88		44.72		93.06		100.16	
OGSt	90.02	34.78	0.902	56.46	0.175	54.83	0.594	96.03	0.727	110.53	0.649
BTOGSt	117.33	41.75		36.48		39.37		79.36		84.56	

Values are represented as MEAN ± S.D, n = 3 replicates. BT – Black Tea, OG – *O. gratissimum*, St – *S. rebaudiana*, BTOG – Black Tea + *O. gratissimum*, BTSt – Black Tea + *S. rebaudiana*, - Black Tea+ *O. gratissimum* + *S. rebaudiana* for looking into correlation of phenolics with EC50 values of DPPH, NO, LPO and Anti-hemolysis. (TPC – Total Flavonoid Content, DPPH – 2,2 – diphenyl 1-1, picryl hydrazyl, ABTS - 2,2'-azino-bis (ethylbenzthiazoline-6-sulfonic acid, NO – Nitric Oxide scavenging activity, LPO – Lipid Peroxidation scavenging activity and Antihemolysis).

Table 40: Correlation between flavonoid level & EC_{50} values of aqueous infusions of BT, OG, St alone and in binary/ternary combination BTOG, BTSt, OGSt and BTOGSt.

SAMPLE EXTRACT	TFC	DPPH	correlation	ABTS	correlation	NO	correlation	LPO	correlation	Anti - hemolysis	correlation
BT	39.96	38.26		29.92		27.53		43.56		68.90	
OG	82.20	47.13	1	45.08	1	49.51	1	50.11	1	76.16	1
St	55.53	26.18		32.05		16.37		21.07		30.75	
BTOG	84.43	29.66		30.42		29.74		53.97		57.43	
BTSt	81.60	39.66		44.88		44.72		93.06		100.16	
OGSt	86.92	34.78	0.713	56.46	0.416	54.83	0.786	96.03	0.830	110.53	0.778
BTOGSt	88.63	41.75		36.48		39.37		79.36		84.56	

Values are represented as MEAN ± S.D, n = 3 replicates. BT – Black Tea, OG – *O. gratissimum*, St – *S. rebaudiana*, BTOG – Black Tea + *O. gratissimum*, BTSt – Black Tea + *S. rebaudiana*, - Black Tea+ *O. gratissimum* + *S. rebaudiana* for looking into correlation of flavonoids with EC_{50} values of DPPH, NO, LPO and Anti-hemolysis. (TPC – Total Flavonoid Content, DPPH – 2,2 – diphenyl 1-1, picryl hydrazyl, ABTS - 2,2'-azino-bis (ethylbenzthiazoline-6-sulfonic acid, NO – Nitric Oxide scavenging activity, LPO – Lipid Peroxidation scavenging activity and Antihemolysis).

A strong correlation was noticed between TPC/TFC and antioxidant parameters (TPC/TFC–DPPH: R^2 = -1/1, TPC/TFC- NO: R^2 = -1/1, TPC/TFC –ABTS: R^2= -1/1, TPC/TFC–LPO: R^2 =-1/1, and TPC/TFC–Anti-haemolysis: R^2 = -1/1) for individual infusions (black tea, *O. gratissimum* & *S. rebaudiana*), but the strength of correlation was strong, weak & very weak in BT combinations: (TPC–DPPH: R^2 = 0.902, TPC-LPO: R2 = 0.727, TPC– Anti-haemolysis: R^2 = 0.649, TPC–NO: R^2 = 0.594 and TPC–ABTS: R^2 = 0.175). However, an almost strong or positive correlation was found between TFC and antioxidant potential of black tea combinations (BTOG, BTSt, OGSt & BTOGSt): (TFC–LPO: R^2 = 0.830, TFC–NO: R^2 = 0.786, TFC–Anti-haemolysis: R^2= 0.778, TFC–DPPH: R^2 = 0.713 and TFC-ABTS: R^2 = 0.416) as depicted in **Table 39** and **40**.

Table 41: The radical quenching capability of the individual (BT, OG & St) and combined infusion (BTOG, BTSt, OGSt and BTOGSt) in comparison to Black Tea.

Infusion type	% Change in DPPH activity	% Change in ABTS activity	% Change in NO activity	% Change in LPO activity	% Change in anti-hemolytic activity	% Average change in scavenging activity
BT	-	-	-	-	-	-
OG	19.48±2.25^{a}**(-)**	30.42±0.17^{a}**(-)**	42.45±0.56^{a}**(-)**	13.10±0.43^{a}**(-)**	9.20±0.39^{a}**(-)**	22.93±0.76**(-)**
St	43.81±1.87^{b}**(+)**	7.91±5.50^{b}**(+)**	68.66±11.28^{b}**(+)**	107.56±0.66^{b}**(+)**	128.66±9.01^{b}**(+)**	71.32±5.66**(+)**
BTOG	178.39±46.60^{c}**(+)**	106.77±3.00^{c}**(+)**	82.90±19.09^{c}**(+)**	62.73±1.76^{c}**(+)**	142.52±4.48^{c}**(+)**	114.66±14.98**(+)**
BTSt	173.40±6.74^{c}**(+)**	107.28±5.98^{c}**(+)**	81.66±12.68^{c}**(+)**	38.86±3.62^{d}**(+)**	104.82±3.45^{c}**(+)**	101.20±6.49**(+)**
OGSt	214.35±2.65^{d}**(+)**	65.62±1.62^{d}**(+)**	49.57±3.99^{d}**(+)**	35.65±2.63^{d}**(+)**	87.12±5.17^{d}**(+)**	90.46±3.21**(+)**
BTOGSt	161.41±4.69^{e}**(+)**	154.37±6.41^{e}**(+)**	109.11±5.98^{e}**(+)**	64.33±3.71^{e}**(+)**	136.55±2.39^{e}**(+)**	125.15±4.63**(+)**

DPPH- 2,2-diphenyl-1-picryl hydrazyl, ABTS- 2,2'-azino-bis (ethylbenzthiazoline-6-sulfonic acid), NO- Nitric oxide scavenging activity, LPO- Lipid Peroxidation and anti-hemolytic assay. BT- black tea, OG- *O. gratissimum* and St- *Stevia rebaudiana*, black tea + *O. gratissimum* (BTOG), black tea + stevia (BTSt), *O. gratissimum* + stevia (OGSt) and black tea + *O. gratissimum* + *Stevia rebaudiana* (BTOGSt). The same alphabets (a,a,b,b….) showed no significant differences between different infusion types at $p \leq 0.05$. Different alphabets (a,b,c,d….) showed significant differences between different infusion types for an antioxidant parameter ($p \leq 0.05$). Letters above a column followed by dissimilar letters above other columns are significantly different at $p \leq 0.05$. Values in parenthesis are arcsine transformed values; a-e represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

In supplementation, different infusions have a major impact on the antioxidant activity of black tea. However, stevia as an additive in BTOG (ternary) combination showed maximum effect with the range of 125%. Whereas, in the binary combination the fortification of OG in black tea (BTOG) showed 114%, BTSt-90%, and OGSt-101% enhancement in the scavenging potential of black tea shown in **Fig. 63** and **Table 41.**

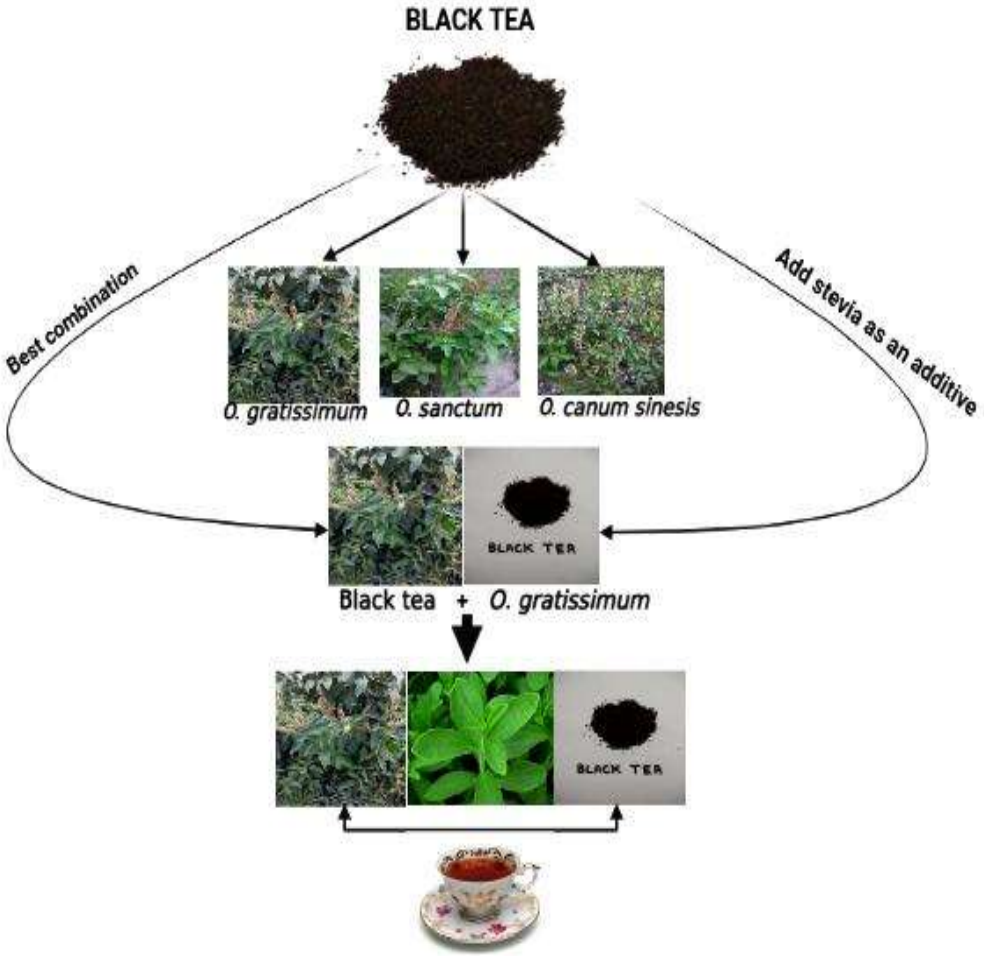


Fig. 65: Stevia supplementation as an additive in Black Tea with *O. gratissimum* (ternary) combination for making a refreshing drink

These results indicated that the antioxidant activity of black tea (BT) and different *Ocimum* spp. (*O. gratissimum*, *O. sanctum* and *O. canum*) alone or in binary/ternary combination (BTOG, BTSt, OGSt, and BTOGSt) are highly contributed by phenolics or flavonoids as depicted in **Fig's. 61(a), (b), 62(a), (b), 63 and Table 38, 39, 40, 41.**

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***6.7* To check the anti-genotoxic effect of Black Tea (BT), *O. gratissimum* (OG), *O. sanctum* (OS), *O. canum* (OC) and *S. rebaudiana* (St) alone or its binary (BTOG, BTOS, BTOCS, BTSt, OGSt, BTOG 3:1, BTOG 2:1, BTOG 1:1, BTOG 1:2 and ternary (BTOGSt) combination**

The results indicate that black tea (75%) showed maximum anti-genotoxic activity as compared to OG, OS, OC & St, respectively.

BTOGSt (87%) expressed highest effect of anti-genotoxicity as compared to other combinations, respectively as shown in **Fig. 66** and **Table 43(a), (b).**

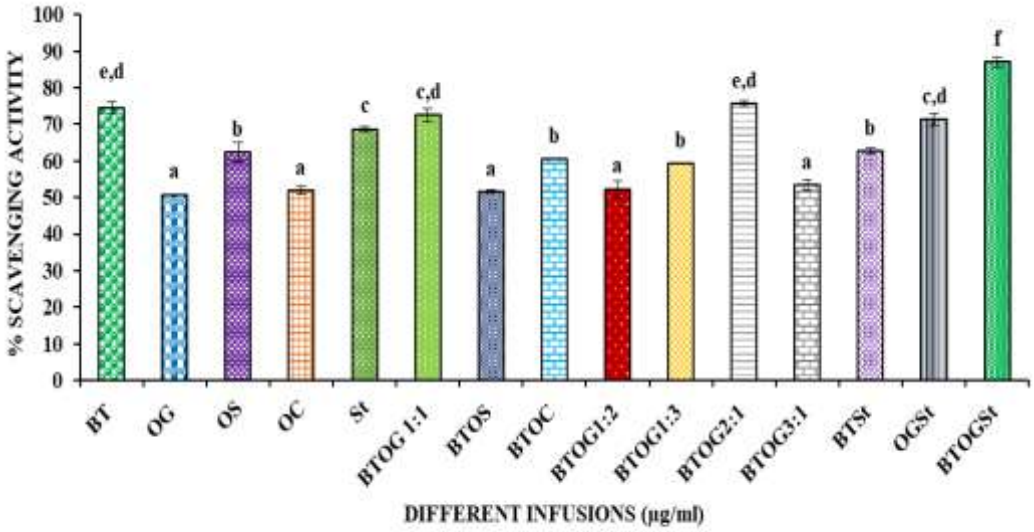


Fig. 66: The anti-genotoxic activity of Black Tea (BT), *O. sanctum* (OS), *O. gratissimum* (OG), *O. canum* (OC), *S. rebaudiana* (St) and its binary (BTOG, BTOS, BTOC, OGSt, BTSt, BTOG 1:2, BTOG 1:3, BTOG 2:1, BTOG 3:1 and ternary (BTOGSt). Values are represented as MEAN± S.D; n = 3 replicates. The dissimilar alphabets illustrated significant difference ($p \leq 0.05$) with respect to the total flavonoid content between different infusion (BT-Black Tea, OG-*O. gratissimum*, St – *Stevia rebaudiana*, BTOS – Black tea + *O. sanctum*, BTOC – Black Tea + *O. canum*, BTOG – Black Tea + *O. gratissimum*, BTSt – Black Tea + *Stevia rebaudiana*, BTOGSt – Black Tea + *O. gratissimum* + *Stevia rebaudiana.* Values in parenthesis are arcsine transformed values; a-e represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

Table 42: The percentage of antigenotoxic activity of black tea (BT), *O. gratissimum* (OG), *O. sanctum* (OS), *O. canum* (OC) and *S. rebaudiana* (St) alone and it's binary (BTOG 1:1, BTOS, BTOC, BTOG 3:1, BTOG 2:1, BTOG 1:1, BTOG 1:2, BTOG 1:3, BTSt & OGSt) and ternary combination (BTOGSt) (mean±S.D).

Sample	Mean±S.D
BT1 (100 µl)	74.4±1.65^{e,d}
OG2 (100µl)	50.8±0.05^{a}
OS3 (100µl)	62.4±2.77^{b}
OC4 (100µl)	51.9±1.03^{a}
St5 (100µl)	68.7±0.68^{c}
BTOG 1:1 (100 µl)	72.5±1.91^{c,d}
BTOS (100µl)	51.6±0.56^{a}
BTOC (100µl)	60.6±0.17^{b}
BTOG 1:2 (100µl)	52.3±2.31^{a}
BTOG 1:3 (100 µl)	59.3±0.22^{b}
BTOG 2:1 (100 µl)	75.7±0.75^{e,d}
BTOG 3:1 (100 µl)	53.4±1.41^{a}
BTSt (100 µl)	62.7±0.69^{b}
OGSt (100 µl)	71.3±1.66^{c,d}
BTOGSt (100 µl)	87±1.16^{f}

Different alphabets represent significant differences at ($p \leq 0.05$) within the same column for a test. Data are shown as MEAN ± S.D for two independent experiments (each with triplicates for each test point). Values in parenthesis are arcsine transformed values; a-e represents the levels of treatments: 'a' = highest effect and 'e' = less effect.

***6.8* To check the cytotoxic activity of Black Tea (BT), *O. gratissimum* (OG) and *S. rebaudiana* (St) in binary (BTOG, BTSt & OGSt) and ternary combination (BTOGSt).**

Adrimycin drug (positive control) showed the lowest percentage of cell viability (9.8%) in A549 cells. Among the aqueous infusions, *O. gratissimum* (OG) exhibited least (36%) cell viability. Whereas, black tea (BT) and stevia demonstrated cell viability of 65 – 69% in the A549 cancer cell line respectively.

The percentage of cell viability was noted in BTSt – 59% & OG + *S. rebaudiana* (St) [BTOGSt] – 66% followed by other mixtures i.e. black tea + *O. gratissimum* (BTOG) – 73% and *O. gratissimum* + *S. rebaudiana* (OGSt) – 75%, respectively as shown in **Fig. 67.**

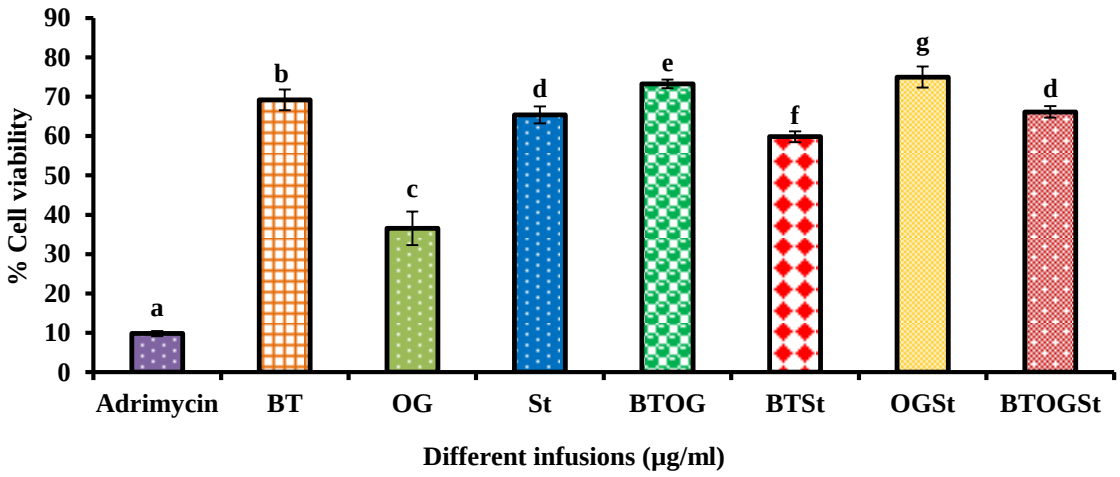
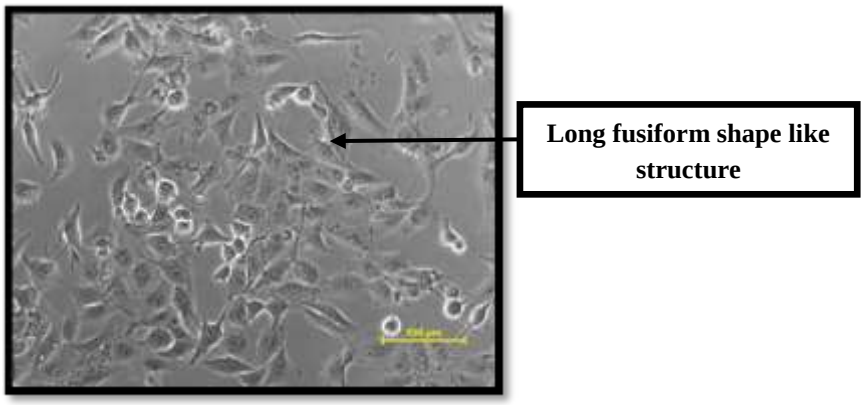


Fig. 67: The cytotoxic activity of aqueous infusions of Black Tea, *O. gratissimum* and *S. rebaudiana* alone and in combination. Data is represented as MEAN ± S.D; n=3 replicates. Same alphabets showed no significant differences (p ≤ .05) between different infusion (BT-Black Tea, OG-*O. gratissimum*, BTOG – Black Tea + *O. gratissimum*, BTSt – Black Tea + *S. rebaudiana*, BTOGSt – Black Tea + *O. gratissimum* + *S. rebaudiana*. Different alphabets showed significant differences (p ≤ .05) between different infusion (BT-Black Tea, OG-O. gratissimum, BTOG – Black Tea + *O. gratissimum*, BTSt – Black Tea + *S. rebaudiana*, BTOGSt – Black Tea + *O. gratissimum* + *S. rebaudiana*. Values in parenthesis are arcsine transformed values; a-g represents the levels of treatments: 'a' = highest effect and 'g' = less effect.

6.8.1 Morphological identification

A549 cells

A549 is a human alveolar basal epithelial cell that showed a long fusiform shape, small size, clear cell boundaries, well-adherent pebble-like growth, placental cytoplasm, and fewer cytoplasmic granules as shown in **Fig. 68.**

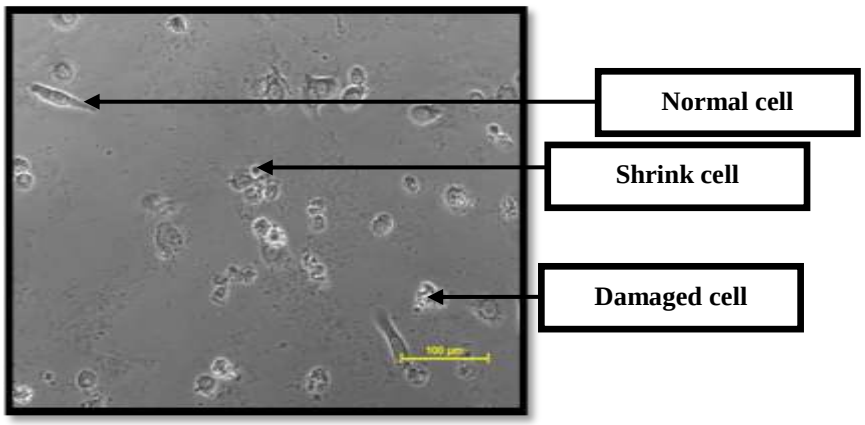


Control

Fig. 68: Morphology of A549 human lung cancer cell line

Adrimycin (Positive Control)

- It is rounded and normal in shape.
- Also, the cells showed a reduction in cell volume and destabilization of the plasma membrane, as shown in **Fig. 69.**



Adrimycin (Positive Control)

Fig. 69: Effect of adrimycin (Adr) Drug on A549 human lung cancer cell line

Morphological identification of samples

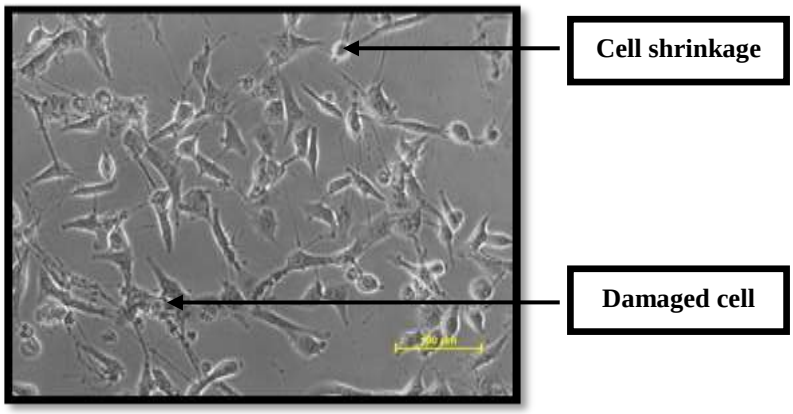


Fig. 70: Effect of aqueous Black Tea extract on A549 human lung cancer cell line

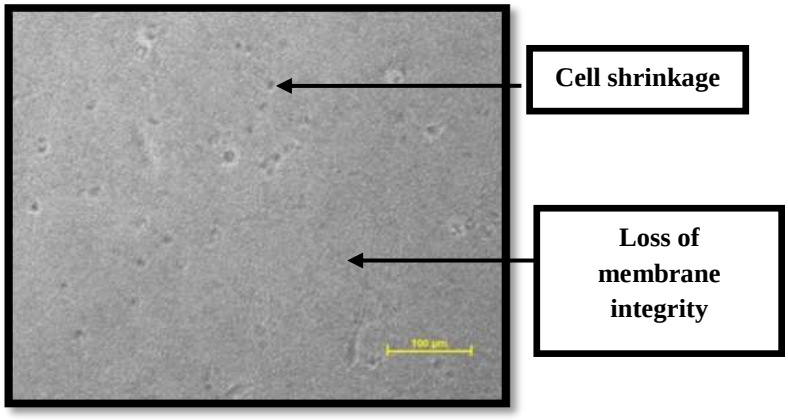


Fig. 71: Effect of aqueous *O. gratissimum* extract on A549 human lung cancer cell line

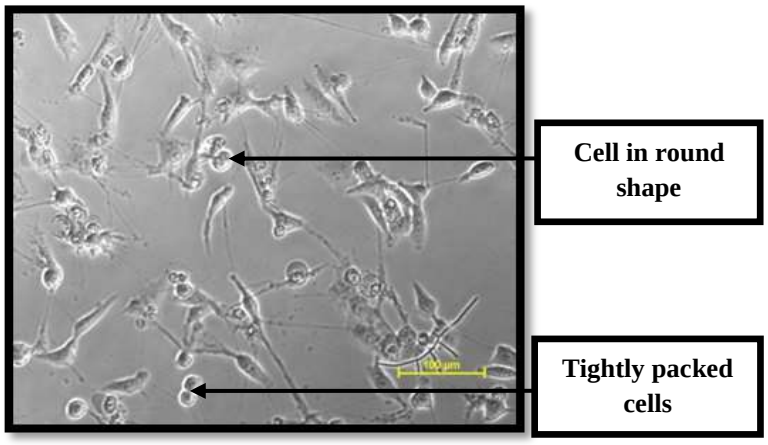


Fig.72: Effect of aqueous *S. rebaudiana* extract on A549 human lung cancer cell line

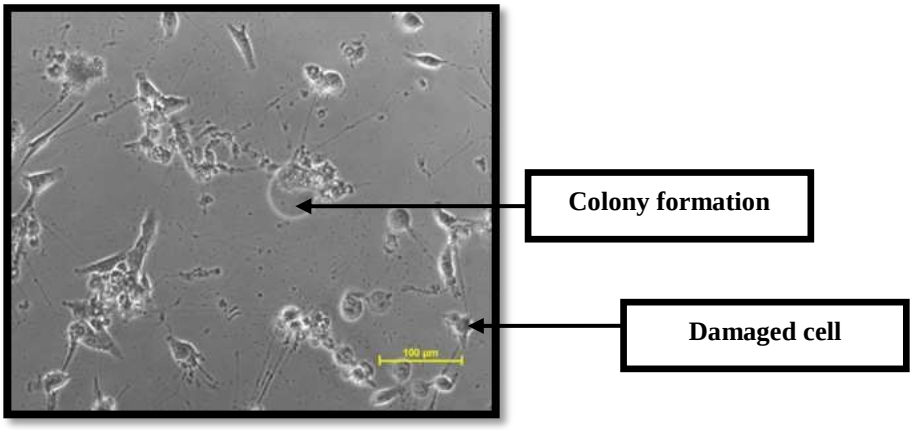


Fig. 73: Effect of aqueous Black tea + *S. rebaudiana* extract on A549 human lung cancer cell line

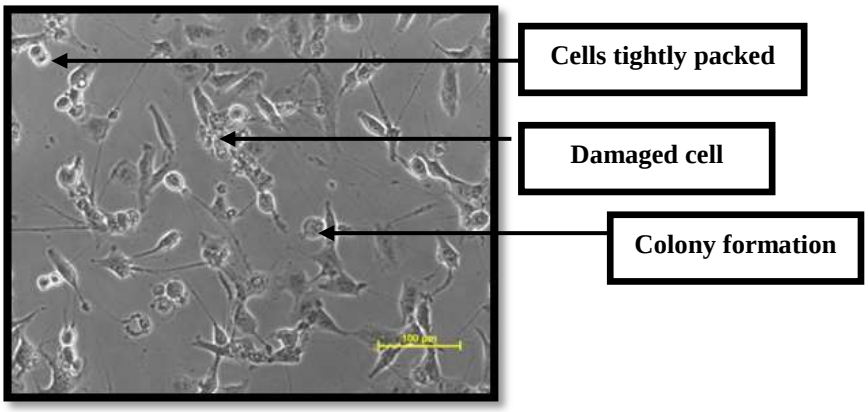


Fig. 74: Effect of *O. gratissimum* + *S. rebaudiana* sample extract on A549 human lung cancer cell line

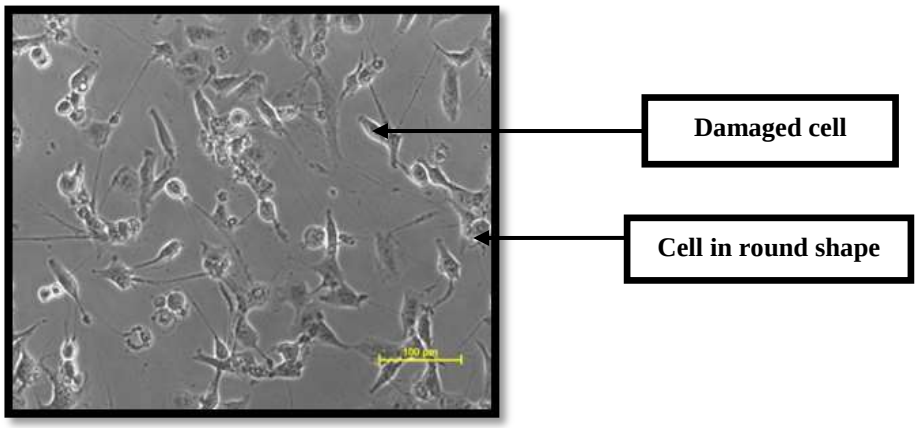


Fig. 75: Effect of Black Tea + *O. gratissimum* sample extract on A549 human lung cancer cell line

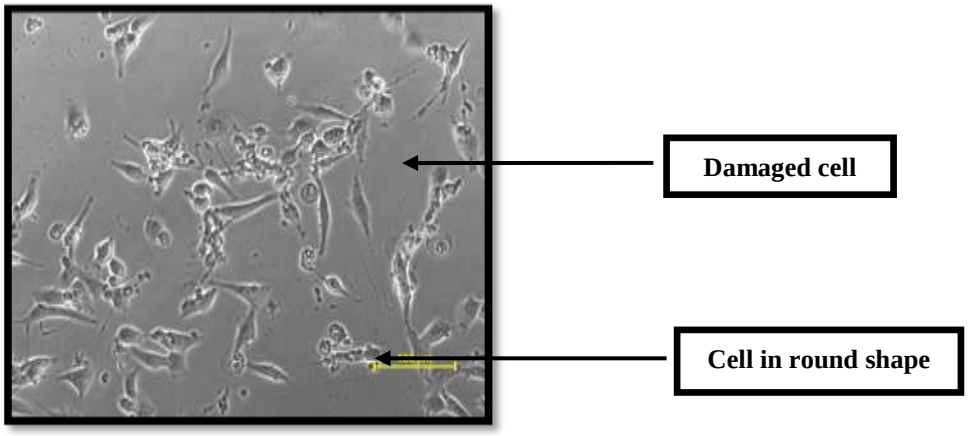


Fig. 76: Effect of Black Tea + *O. gratissimum* + *S. rebaudiana* sample extract on A549 human lung cancer cell line

- Uniform detachment of cells, inhibition of cell growth, membrane integrity, and cell shrinkage were observed in the aqueous infusions in comparison to adrimycin (positive control), as shown in **Fig. 70-76**.

The preliminary studies conducted in our laboratory employing chemical and *ex vivo* models to determine the antioxidant activity of black tea alone and in combination with *O. canum* Sims or *O. gratissimum* or *O. sanctum* revealed that black tea and *O. gratissimum* showed higher radical quenching ability as compared to a binary combination of black tea with other species. Moreover, it was also found that a ternary combination of black tea with *O. gratissimum* and *S. rebaudiana* (St) expressed additive interaction and maximum free radical activity. Furthermore, to confirm the chemopreventive potential of the above-mentioned ternary combination, an *in vivo* study was proposed.

6.9 Appraisal of cancer chemopreventive potential of the ternary combination of Black Tea, *O. gratissimum,* and *Stevia rebaudiana*

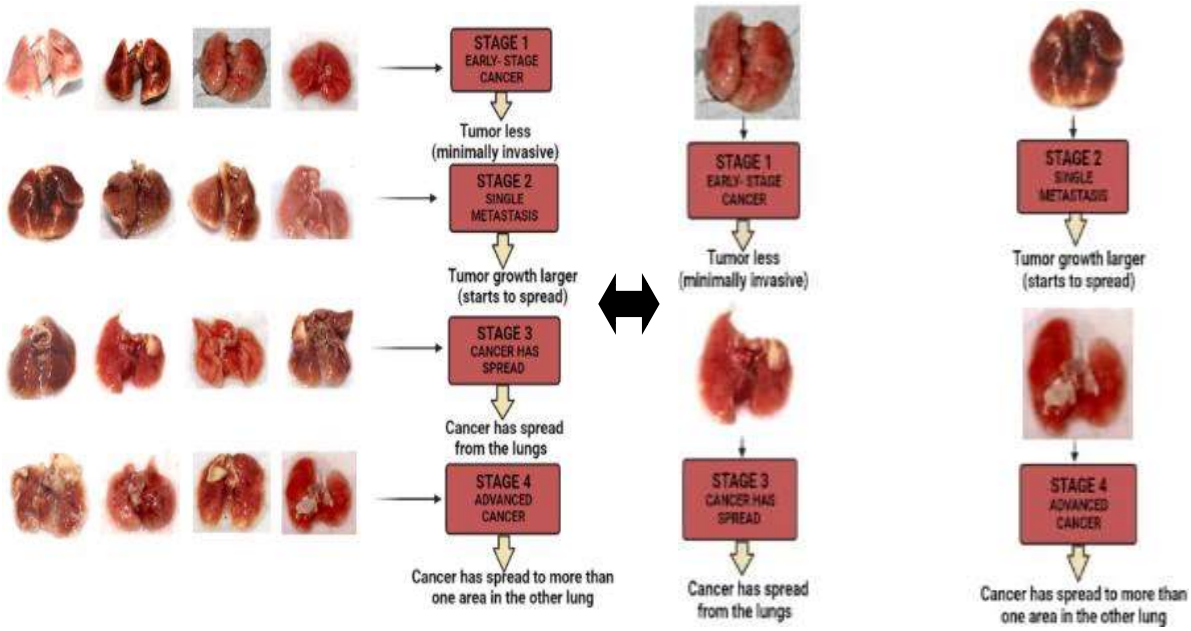


Fig. 77: Different stages of lung tumor observed in mice after sacrificing them

After sacrificing the mice at the end of the complete study, tumors were observed in the lungs of the mice. It was identified that the tumor in the first stage could start to generate and then grow larger (second stage) and finally it can spread to whole lung (3 and 4 stage) as illustrated in **Fig. 77.**

Table 43: Effect of benzo(a)pyrene and aqueous infusions on the body weight (gm) of control and experimental groups of animals during the experimental period.

No. of groups	Nov-Dec 2021	Jan - 2022	Feb - 2022	Mar - 2022
Group 1	25.90 ± 0.95^{a}	25.30 ± 0.75^{b}	24.92 ± 0.51^{d}	23.64 ± 0.94^{e}
Group 2	25.83 ± 0.75^{a}	25.20 ± 0.63^{b}	24.50 ± 0.75^{d}	24.72 ± 0.23^{f}
Group 3	25.50 ± 0.83^{a}	25.00 ± 0.69^{b}	24.87 ± 0.98^{d}	26.76 ± 0.91^{g}
Group 4	25.79 ± 0.51^{a}	25.20 ± 0.89^{b}	25.17 ± 0.98^{d}	27.78 ± 0.83^{h}
Group 5	25.53 ± 0.40^{a}	25.80 ± 0.70^{b}	24.80 ± 0.75^{d}	25.88 ± 0.54^{i}
Group 6	25.76 ± 0.98^{a}	25.60 ± 0.81^{b}	24.75 ± 0.54^{d}	27.96 ± 0.70^{h}
Group 7	25.66 ± 0.81^{a}	25.00 ± 0.51^{b}	24.72 ± 0.51^{d}	26.04 ± 0.83^{j}
Group 8	25.43 ± 0.54^{a}	24.80 ± 0.54^{c}	24.50 ± 0.63^{d}	25.82 ± 0.91^{i}

Data are shown as mean ± s.d (n=8). Group 1- Vehicle control (drinking water only), Group 2- B(a)P drug only, Group 3- BT infusion, Group 4- OG infusion, Group 5- St infusion, Group 6- BTOG mixture, Group 7- BTSt mixture & Group 8- BTOGSt mixture. Different alphabets (a,b,c,d…) represented significant differences at p≤0.05 for a test. Same alphabets (a,a,b,b…) represented no significant difference at p≤0.05 for a test. Data are shown as mean ± s.d. Different color of alphabets showed different months ($P \leq 0.05$). Values in parenthesis are arcsine transformed values; a-j represents the levels of treatments: 'a' = highest effect and 'j' = less effect.

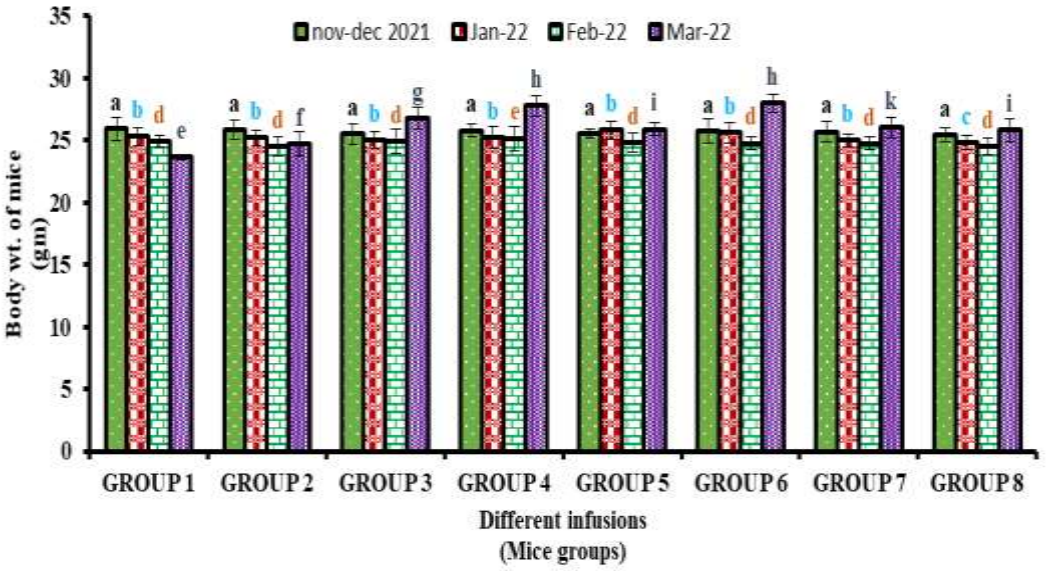


Fig. 78: Effect of the drug (Benzo(a)pyrene) and aqueous infusions on control and experimental groups of the animals.

6.9.2.1 Body status, food, and infusion intake

The table displays how different groups of mice (control and experimental) responded to aqueous infusions and benzo(a)pyrene on the body weight and lung weight of the mice. Up to the 12th week of the trial (from December 2021 to

February 2022), the inter-group disparities in body weight were not seen (p 0.05). In contrast to the control group (Group 1), the B(a) P-administered mice (Group 2) showed a decrease in body weight by the 16th week, whereas the aqueous infusion groups (Groups 3-8) showed an increase, as shown in **Fig. 78** and **Table 43**.

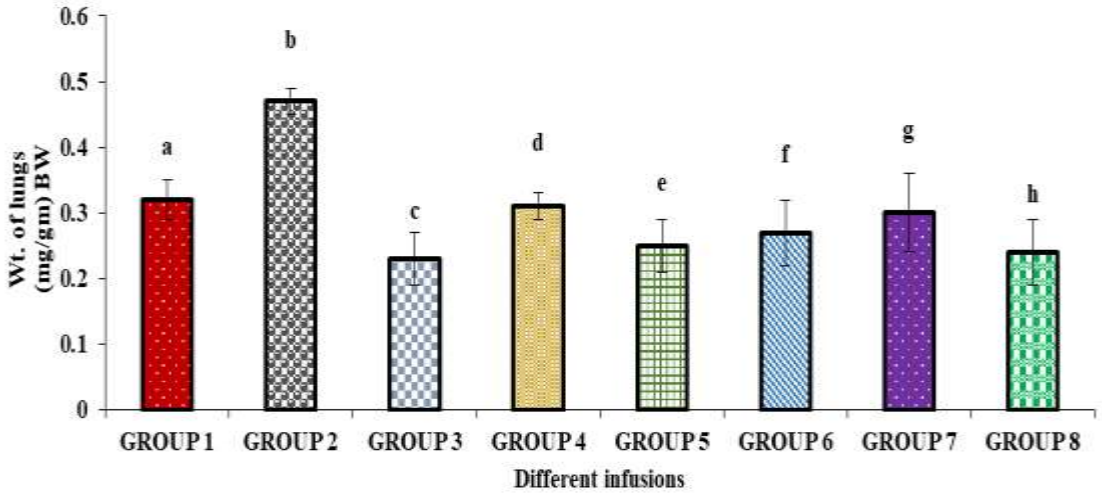


Fig. 79: Total lung weight (mg/gm) BW of the control and experimental group of the animals

Whereas the aqueous infusion intake and water consumption were observed to be similar in all the studied groups of animals ($p \geq 0.05$), as shown in **Fig. 78, 79, and 80 and Tables 43 and 44**. The food intake was found to be similar within the respective group during the experimental duration, whereas some inter-group variations were observed, as shown in **Table 44.**

Table 44: Total food intake (gm/mouse/day) by control and experimental groups of animals during study.

No. of groups	Nov -Dec 2021	Jan - 2022	Feb - 2022	Mar - 2022
Group 1	164.21 ± 15.84[a]	181.40 ± 26.85[a]	169.00 ± 10.77[a]	177.25 ± 14.68[a]
Group 2	177.26 ± 15.50[b]	173.93 ± 13.77[b]	165.12 ± 12.77[a]	177.37 ± 14.68[a]
Group 3	128.78 ± 30.23[c]	95.46 ± 12.78[c]	101.92 ± 14.00[b]	146.87 ± 25.53[b]
Group 4	166.42 ± 17.82[d]	165.20 ± 20.11[d]	162.50 ± 12.40[c]	173.62 ± 13.71[c]
Group 5	155.78 ± 15.12[e]	164.80 ± 12.30[d]	161.78 ± 11.97[c]	153.87 ± 20.46[d]
Group 6	158.36 ± 16.49[e]	146.40 ± 2.26[e]	142.21 ± 15.75[d]	149.87 ± 22.03[e]
Group 7	140.06 ± 30.66[f]	146.66 ± 1.91[e]	131.21 ± 19.70[e]	149.79 ± 20.23[e]
Group 8	137.73 ± 24.23[g]	146.66 ± 15.26[e]	126.35 ± 21.35[f]	134.00 ± 22.4[f]

Data are shown as mean ± s.d (n=8). Group 1- Vehicle control (drinking water only), Group 2- B(a)P drug only, Group 3- BT infusion, Group 4- OG infusion, Group 5- St infusion, Group 6- BTOG mixture, Group 7- BTSt mixture & Group 8- BTOGSt mixture. Different alphabets (a, b, c, d….) represented significant differences at p≤0.05 for a test. Data are shown as mean ± s.d. Same alphabets (a, a, b, b…) showed no significant difference (P ≤ 0.05). Different color of alphabets showed different months at p≤0.05. Values in parenthesis are arcsine transformed values; a-d represents the levels of treatments: 'a' = highest effect and 'f ' = less effect.

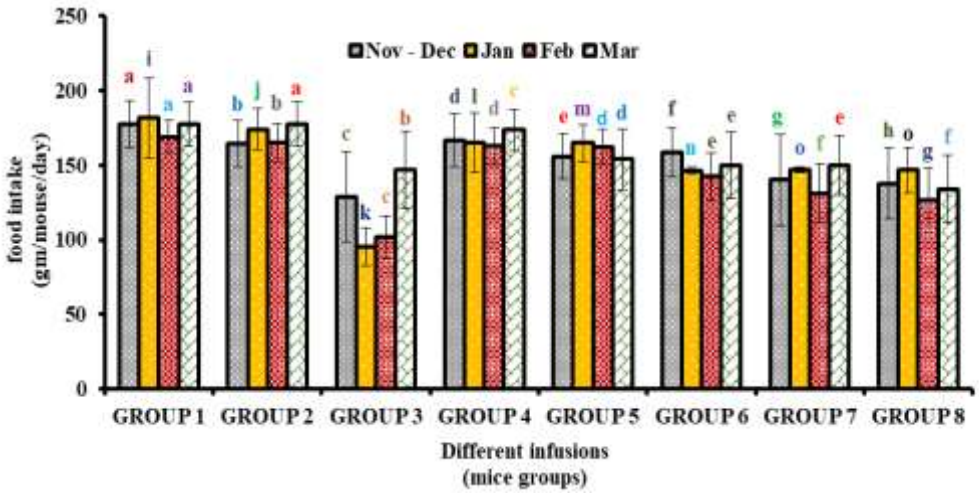


Fig. 80: Total food intake (gm/mouse/day) of the control and experimental group of the animals

Table 45: Total fluid intake (ml/mouse/day) by control and experimental groups of animals during study.

No. of groups	Nov-Dec 2021	Jan - 2022	Feb - 2022	Mar - 2022
Group 1	60.22 ± 9.35[a]	74.60 ± 9.78[a]	83.71 ± 3.61[a]	89.20 ± 4.63[a]
Group 2	54.05 ± 5.95[b]	68.73 ± 10.13[b]	78.78 ± 3.25[b]	83.13 ± 3.61[b]
Group 3	54.11 ± 6.29[b]	71.86 ± 7.52[c]	74.85 ± 3.04[c]	80.13 ± 3.11[c]
Group 4	64.00 ± 2.80[c]	73.66 ± 7.77[d]	71.14 ± 5.03[d]	80.26 ± 2.83[c]
Group 5	61.88 ± 3.00[d]	76.13 ± 8.47[e]	76.21 ± 3.11[e]	77.26 ± 3.48[d]
Group 6	61.61 ± 4.90[d]	68.53 ± 6.66[b]	73.78 ± 4.49[f]	85.46 ± 1.97[e]
Group 7	62.66 ± 5.29[e]	74.73 ± 6.53[f]	77.57 ± 2.30[g]	77.80 ± 2.17[d]
Group 8	53.38 ± 5.83[f]	72.06 ± 4.04[g]	72.21 ± 2.12[h]	76.26 ± 2.28[f]

Data are shown as mean ± s.d (n=8). Group 1- Vehicle control (drinking water only), Group 2- B(a)P drug only, Group 3- BT infusion, Group 4- OG infusion, Group 5- St infusion, Group 6- BTOG mixture, Group 7- BTSt mixture & Group 8- BTOGSt mixture. Same alphabets (a,a,b,b…) represented no significant differences at p≤0.05 for a test. Data are shown as mean ± s.d. Different alphabets (a,b,c,d…) showed a significant differences (P ≤ 0.05). Different color of alphabets showed different months at p≤0.05. Values in parenthesis are arcsine transformed values; a-h represents the levels of treatments: 'a' = highest effect and 'h' = less effect.

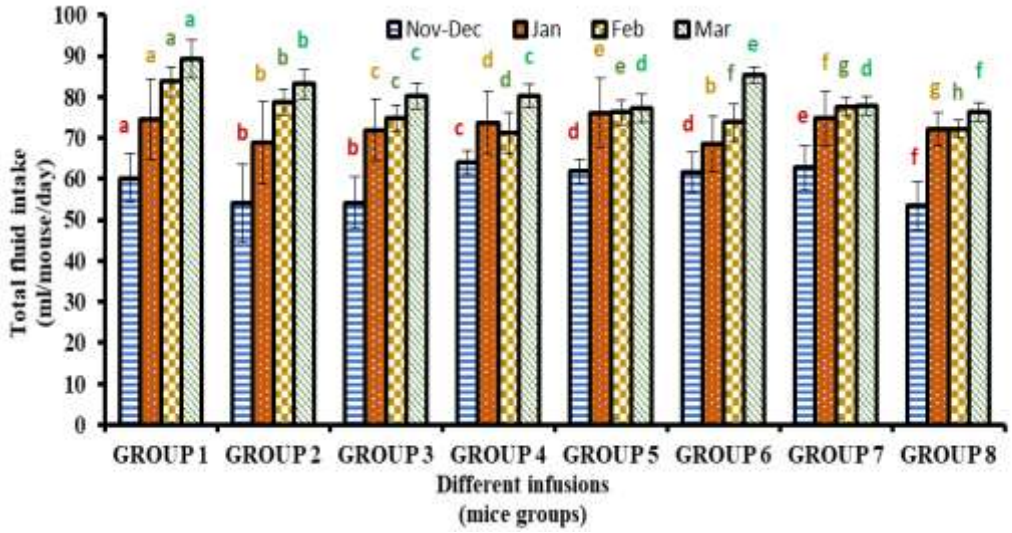


Fig. 81: Total fluid intake (ml/mouse/day) by the control and experimental group of the animals

Table 46: Total lung weight (mg/gm BW) of control and experimental groups of animals during study.

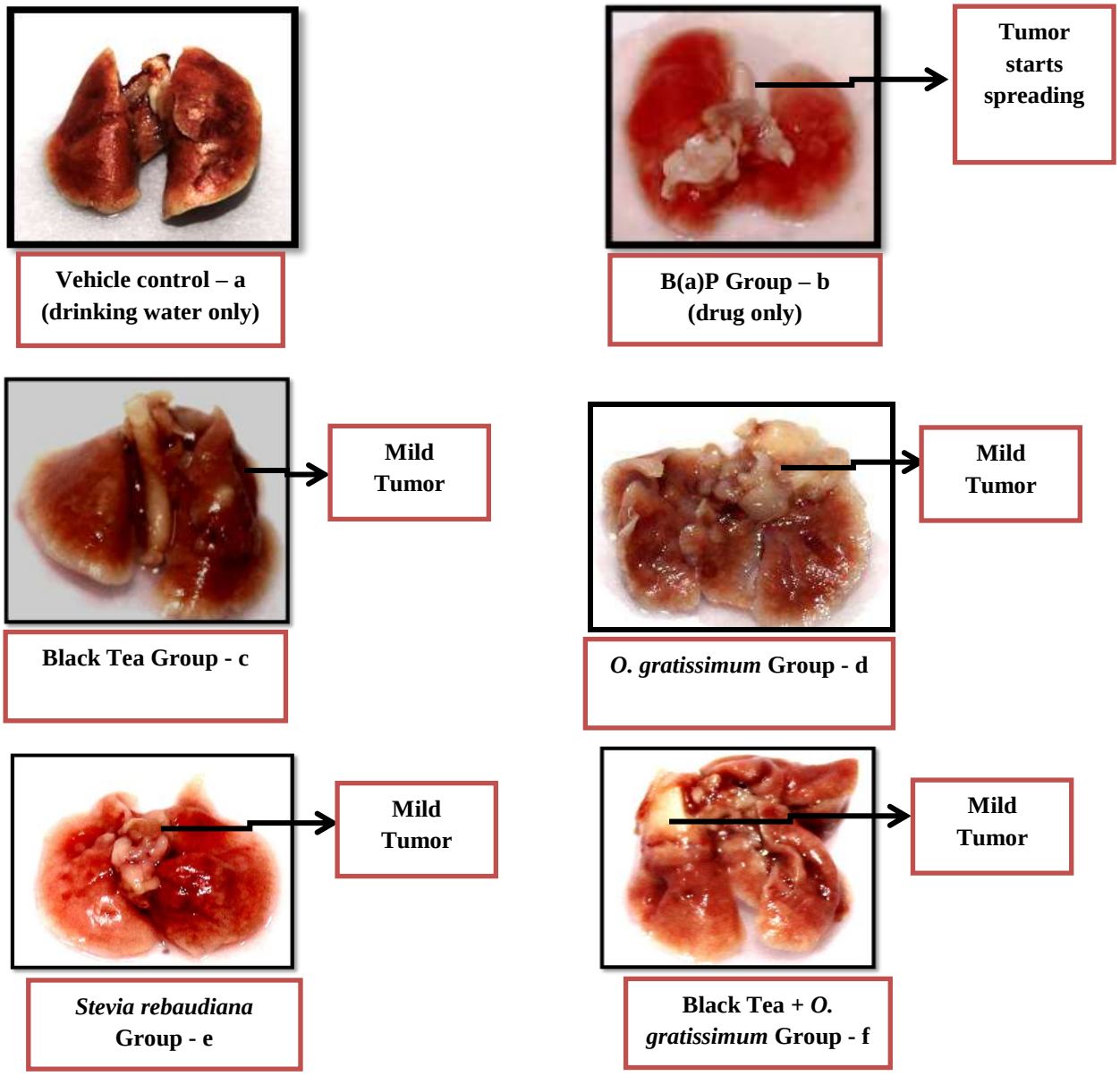
No. of groups	Total Lung Weight (mg/gm BW)
Group 1	0.32 ± 0.03^{a}
Group 2	0.47 ± 0.02^{b}
Group 3	0.23 ± 0.04^{c}
Group 4	0.31 ± 0.02^{d}
Group 5	0.25 ± 0.04^{e}
Group 6	0.27 ± 0.05^{f}
Group 7	0.30 ± 0.06^{g}
Group 8	0.24 ± 0.05^{h}

Data are shown as mean ± s.d (n=8). Group 1- Vehicle control (drinking water only), Group 2- B(a)P drug only, Group 3- BT infusion, Group 4- OG infusion, Group 5- St infusion, Group 6- BTOG mixture, Group 7- BTSt mixture & Group 8- BTOGSt mixture. Same alphabets (a,a,b,b…) represented no significant differences at $p \leq 0.05$ within the same column for a test. Data are shown as mean ± s.d. Different alphabets (a,b,c,d…) showed a significant difference ($P \leq 0.05$). Values in parenthesis are arcsine transformed values; a-h represents the levels of treatments: 'a' = highest effect and 'h' = less effect.

6.9.2.2 Relative Lung Weight

The external morphology of the lungs showed normal architecture for vehicle control (drinking water only) (Gp. 1), as depicted in **Fig's. 81** and **82**. The lung weight was more noticed in the animals of B(a)P (Gp. 2) because of more tumor size, as shown in **Fig's. 81** and **82**. Whereas the lung weight was less and was found to be similar in the group treated animals with aqueous infusions [Black Tea (Gp. 3), *O. gratissimum* (Gp. 4), *S. rebaudiana* (Gp. 5) and their binary (Gp. 6 & 7) and ternary combination (Gp. 8)] as illustrated in **Fig's. 82(c-h)** and **Table 46**. The formula for relative lung weight is:

Relative organ weight = organ weight (g) × 100 / body weight (g)



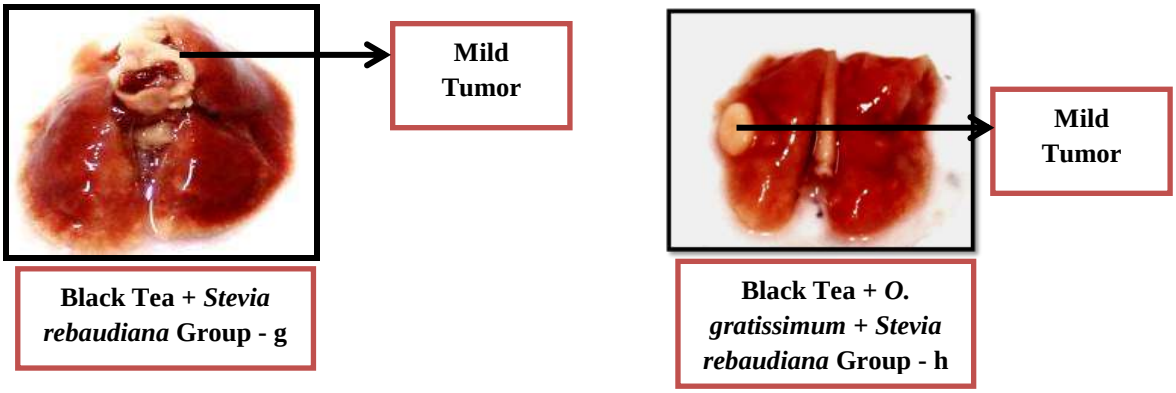


Fig. 82: Effect of BT, OG, St and their combination on external morphology of lungs against B[a]P- induced lung carcinogenesis: (a) Gp.1 (Vehicle control – drinking water only), (b) Gp. 2 (B(a)P – drug only), (c) Gp. 3 (Black Tea), (d) Gp. 4 (*O. gratissimum*), (e) Gp. 5 (*S. rebaudiana*), (f) Gp. 6 (Black Tea + *O. gratissimum*), (g) Gp. 7 (Black Tea + *Stevia rebaudiana*) & (h) Gp. 8 (Black Tea + *O. gratissimum* + *S. rebaudiana*).

Table 47: Effect of Benzopyrene along with BT, OG, St and their binary (BTOG, BTSt) and ternary (BTOGSt) combination on body weight and relative lung weight (mg/gm b.wt.) of various groups of animals.

Group of mice	Body Weight (gm)	Relative Lung Weight (mg/gm b.wt.)
Group 1	23.64 ± 0.94	1.35 ± 0.75^{g}
Group 2	24.72 ± 0.23	1.90 ± 0.63^{h}
Group 3	26.76 ± 0.91	0.85 ± 0.69^{i}
Group 4	27.78 ± 0.83^{d}	1.11 ± 0.89^{j}
Group 5	25.88 ± 0.54^{e}	0.96 ± 0.70^{k}
Group 6	27.96 ± 0.70^{d}	0.96 ± 0.81^{k}
Group 7	26.04 ± 0.83^{f}	1.15 ± 0.51^{j}
Group 8	25.82 ± 0.91^{e}	0.92 ± 0.54^{l}

Whereas Group 1: B(a)P, Group 2: no B(a)P, Group 3: Black tea, Group 4: *O. gratissimum*, Group 5: *Stevia rebaudiana*, Group 6: Black tea + *O. gratissimum*, Group 7: Black tea + *Stevia rebaudiana*, Group 8: Black tea + *O. gratissimum* + *Stevia rebaudiana* combination. Values represented mean ± S.D for six mice in each group. Both body weight and relative lung weight

is expressed in grams (g and mg/g). BW (body weight). Values in parenthesis are arcsine transformed values; a-f represents the levels of treatments: ‘a’ = highest effect and ‘f ’ = less effect.

6.9.2.3 Evaluation of lung histological changes

Microscopically, lung tissue sections stained with hematoxylin and eosin were analyzed. Mice's lung's internal anatomy revealed the presence of bronchioles, the terminal bronchiole, alveolar ducts, alveolar sacs, and alveoli. Simple cuboidal cells and many blood vessels were discovered in the bronchiolar epithelium.

Terminal bronchioles, a division of bronchioles that enters alveolar ducts, have been noted. Alveolar ducts are first formed by the alveoli and smooth muscles. The alveolar sacs, which are a sphincter-like structure seen in the lung tissue, are created by the alveoli.

The respiratory region's final segment is this one. Through a pore in the alveolar septa, the alveoli coming from the surrounding alveolar ducts and respiratory sacs are joined. The structural and functional component of the respiratory region, called the alveolus, is also present in the lung tissue. The lung lesions are classified as alveolar (tiny air-sacs at the end of bronchioles), bronchiolar (smaller and main airways), alveolar/bronchiolar hyperplasia (morphologic change that occurs in the bronchiolar/alveolar epithelium), emphysema (shortness of breath), dysplasia (presence of abnormal cells in the tissues), mononuclear cell infiltration (chronic inflammatory reaction) and early stage of carcinoma (stage 1 – that has not spread to the other parts of the body). mononuclear cell infiltration (MNC), emphysema, hyperchromatic nuclei (HCN) regions. Whereas, BTSt and BTOG showed less reduction in the pulmonary lesions [hyperplastic, dysplastic, (early stage of carcinoma – starting of abnormal cells), mononuclear cell infiltration (MNC), emphysema, hyperchromatic nuclei (HCN) regions]. Therefore, these results indicate that these substances could potentially act as chemopreventive agents against the progression of lung. The study found that BT, OG, St, and BTOGSt resulted in a reduction in hyperplastic, dysplastic, (early stage of carcinoma – starting of abnormal cells),

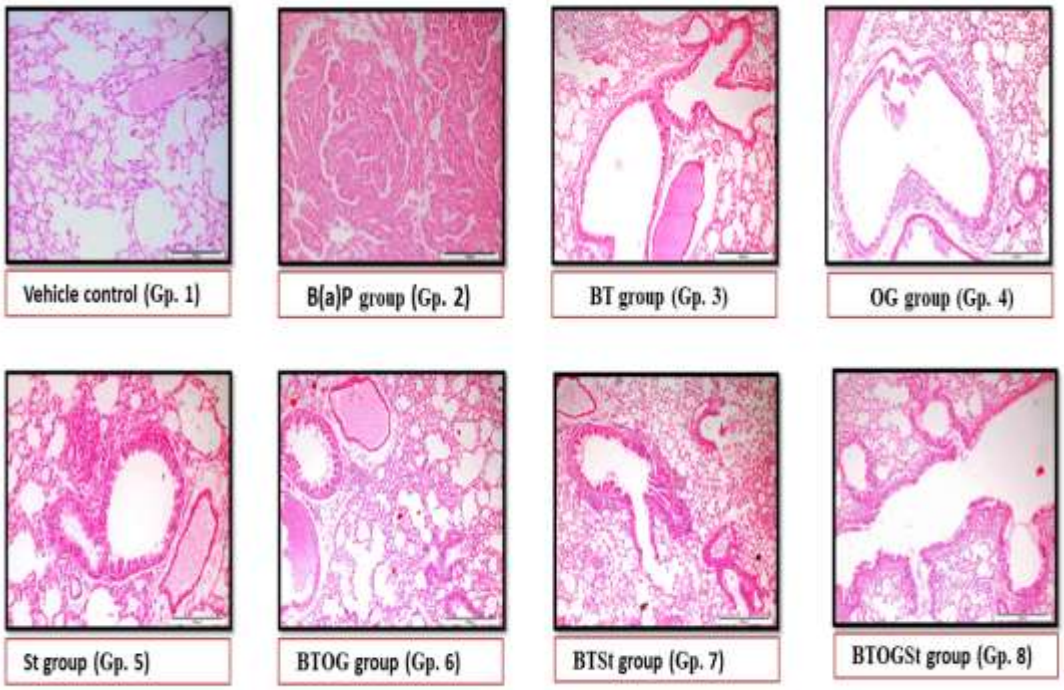


Fig. 83: The morphological appearance of lungs (mg/gm b.wt.) of different groups of animals after completion of 16th weeks of study

Gp. I (Vehicle control) animals demonstrated normal architecture of lung sections showing alveolar sac (tiny air sacs), alveolar space (tiny air sacs), blood capillaries, alveolar duct, terminal bronchiole (contain a smaller number of alveoli), and blood vessels **(Fig's. 72a-d).**

Gp. 2 [B(a)P] animals showed changes to the lung's histoarchitecture. At other locations, the alveolus, bronchiolar hyperplasia, hyperchromatic nuclei (darkly stained nuclei), widespread proliferation of bronchiolar epithelium giving rise to papillary progression, early stage of carcinoma was detected in B(a)P injected (Gp. 2) lung tissues. Hyperplasia is the replacement of a single layer of bronchiolar and alveolar cells in certain areas by numerous layers of cells in **Fig's. 83 - 91**. The alveolar sac, alveolar duct, bronchiolar duct, and terminal bronchiolar structure were found to be distorted.

Gp. 3 (Black Tea), 4 (*O. gratissimum*), 5 (*S. rebaudiana*), and 8 (Black Tea + *O. gratissimum* + *S. rebaudiana*) treated animals exhibited mild hyperplasia, dysplasia, emphysema, mononuclear cell infiltration, decrease the number of pulmonary lesions, diminished the alveolar wall cells, hyperchromatic & uneven nuclei as compared to B(a)P gp. of animals (Gp. 2). Whereas, Gp. 6 (Black Tea + *O. gratissimum*) & 7 (Black Tea + *S. rebaudiana*) treated animals determined moderate

emphysema, mild hyperplasia with mild focal mononuclear cell infiltration was observed as depicted in **Fig's. 83 - 91**.

The study found that BT, OG, St, and BTOGSt resulted in a reduction in hyperplastic, dysplastic, (early stage of carcinoma – starting of abnormal cells), mononuclear cell infiltration (MNC), emphysema, hyperchromatic nuclei (HCN) regions. Whereas, BTSt and BTOG showed less reduction in the pulmonary lesions [hyperplastic, dysplastic, (early stage of carcinoma – starting of abnormal cells), mononuclear cell infiltration (MNC), emphysema, hyperchromatic nuclei (HCN) regions]. Therefore, these results indicate that these substances could potentially act as chemopreventive agents against the progression of lung carcinogenesis, as illustrated in **Fig's. 83 – 91.**

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6.9.2.3 Histopathological analysis (detailed form)

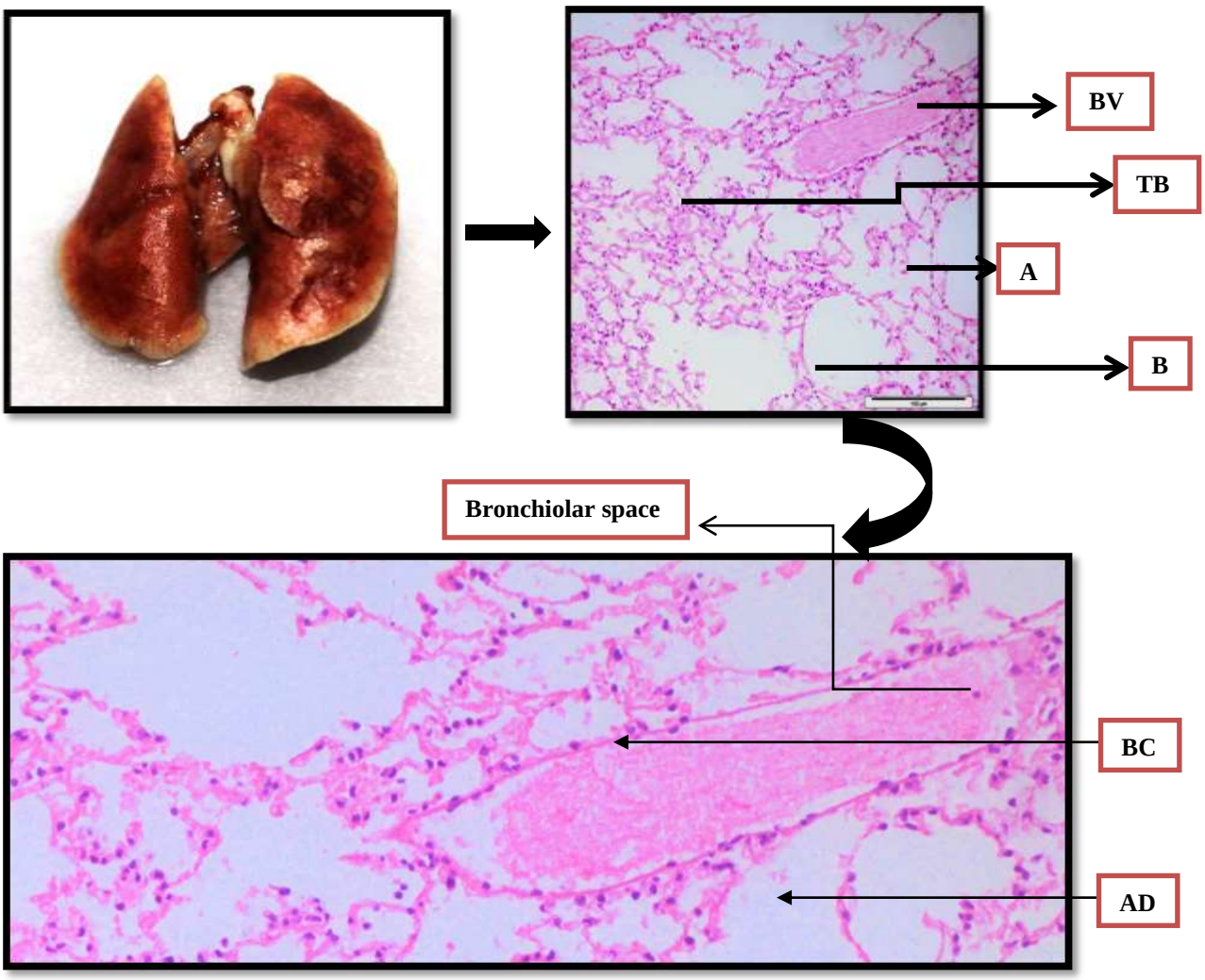

Fig. 84: The histology of the lungs [Vehicle control (drinking water only)] using staining hematoxylin and Eosin. A – Alveoli Space, B – Bronchiolar Space, BC – Blood Capillaries, AD – Alevolar Duct, TB – Terminal Bronchiole & BV – Blood Vessels

Morphological features identified

- Normal lung tissue structure. The normal architecture of lung sections shows a single layer of bronchiolar and alveolar epithelium.

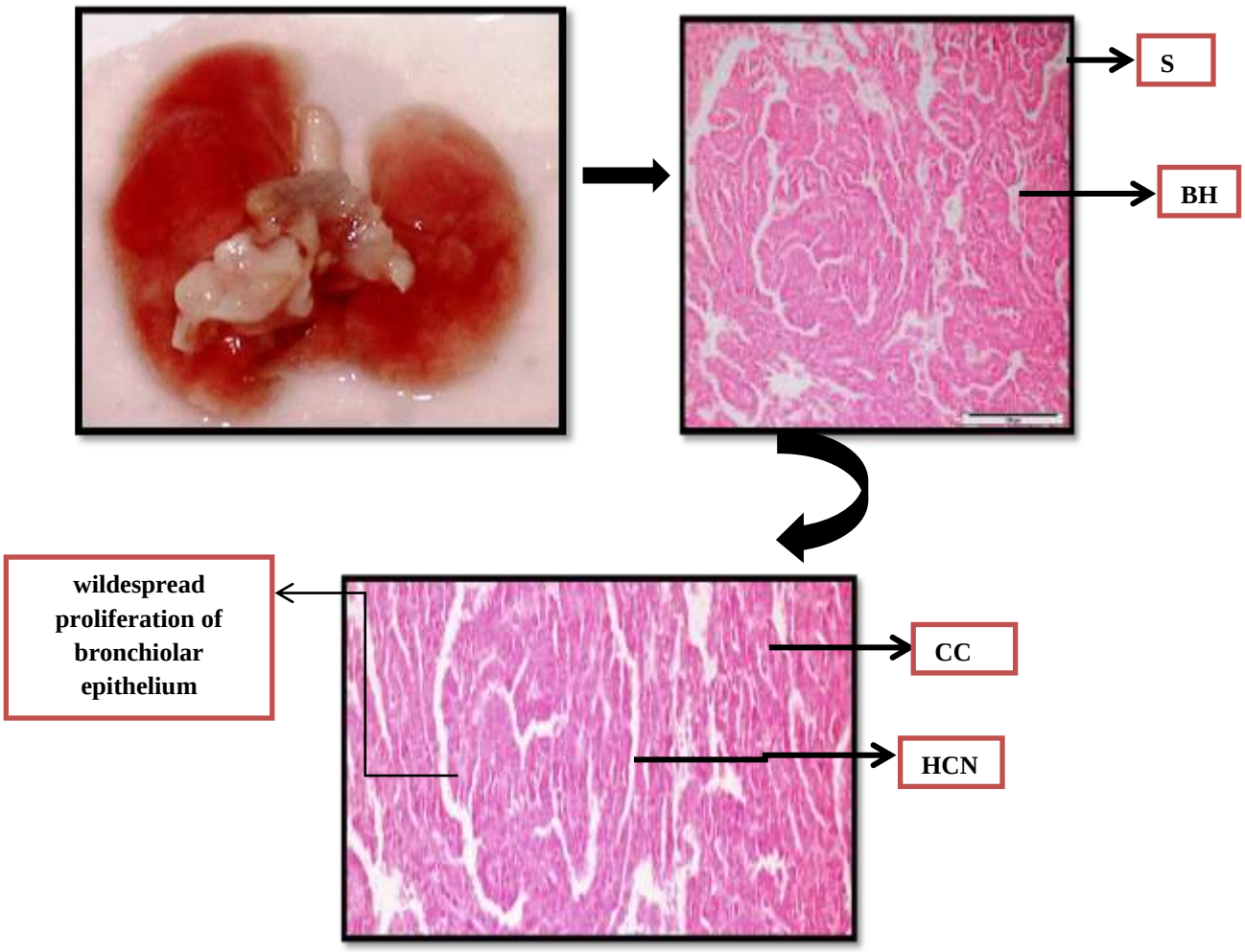

Fig. 85: The histology of the lungs [B(a)P - Group (drug only)] using staining hematoxylin and eosin. A – Alveolus, BH – Bronchiolar Hyperplasia, CC – Clara cells, HCN – Hyperchromatic nuclei & BV – Blood Vessels

Morphological features identified

- The widespread proliferation of bronchiolar epithelium gives rise to papillary progression.
- Starting of Early carcinoma. It has an even distribution of epithelial cells and nuclei with chromatin that is clumped in an uneven pattern.

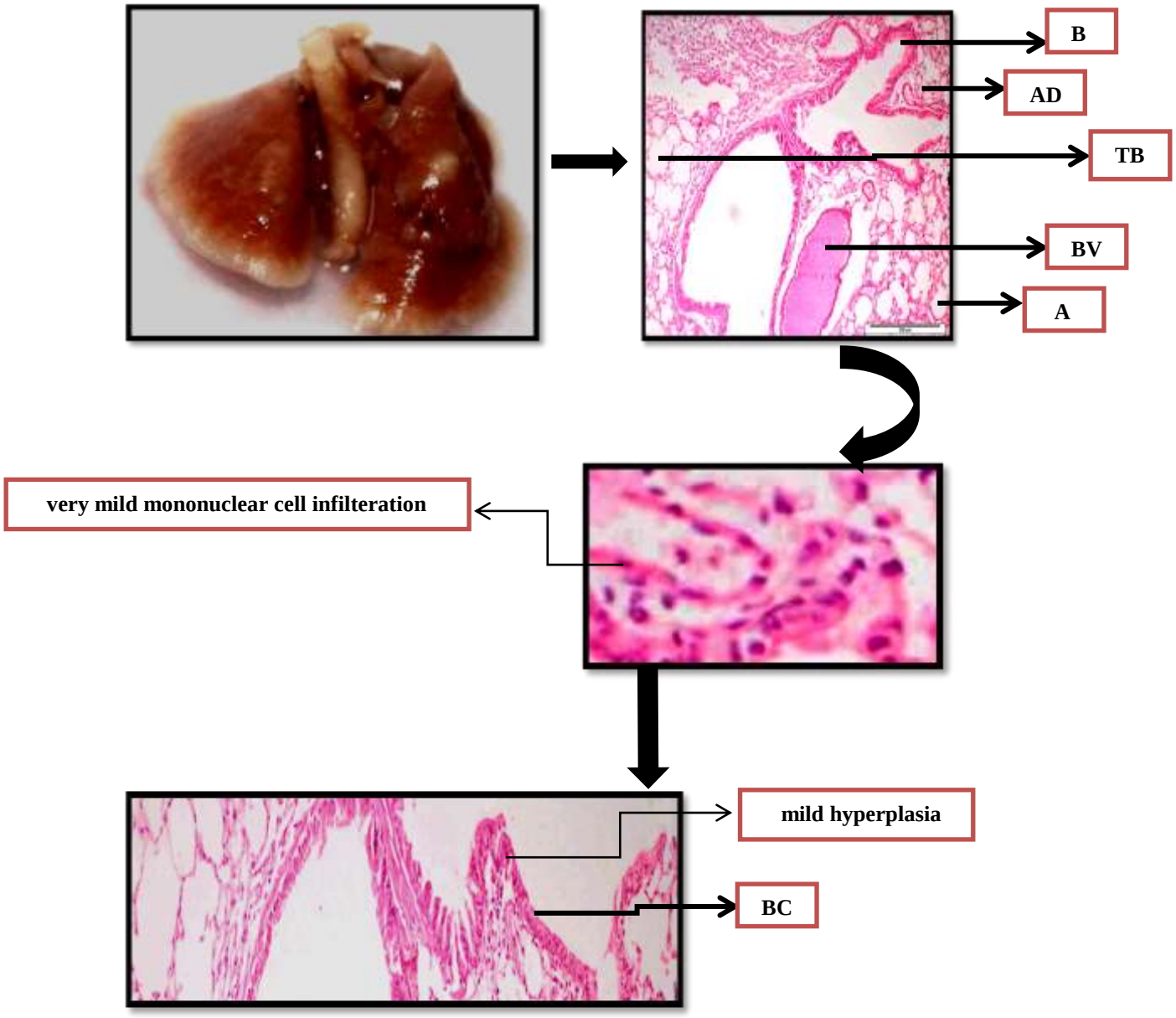

Fig. 86: The histology of the lungs [Black Tea (BT) Group] using staining hematoxylin and eosin. A – Alveolus, B – Bronchiolar Space, BC – Blood Capillaries, AD – Alevolar Duct, TB – Terminal Bronchiole & BV - Blood Vessels

Morphological features identified

- Moderate emphysema.
- Mild hyperplasia.
- Very mild mononuclear cell infiltration (MNC).

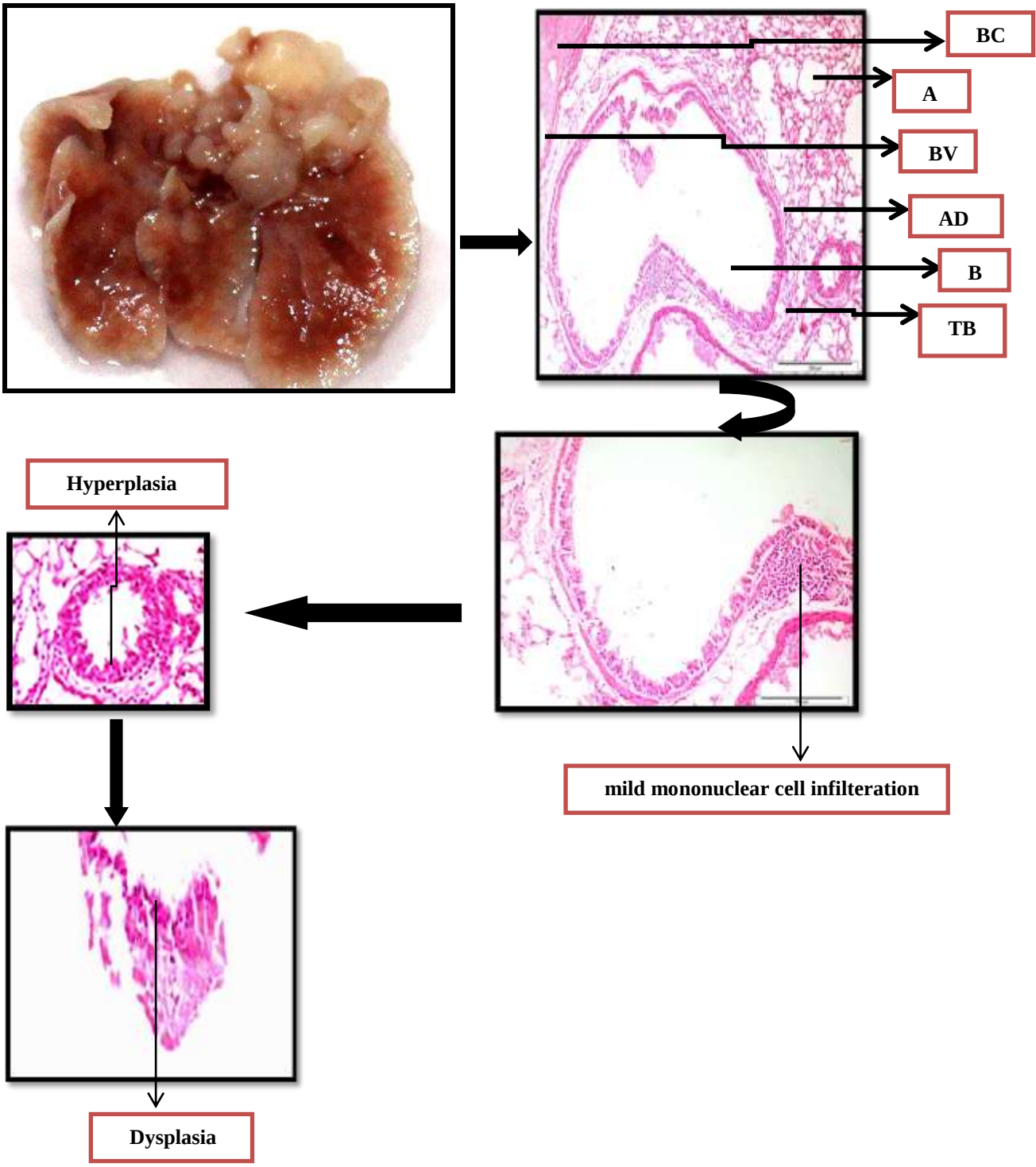

Fig. 87: The histology of the lungs [*O. gratissimum* (OG) group] using staining hematoxylin and Eosin. A – Alveolus, B – Bronchiolar Space, BC – Blood Capillaries, AD – Alevolar Duct, TB – Terminal Bronchiole & BV – Blood Vessels

Morphological features identified

- Very mild emphysema.
- Mild hyperplasia & dysplasia.
- Mild mononuclear cell infiltration.

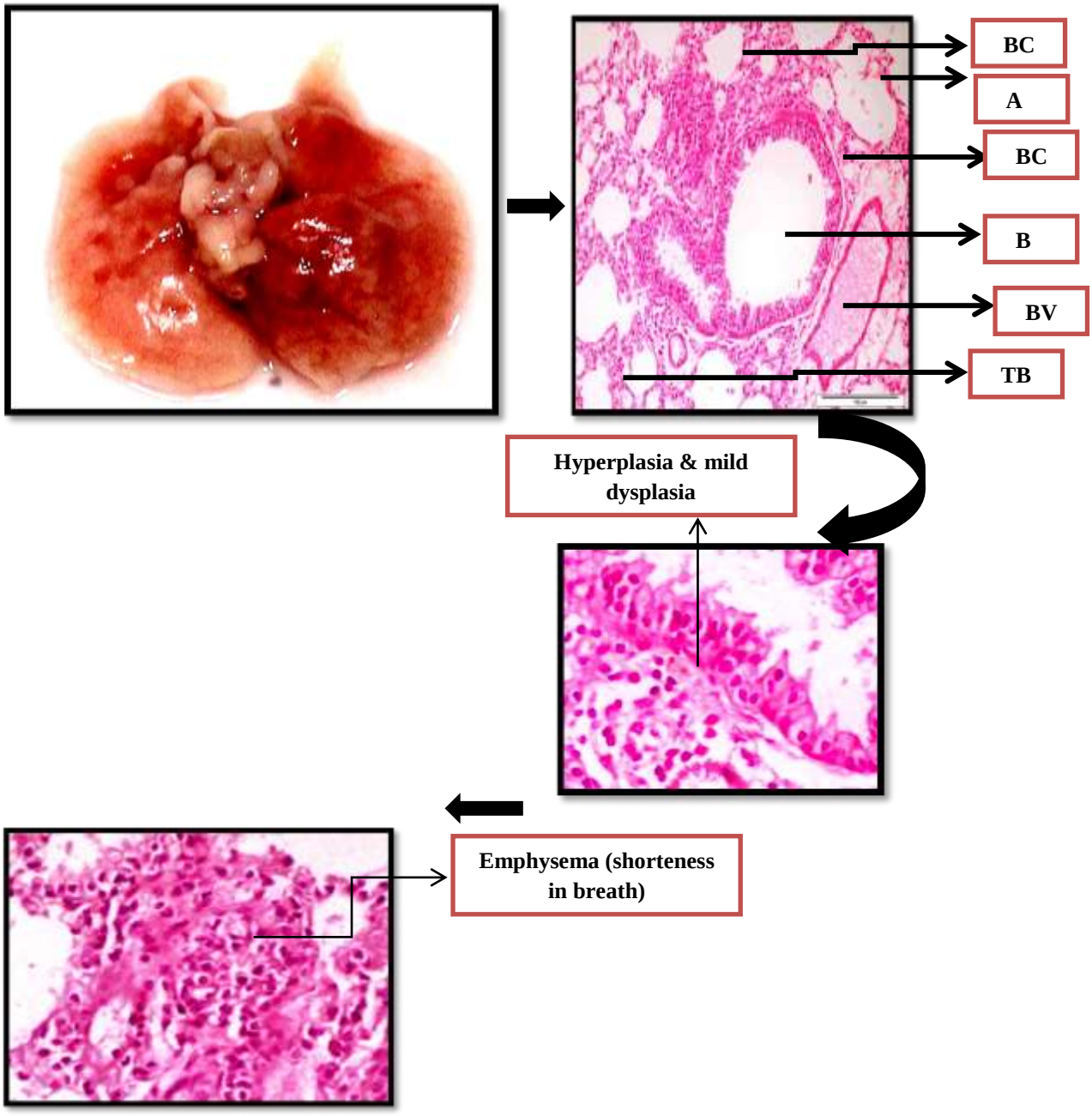

Fig. 88: The histology of the lungs [*S. rebaudiana* (St) Group] using staining hematoxylin and Eosin. A – Alveolus, B – Bronchiolar Space, BC – Blood Capillaries, AD – Alevolar Duct, TB – Terminal Bronchiole & BV – Blood Vessels

Morphological features identified

- Moderate emphysema.
- Very mild hyperplasia, dysplasia.
- Mild peribronchiolar mononuclear cell infiltration (MNC).

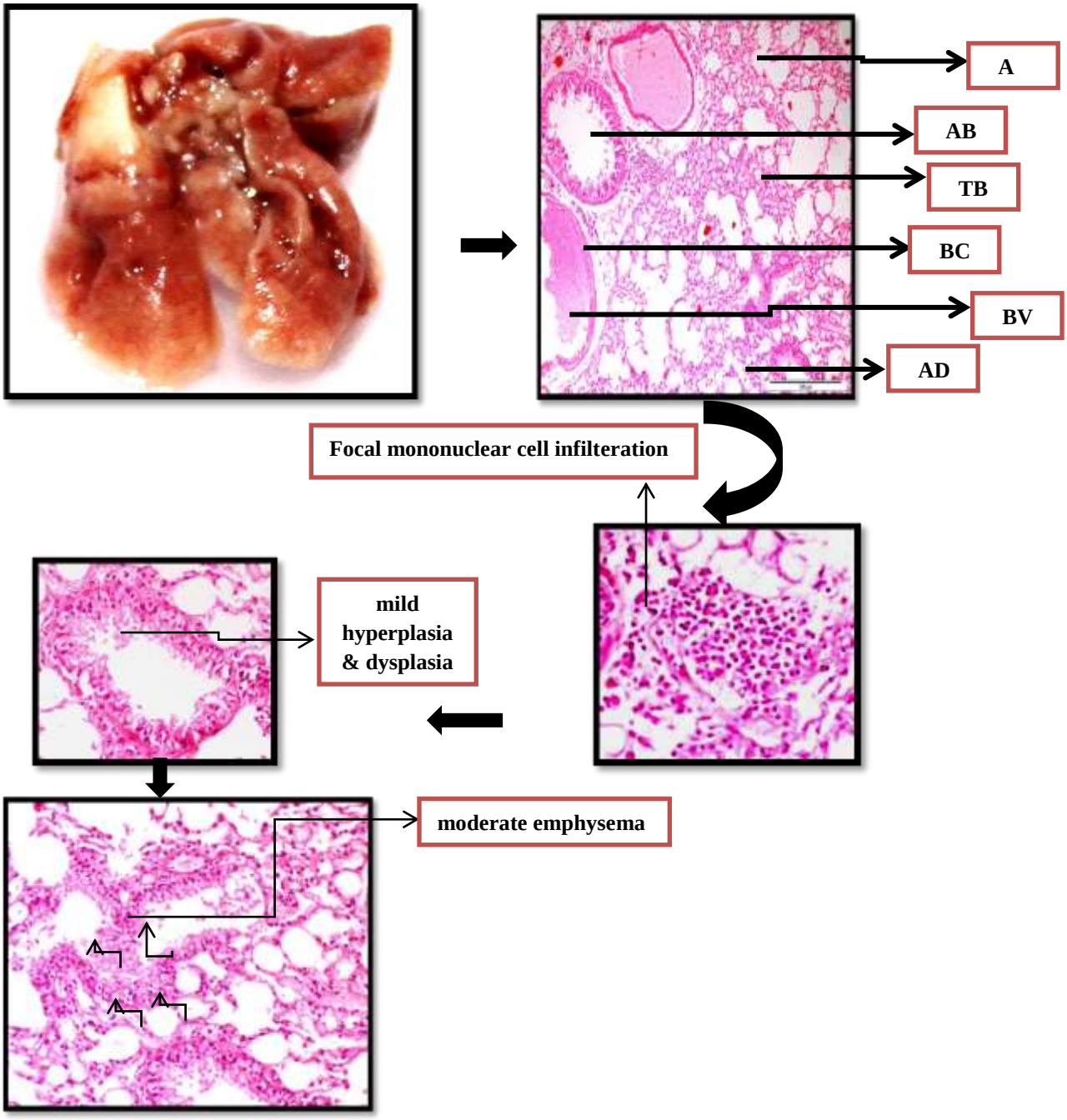

Fig. 89: The histology of the lungs [BTOG Group] using staining hematoxylin and eosin. A – Alveolus. B – Bronchiolar Space, BC – Blood Capillaries, AD – Alevolar Duct, TB – Terminal Bronchiole & BV – Blood Vessels

Morphological features identified

- Moderate emphysema.
- Mild hyperplasia with focal mononuclear cell infiltration (MNC) (small place).

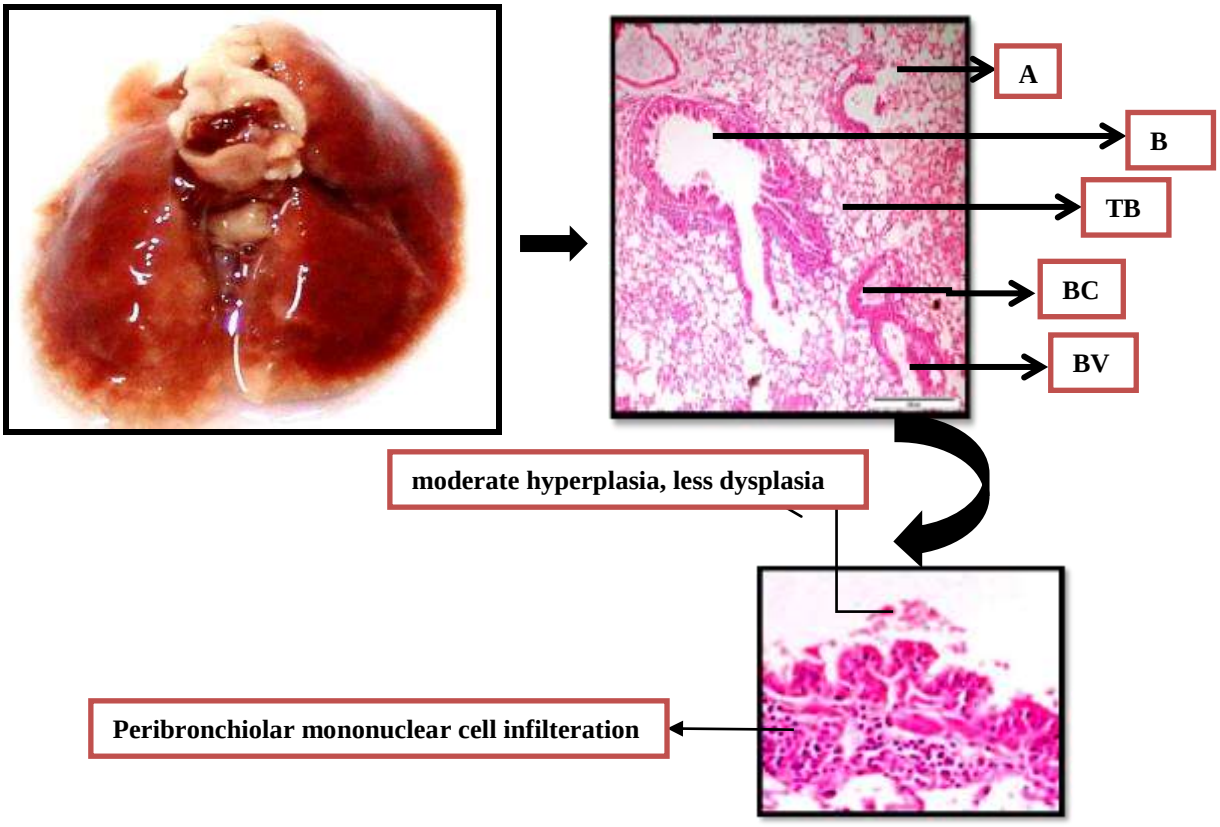

Fig. 90: The histology of the lungs [BTSt Group] using staining hematoxylin and eosin. A – Alveolus, B – Bronchiolar Space, BC – Blood Capillaries, AD – Alevolar Duct, TB – Terminal Bronchiole & BV – Blood Vessels

Morphological features identified

- Moderate hyperplasia, less dysplasia.
- Mild to moderate emphysema along with peribronchiolar mononuclear cell infiltration.

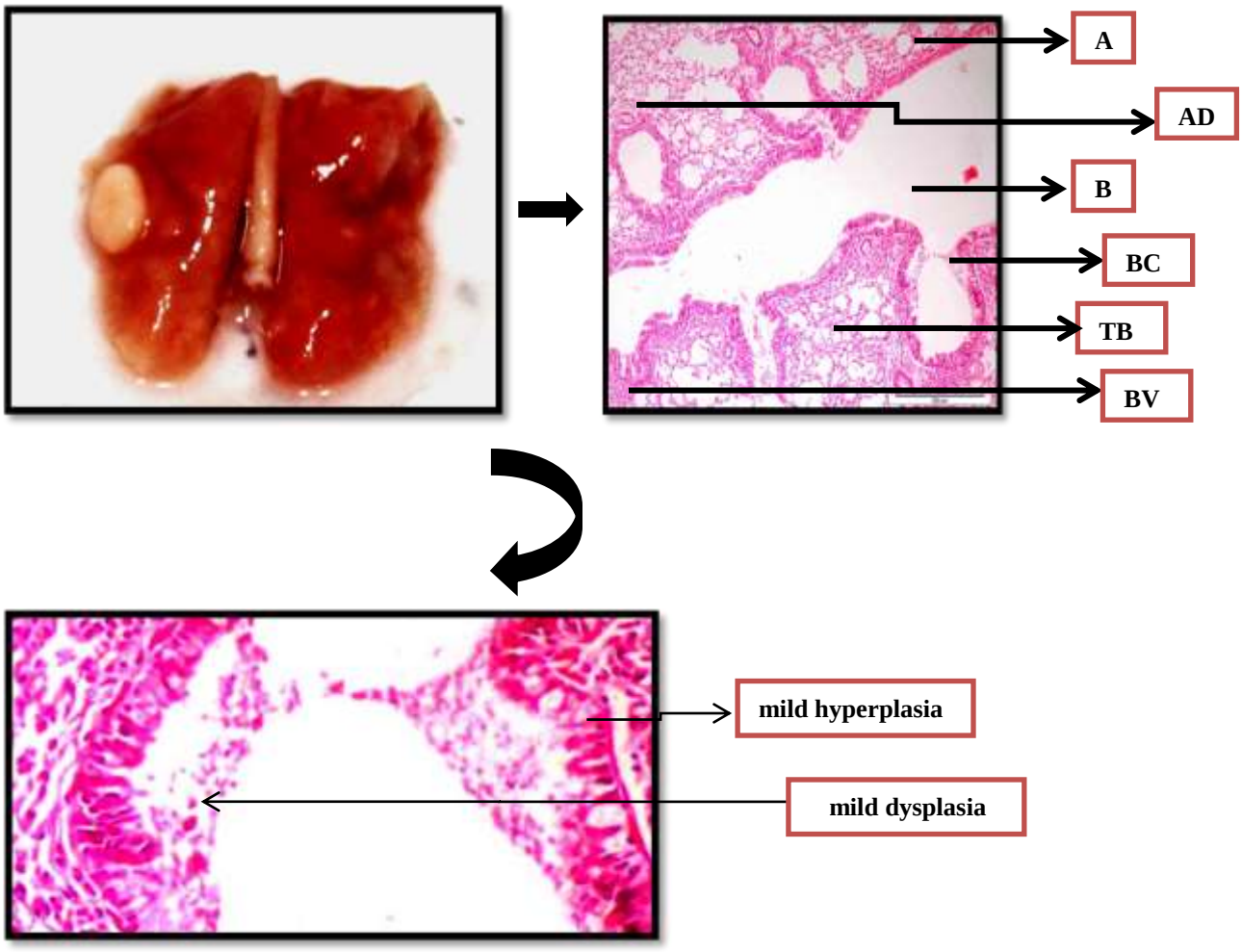

Fig. 91: The histology of the lungs [BTOGSt Group] using staining hematoxylin and eosin. A – Alveolus, B – Bronchiolar Space, BC – Blood Capillaries, AD – Alevolar Duct, TB – Terminal Bronchiole & BV – Blood Vessels

Morphological features identified

- Mild emphysema.
- Mild hyperplasia, dysplasia along with mild inflammatory cell infiltration (MNC).

CHAPTER 7

DISCUSSION

DISCUSSION

Tea is a significant component of most people's daily diets and a strong natural source of antioxidants. Categorized into *C. sinensis* and non- *C. sinensis* teas; the former ones are also known as true teas, such as black, oolong, green, and white tea, whereas the latter ones are called herbal teas or tisanes (Cabrera *et al*., 2003; Ivanova *et al*., 2005; Kumar *et al*., 2018; Guleria *et al*., 2022). Teas are divided into non-fermented, semi-fermented, and completely fermented categories depending on the manufacturing procedure (Xu and Chen, 2002; Zhang *et al*., 2019; Liu *et al*., 2023). Catechins, also referred to as polyphenols, are the active components that play significant roles related to biological activitiy of teas. Additionally, their antioxidant activity is widely recognized (Nibir *et al*., 2017; Brodowska, 2017; Zhang *et al*., 2021). Teas (green and black) contain various forms of catechins and their derivatives, which make them capable of functioning as potential antioxidants (Soni *et al*., 2015; Nibir *et al*., 2017; Guleria *et al*., 2022).

It was revealed from various studies that tea extract, catechins, and polyphenols found in tea may help combat oxidative stress occurs and reduce *in vitro* DNA damage, caused by factors like arsenic exposure (Nibir *et al*., 2017; Singh *et al*., 2021). When the production of ROS and RNS exceeds the capacity of these defense mechanisms to neutralize them, oxidative stress. This imbalance can damage to cellular components, including lipids, proteins, and DNA. Over time, chronic oxidative stress has been associated with various health issues, including cancer, cardiovascular and neurodegenerative diseases (Valko *et al*., 2006; He *et al*., 2017; Ho *et al*., 2022).

Under normal conditions, the body has a well-coordinated antioxidant defense system to counterbalance the formation of reactive species including free radicals. This defense system is essential for maintaining cellular and tissue health. The body’s natural defense systems, including antioxidants and repair enzymes, play a critical role in minimizing the damage caused by oxidative stress (Poljsak *et al*., 2013; Adwas *et al*., 2019; Demirci *et al*., 2022). Numerous reports have shown that balance between oxidative stress and antioxidant defenses is disrupted, which can lead to chronic inflammation, tissue damage, and various health problems such as cancer and

atherosclerosis, aging, arthritis, inflammatory and neurodegenerative diseases, etc (Phaniendra *et al*., 2015; Sharifi *et al*., 2020; Wang *et al*., 2023).

Antioxidant activity prevents the oxidation of proteins, lipids, DNA, or other substances. This inhibition stops the oxidative chain reaction's propagation step, scavenging reactive species, and chelating metal ions (Lee *et al*., 2004; Pisoschi and Pop, 2015; Aureliano *et al*., 2023). One strategy to protect organisms from oxidative damage and inflammation and combat chronic diseases is to consume foods that are rich in antioxidants.

Herbal teas possess properties that can suppress reactive species, adding to their potential as a source of antioxidants for health benefits (Marnewick *et al*., 2005; Bhattacharya *et al*., 2011; Loganathan and Mohan, 2023). Various studies have reported leafy herbal teas (rooibos, rosemary, lemongrass, black tea, peppermint, lotus, mate, bamboo, and mulberry and persimmon leaf) exert a positive impact on lifestyle-related diseases because of their antioxidant, antiatherogenic, antimicrobial and chemopreventive activities. Herbal teas can be obtained from herbs, spices, or plants other than *C. sinensis*. The basil or tulsi plant leaves are a common medicinal plant in India used to supplement black tea. In this study, a comparison of the antioxidant potential of aqueous infusions of black tea granules or three species of basil (*O. gratissimum*, *O. sanctum*, and *O. canum*) alone and as a binary combination of black tea with different *Ocimum* spp. was undertaken.

The activity was measured regarding EC_{50} (half maximal effective concentration); the lower the EC_{50} higher the antioxidant potential. BT and other herbal teas (OS, OG and OC) illustrated applicable antioxidant quenching capability. To determine the antioxidant activity, chemical-based models (DPPH, ABTS and NO) and *ex vivo* assays (hemolysis and lipid peroxidation) were used, as shown in **Fig. 28 - 32** and **Table 10 - 16.** DPPH is a free stable radical, and ABTS is a colored free radical reagent that is dissolved in organic and aqueous solutions (Kim *et al.*, 2002; Mathew and Abraham, 2006). Excess nitric oxide radical (NOR) interacts with oxygen to form free radicals called nitrite and peroxy nitrite anions (Clancy et al., 1992). Iron and catalyzed oxidation processes that result in hydroxyl radicals are modulated by NO (Dadashpour *et al*., 2011; Rana and Suttee, 2012).

These free radical scavengers are used for measuring and analyse the antioxidant capacity of food items, phytochemicals, plant extracts (herbal remedies) and drinks.

All assays (DPPH, ABTS, NO, LPO, and hemolysis) performed on the separate infusions showed that BT had the lowest EC_{50} (greatest antioxidant capability), as demonstrated in **Fig. 28 – 32** and **Table 10 - 16.** Previous studies also reported the high radical scavenging ability of black tea in comparison to some herbal teas (chamomile tea, peppermint tea, verbena tea, rosemary tea, lemongrass tea, and sage tea) (Chan *et al*., 2010; Abbasian *et al*., 2013). Black tea expressed a maximum anti-lipid peroxidation effect as compared to other infusions. Food color, texture, nutritional value and flavor are all affected by lipid peroxidation. Many studies showed black tea and *Ocimum* species can prevent oxidant-induced lipid peroxidation and hemolysis (Guleria and Sehgal, 2020). RBCs are frequently employed, as model system to explore whether plant extracts affect H_2O_2–induced damage. It is well known that the oxidation of PUFA in biological membranes can result in the production and dispersion of lipid radicals, oxygen uptake, double bond reconfiguration in unsaturated lipids, and even membrane lipid destruction. For a wide range of varieties of cells, many of these metabolic processes can produce very toxic breakdown products (Gavahian *et al*., 2018; Guleria and Sehgal, 2020).

The phenolic and flavonoid compounds found in plants are crucial for free radical scavenging, stabilizing lipids to prevent peroxidation and inhibiting different types of oxidizing enzymes (Kahkonen *et al*., 1999; Karimi *et al*., 2010; Chandra *et al*., 2014; Manssouri *et al*., 2020; Royani *et al*., 2023). Extracts from plants have many therapeutic qualities, such as antidiabetic, anticarcinogenic, and antioxidant properties, which can be ascribed to their phenolics and flavonoid compounds (Karak, 2019; Reddy *et al*., 2020; Bouslamti *et al*., 2023). Due to their capacity to neutralize free radicals phenolics can scavenge them. They can prevent the negative effects of oxidative stress (Hidalgo *et al*., 2010; Aziz *et al*., 2019). The potency of antioxidant activity in flavonoids relies on the free OH number and position (Panche *et al*., 2016). Maximum TFC was found in Black Tea, followed by *O. gratissimum, O. sanctum* and *O. canum*. In comparison, *O. gratissimum* leaves have higher phenolic content than other *Ocimum* species (*O. americanum, O. minimum, O. citridorum, O. lamblifolium,* and *O. selloli*) (Shannon *et al*., 2017). The differences found in the distribution of phenolic and flavonoid compounds among different *Ocimum* species may be attributed to genetic variations (Rababah *et al*., 2011; Shannon *et al*., 2017). A previous study showed that *Camellia sinensis* (black, green and white tea) and herbal tea (such as chamomile) are rich sources of phenolic and flavonoid compounds. The

highest amount of flavonoid compound was identified in black tea (Li *et al*., 2013). According to a previous study, black tea contains more flavonoids than berry and chamomile tea (Shannon *et al*., 2017).

Black tea alone and in binary combinations (BTOG) showed a strong negative correlation between quenching activity (EC_{50}) and TPC/TFC, respectively. Another study reported a similar relationship between tea phenolic content and antioxidant activity in green, white and black tea with an increase in TPC resulting in an increased radical DPPH scavenging potential of tea infusions (Pereira *et al*., 2014). The variations in the antioxidant activity of black tea and other teas may be due to differences in various factors such as cultivation, agricultural practices, cultivar, leaf age, grade, topographical conditions, storage and processing, and the number of polyphenols found in different teas (Damiani *et al*., 2014; Farooq and Sehgal, 2018; Guleria and Sehgal, 2022).

Herbal products are used in various regions and cultures as a single herb, a combination of herbs, or a mixture of herbs and drugs (Wills *et al*., 2000; Che *et al*., 2013). The effects can be complicated when herbs are combined because various interactions between the individual components can occur. Interactions resulting additional therapeutic benefits are the most desirable (Colalto, 2010; Che *et al*., 2013). When using combination therapy, this is frequently the desired or expected outcome. However, because herbal products contain multiple components, the effects of herb-herb or herb-drug interactions are frequently unpredictable and complicated (Zhou *et al*., 2005; Gurley *et al.*, 2012).

Different forms of pharmacokinetic and pharmacodynamic interactions resulting from herb-drug combinations have been well described and documented in literature. Herb-herb interaction, on the other hand, has received far less attention even though the use of herbal medicine as a therapeutic approach has been established and considered desirable since time immemorabale (Huangdi Neijing) China, more than 2,000 years ago (Che *et al*., 2013; Liu *et al*., 2014).

Combination therapies are currently employed to achieve improved therapeutic effects in the treatment of deadly ailments like AIDS, cancer and pulmonary tuberculosis (Che *et al*., 2013). The interactions between different phytoconstituents present in it may influence the total antioxidant capacity of a mixture. These interactions can be classified into three categories: synergistic, additive and

antagonistic (Wang *et al*., 2011; Chen *et al*., 2022). When blended with *O. gratissimum*, black tea, it exhibited an additive interaction and maximum antioxidant potential. However, the supplementation of *O. sanctum* or *O. canum* resulted in antagonistic interaction and a lower radical scavenging ability (Guleria and Sehgal, 2022). An additive interaction is due to an increase in the concentration of phytochemicals responsible for antioxidant potential, whereas antagonistic interaction may emerge because of alteration in structural characteristics of phytochemicals that may decrease the functional properties of a mixture (Hidalgo *et al*., 2010). The flavored black teas containing fruit or other plant extracts exhibited higher DPPH scavenging activity, followed by black tea and fruit teas (Pekal *et al*., 2011). The interaction between the antioxidant activity of tea (black and green tea extracts) and ascorbic acid in different weight ratios (10:1, 5:1, 2:1, 1:1, 1:2, 1:5 and 1:10) ranged from antagonism to additive (Enko and Swigło, 2015). The antioxidant potential of aqueous and methanolic extract of a polyherbal combination of some herbs [(tea + ginger (1:1), tea + black pepper (1:1), tea + tulsi (1:1), tea + ginger + black (1:1:1), tea + black pepper + tulsi (1:1:1), and tea + ginger + black pepper + tulsi (1:1:1:1)] showed synergistic effect (Gupta *et al.,* 2014). The binary combination of black tea and *Ocimum* spp. exhibited higher phenolic and flavonoid contents due to their higher concentration (2% w/v) than single infusions (1% w/v). The combination of black tea and *O. gratissimum* (BTOG) manifested maximum phenolic and flavonoid, followed by BTOS and BTOC. The binary combination of black tea and *O. gratissimum* was found to be the best among the studied pairs their antioxidant activity (Guleria and Sehgal, 2022).

Tea research is currently significant attention and recognition globally because it contains different important secondary metabolites with antioxidant activity. because they conatin different important secondary metabolites with antioxidant activity. Tea leaves contain polyphenolic compounds (phenols and flavonoids) which are widely held for their pharmacological properties (Chaturvedula *et al*., 2011; Samarina *et al*., 2022; Guleria *et al*., 2022). Antihaemolytic, scavenging potential, and anti-lipid peroxidation were found maximum in the aqueous infusion of black tea in comparison to OG. This observation was in line with (UlHaq and Sehgal, 2020). BT had a higher flavonoid concentration than OG, although OG had a higher phenolic content. Blended black teas have higher amount of falavonoids in comparsion to unblended form diffrernt region of world like Assam, China and Ceylon (Peterson *et*

al., 2005; Shannon *et al*., 2017). According to a different study, black tea was found to hinder (copper-induced) lipid peroxidation and the production of thiobarbituric acid, while also demonstrating protection against AAPH-induced oxidative hemolysis of erythrocytes in a dosage-dependent manner (Liu and Huang, 2015). According to a study by Rababah *et al.,* 2011, the fresh leaf extract of *O. gratissimum* had the highest level of phenolic content when compared to extracts from other species of *Ocimum*, including *O. americanum, O. minimum, O. citridorum, O. lamlifolium*, and *O. selloi*. The extract was obtained using aqueous, methanolic, and ethanolic solvents. Changes in the antioxidant capacity were observed by modifying the ratio of black tea and *O. gratissimum* in a combined mixture. According to a study, the quenching ability can also be impacted by combining different herbs in different ratios (Enko and Swiglo, 2015; Muhammad *et al*., 2017). Research conducted on BTOG combinations revealed that they exhibited additive antioxidant interactions in many different tests. It was reported that the number of bioactive chemicals, the antioxidant's reaction rates, and their effective concentration at the oxidation site all impacted on how the antioxidant potential changed (Jiang *et al*., 2015; Sonam and Guleria, 2017). A modification of the antioxidant potential was seen by varying the ratio of BT and OG in a binary mixture. According to a study, the ability to quench can also be affected by combining different herbs in varying ratios (Enko *et al*., 2015; Muhammad *et al*., 2017). At a 3:1 ratio, the combination of BTOG displayed the strongest antioxidant potential and additive interaction, according to Guleria *et al.* (2022). The nature of additive, synergistic and antagonistic interactions between a mixture of medicinal plants, such as plant-based meals, beverages as well as herbal remedies and its phytoconstituents, can affect the mixture's antioxidant interaction (Wang *et al*., 2011; Enko and Swiglo, 2015; Muhammad *et al*., 2017). Variations in structure, the deterioration of less potent antioxidants, the kind of radicals involved, and the production of radical adducts are some of the aspects that contribute to the interaction between phytochemicals (Han *et al*., 2012; Farooq and Sehgal, 2020).

Gupta *et al.,* 2019 found that while combining black tea in the ratio of 1:1:1:1 with tulsi, ginger and black pepper has the maximum antioxidant capacity compared to their individual activity. Farooq and Sehgal, 2019 conducted a study revealing that green tea and *O. gratissimum* combined in the ratio of 1:1 possessed major synergistic interaction and ultimately good effect on radical quenching followed by 3:1, 2:1, 1:2 and 1:3. Nedamani *et al*., 2015 documented that, in comparison to oak and rosemary,

green tea alone showed the highest scavenging activity. In the case of binary mixture, green tea: rosemary: oak (50:50:50) demonstrated the greatest capacity for quenching in comparison to the other ratios (50:50:100, 50:100:50, 100:50:100, and 100:100:50). All three types of interactions (additive, synergistic, and antagonistic) were noted in the mixed extract, however, they all displayed diverse behaviors.

The antioxidant activities and interactions are affected by several factors, such as the proportion of bioactive compounds, the structural properties of compounds, the type of radicals involved, and the reaction mechanisms observed in the various assays e.g. LPO, hemolysis, ABTS, and DPPH (Enko and Swiglo, 2015; Muhammad *et al*., 2017; Milani *et al*., 2020). By altering the ratio between the two, it is possible to alter the binary mixture of BT and OG's capacity to quench radicals. The results of the investigation showed that an additive interaction between the two components, at a 3:1 ratio, produced the best radical quenching efficacy in both *in vitro* and *ex vivo* tests. These findings may assist in the developing future designs or formulations for combining black tea with *O. gratissimum* in infusions (Guleria *et al*., 2022).

The rise of obesity cases and the diabetes epidemic led to the development and commercialization of tea-based beverages supplemented with low-calorie artificial sweeteners such as aspartame, sorbitol, sucralose, and stevia (Ahmad *et al*., 2020). Among these, interest is generated for *Stevia* whole leaf extract as it not only acts as a sweetener but also contains phytochemicals with an antioxidant property that may further enhance biological activities of black tea.

Stevia rebaudiana plant leaves are used to make the sweet sugar herb. It has a sweet-tasting component called steviol glycosides, which is naturally present in *Stevia* leaves (Singh *et al*., 2017). This study investigated the effect of *S. rebaudiana* (leaves), supplementation on the antioxidant potential of black tea, and *O. gratissimum* (3:1) was investigated. The antioxidant activity of various infusions was determined using DPPH, ABTS, NO, LPO and hemolysis assays. The activity was measured in terms of EC_{50} (half maximal effective concentration), the lower the EC_{50} higher the antioxidant potential. For single infusions, *S. rebaudiana* showed the highest scavenging potency (DPPH, NO, LPO, and hemolysis), followed by Bt and OG. In the ABTS assay, black tea and *S. rebaudiana* (St) confined a similar effect to *O. gratissimum*. In some research reports, it was demonstrated that the aqueous extract of *Stevia* contained more antioxidant capacity and antihaemolytic capability than other

extracts (ethanol and methanol) (Kim *et al*., 2011; Viedma *et al*., 2017). Earlier studies also investigated the maximum radical quenching ability in black tea than other teas and herbs (chamomile tea, peppermint tea, sage tea, and Echinacea tea) (Cleverdon *et al*., 2018). It indicates that this variation occurred between the tea infusions in antioxidant parameters may be due to the presence of different phytoconstituents in different sample extracts like in *Stevia* (ascorbic acid, terpenes and steviol glycosides, etc.) and black tea (theaflavins & thearubugins), chemical nature, the reactivity of compounds and many antioxidant components (Nimse *et al*., 2015; Sanchez *et al*., 2020).

In combination, black tea + *O. gratissimum* (BTOG) and black tea + *O. gratissimum* + *S. rebaudiana* (BTOGSt) expressed maximum quenching ability. Earlier reports expressed the effect of various additives like sugar, lime juice, citric acid and milk in black tea infusion. Black tea with lime juice illustrated maximum free radical scavenging potential in comparsion to other additives (Muthuiah *et al*., 2009). The interaction between Black Tea and its binary (BTOG, BTSt) and ternary (BTOGSt) mixture ranged from nearly additive to antagonism. BTOG (black tea + *O. gratissimum*) and BTOGSt (Black Tea + *O. gratissimum* + *S. rebaudiana*) expressed maximum scavenging effect. Whereas, in other combinations, black tea + *S. rebaudiana* (BTSt) and *O. gratissimum*+ black tea (OGSt) displayed less effect may be due to the presence of some other phytochemicals in the mixture that inhibit the effect of the active component. From the previous studies, it was proved that each herb contains many constituents that possibly belong to different structural types (Che *et al*., 2013). The effects of herb-herb or herb-drug interactions can be complex and difficult to predict, likely due to the presence of multiple components in herbal products (Gu *et al*., 2014; Parasuraman *et al*., 2014).

In quantifying total phenolics/flavonoids, black tea contained more phenolic content than stevia and OG. The earlier report demonstrated that *Camellia sinensis* (black, green, and white tea) and herbal tea (chamomile) contained a high amount of TPC and TFC as compared to fruit juices (apple juices and orange juices) (Shannon *et al*., 2017). In the case of flavonoids, OG displayed maximum flavonoid content, followed by BT and stevia. The literature revealed that the *O. gratissimum* leaves contained a high number of phytochemical constituents like glycosides, steroids, alkaloids, terpenoids, tannins, and flavonoids (Alexander *et al*., 2016). At the same time, black tea in a ternary combination (BTOGSt) displayed maximum phenolic

content as compared to other combinations (BTSt, OGSt, and BTOG). However, in the case of TFC BTOG, BTSt, OGSt and BTOGSt (aqueous infusions) showed a similar effect. Another study revealed that the flavonoid, phenolic, and free radical potency of black tea with the addition of different additives like ginger, cinnamon, saffron, and coriander showed maximum antioxidant, polyphenol, and flavonoid effect (Amoozadeh *et al*., 2016). It was indicated that this variation is expected in plant extracts due to the presence of other constituents as well as in the types of phenolics/flavonoids involving its structure (number of aromatic/hydroxyl groups) and molecular size, different genotypes, distinct environmental and climatic conditions like temperature, light or drought, etc. (Hakkim *et al*., 2008; Rababah *et al*., 2011 and Tungmunnithum *et al*., 2018). Overall, the supplementation of stevia as an additive [BTOGSt (ternary) combination] showed a maximum free radical scavenging potential effect. Another report investigated that stevia with other polyherbal formulations (*Nephrodiuminophyllum, Polygonum minus, Aunone squamosal* L.) showed the highest antioxidant activity (DPPH & LPO), TPC and TFC (Rahim *et al*., 2019). This indicates that different herbs have a variety of bioactive constituents with contrasting antioxidant abilities. When consumed together, total antioxidant capacity may be affected by synergistic, additive as well and antagonistic effects that may modulate physiological properties (Gupta *et al*., 2017). Results revealed antioxidant activity of black tea (BT) and different *Ocimum* spp. (*O. gratissimum, O. sanctum,* and *O. canum* Sims) alone or in binary/ternary combinations (BTOG, BTSt, OGSt, and BTOGSt) are highly contributed by phenolics or flavonoids. A strong correlation was noted between ABTS, DPPH and reducing power and phenolic or flavonoid content in aqueous leafy herbal tea extracts (rooibos, green, black, rosemary, lemongrass, mulberry leaf, bamboo leaf, and mate tea) (Oh *et al*., 2013).

Another study displayed a significant correlation between antioxidant capacity (DPPH and ABTS) and TPC or TFC for African green, white, and black orthodox tea, which implies that phenolic or flavonoid content could be the main components responsible for acting against oxidative damage (Carloni *et al*., 2013).

Human exposure to genotoxic agents has increased dramatically. Exogenous and endogenous factors can impair genomic stability (Lutz, 1990; Hartwig *et al*., 2020; Rietjens *et al*., 2022). Accumulated DNA damage can lead to mutations that contribute to the onset and development of cancer as well as other diseases (immune deficiencies, cardiovascular neurodegenerative diseases, aging, metabolic syndrome,

and infertility) (Moraes *et al*., 2012; Ambekar *et al*., 2017; Kumari *et al*., 2021). Black tea showed maximum antigenotoxic activity as compared to OG, OS, OC, and St. At the same time, BTOGSt expressed the highest effect of antigenotoxicity as compared to other combinations, respectively as shown in **Fig. 64** and **Table 42.** Previously conducted studies have demonstrated that the antioxidant effects of tea flower extract (cinnamon, jasmine, daisy, rose and lotus) significantly inhibited the hydroxyl radical-induced DNA damage as compared to 75% ethanol extract of fresh tea leaves (green, black and oolong) in LPS-induced RAW 264.7 cells (Lin *et al*., 2003). Earlier studies also investigated the maximum radical quenching ability in black tea than other teas and herbs (chamomile tea, peppermint tea, sage tea, and Echinacea tea) (Cleverdon *et al*., 2018). Previous literature determined that the five extracts (Petroleum ether, ethyl acetate, absolute ethanol, 95% ethanol and water) of Folium *Sennae* (FS) could effectively protect against hydroxyl radical–induced DNA damage and also scavenge hydroxyl radical (.OH) scavenging, superoxide anion ($.O_2^-$), DPPH, ABTS & Cu^{2+} reducing power assays (Lin *et al*., 2014). It was found that natural phenolic antioxidants can shield against oxidative DNA damage through two methods. Firstly, they can scavenge the •OH radical before it causes any DNA damage, and secondly, they can hinder the production of DNA radicals resulting from the •OH radical attack (Lin *et al*., 2014).

Cytotoxicity refers to the ability to harm or damage cells. When assessing the efficacy of a substance as a chemo-preventive agent, it is crucial to find a balance between its therapeutic benefits and potential harmful effects on cells caused by its various infusions. Different methods are used to evaluate toxicity screening. MTT (3-(4, 5-dimethylthiazolyl-2)- 2, 5-diphenyltetrazolium bromide) and SRB tests are commonly used to screen for cytotoxicity in various materials.

The SRB (Sulforhodamine) assay was created by Skehan *et al*., 1990 to precisely and reliably determine the amount of protein within a cell. This assay relies on the ability of the pink amino xanthine SRB dye to attach to the basic amino acid residues of the proteins found within the cell. The SRB assay is a more sensitive cytotoxicity method than the MTT assay, and the relationship between absorbance and cell number is linear, even with high cell numbers. Furthermore, unlike the MTT test, the SRB test does not rely on metabolic activities and provides results independent of a variety of alternative conditions. Considering, the *in vitro* cytotoxicity effect of aqueous infusion of black tea (BT), *O. gratissimum* (OG), *S. rebaudiana* (St) alone or in binary/ternary combination were tested against A549 human lung cancer cell line

using SRB (Sulforhodamine) assay was evaluated. Because the above-mentioned infusions needed to be tested further for *in vivo* lung cancer models, a lung cancer cell line was chosen.

The results demonstrated that all aqueous individual and combined infusions (OG, St, BT, BTOG, BTSt and BTOGSt) showed reduced cell viability and percentage of growth inhibition of A549 cells at 48h. These aqueous infusions illustrated greater inhibition at lower concentrations. In the previous studies, black tea constituents such as theaflavins and thearubigins and their combination cause inhibition in cell viability against HCT-116, HT 460, HT-29, MCF-7, A549, and NIH-3T3 (colon cancer cells, lung cancer cells, human colon carcinoma, human breast carcinoma, human alveolar carcinoma and healthy cell lines) respectively (Konarikova *et al*., 2015; Imran *et al*., 2019). Whereas, in other reports, stevia (crude dichloromethane) and *O. gratissimum* (aqueous) leaf extract significantly showed a percent of inhibition as compared to hexane and methanol extract in A549 cells and NCI-H 187 lung cancer cell line (Chen *et al*., 2010). A549 is a lung carcinoma epithelial cell that constitutes a cell line. These cells were isolated from the lung tissue of a White, 58-year-old male with lung cancer. This cell line can be used in cancer, immuno-oncology, and toxicology research.

It is a human alveolar basal epithelial cell that shows a long fusiform shape, small size, clear cell boundaries, well-adherent pebble-like growth, placental cytoplasm, and fewer cytoplasmic granules. Adriamycin is a drug that comes from the bacterium *Streptomyces peucetius* and is used alone or with other drugs to treat many types of cancer, including leukemia, lymphoma, and certain cancers of the lung. It slows or stops the growth of cancer cells by blocking an enzyme called topoisomerase (Chen *et al*., 2019). It is round and normal in shape. The cells showed a reduction in cell volume and destabilization of the plasma membrane. Meanwhile, aqueous infusions inhibit cell growth, uniform detachment of cells, cell shrinkage, and membrane integrity. Morphological appearance is not as clear in OG-treated cells, which could be attributed to the consistency and composition of the *O. gratissimum* sample.

The respiratory system, specifically the lungs, is susceptible to inhaled contaminants such as pollutants and carcinogens like B(a)P. These compounds have been recognized as essential factors in the growth of lung cancer (Bostrom *et al*., 2002; Valavanidis *et al*., 2013; Vargas *et al*., 2023). Most lung cancer cases are not

diagnosed until they have reached advanced stages, at which point treatment options tend to be primarily focused on providing palliative care (Collins *et al.*, 2007; Ferrell *et al*., 2015; Wachter *et al*., 2022). An extremely potent procarcinogen called B(a)P is transformed into a variety of metabolites by enzymes, which can start the tumorigenic process (Goyal *et al*., 2010; Thirunavukkarasu *et al*., 2013; Bhardwaj *et al*., 2022). ROS are generated through metabolic processes in the form of hydrogen peroxide, hydroxyl radicals, and superoxide anion. However, when their production exceeds the body's capacity to neutralize them, it can lead to elevated ROS levels in cells.

Oxidative stress occurs when there is imbalance between the production of ROS and the ability of the cell to scavenge & neutralize these harmful molecules (Elera *et al*., 2012; Basak *et al*., 2020). These imbalances can be caused by several factors, including exposure to environmental toxins (like carcinogens), mitochondrial dysfunction, and inflammation (Ziech *et al*., 2010; Azzam *et al*., 2012). Abnormal cancer cell growth is often associated with oxidative stress. High levels of ROS can cause damage to cellular components, including DNA, proteins, and lipids (Basak *et al.,* 2020). Recent cancer chemopreventive agents have been employed to prevent different forms of cancer based on findings from animal experiments and epidemiological data (Kotecha *et al*., 2016; Menter and Bresalier, 2023). Based on the insights gained from animal experiments and epidemiological data, researchers may identify promising compounds or strategies for cancer prevention (Esmeeta *et al*., 2022). These can include natural compounds found in foods, dietary components (vegetables, fruits, spices, teas) and medicinal plants (Huang *et al*., 2009; Roy *et al*., 2018; Esmeeta *et al*., 2022).

Chemoprevention has the potential to be an essential method of preventing cancer for both the general population and, more importantly, for individuals at high risk (Wattenberg, 1997; Reddy, 2000; Gu and Li, 2020). (Gullett *et al*., 2010; Feitelson *et al*., 2015; Dutta *et al*., 2019; Li *et al*., 2023). Therefore, it is increasingly important to identify natural compounds that can stop, slow down, or stop the development of cancer, which is becoming more and more crucial (Baker *et al*., 1995; Gullett *et al*., 2010; Tewari *et al*., 2022).

Phenolic compounds, such as flavonoids and polyphenols derived from plants have been shown to play an essential role in controlling the levels of reactive oxygen species in the body, which are associated with cancer development, reducing the risk

of cellular damage and mutations, inducing apoptosis, blocking signaling pathways, slow down the rate of cell division and proliferation, especially in cancer cells target specific enzymes as well as proteins involved in the cell cycle preventing uncontrolled cell growth and inhibition of angiogenesis (depriving cancer cells of their blood supply and impeding tumor growth (Hadi *et al*., 2000; Miekus *et al*., 2020; Alotaibi *et al*., 2021). In recent years, extensive endeavors have been undertaken to enhance natural or synthetic chemopreventive agents that can block or modulate these specific molecular targets (associated with different types of cancer) which may be specific genes, proteins, or pathways involved in cancer development (Mehta *et al*., 2010; Karikas, 2010; Perez *et al*., 2019; Thompson and Lutsiv, 2023). For the *in vivo* trial in this investigation, black tea and *O. gratissimum* were combined because, at a 3:1 ratio, it showed the best antioxidant potential and maximum additive effect (Guleria *et al*., 2022). Thus, aqueous infusions of black tea, *O. gratissimum*, and their combination with *S. rebaudiana* (ternary mixture) as an additive were tested against benzo(a)pyrene-induced lung histological changes in various experimental groups. The observations of the present study illustrated significant differences in water consumption and food intake in all experimental groups ($p \geq 0.05$) **Fig's. 78, 79,** and **80.** A recent study reported, no difference in water and food consumption for B(a)P administration animals as compared to control group animals (Mohan and Koul, 2018). The average body weight in B(a)P-activated mice (Group 1) was decreased, and the lung weight augmented when compared with a normal group of animals. In the treatment of black tea (different dietary infusions) to B(a)P – induced mice, the body weight was improved/increased and lessened lung weight as compared to non-treated groups (Kamaraj *et al*., 2009; Ravichandran *et al*., 2011). On the other hand, a tumor was noted in B(a)P-injected mice (Gp. 1) while both treated groups (3,4,5,6,7 and 8) showed a reduction in the tumor size gradually as shown in **Table 46** and **47** and **Fig. 81 - 83.**

Early carcinoma was identified in B(a)P-group animals (Gp.1), as shown in **Fig. 85.** It has been reported that B(a)P-induced lung histological changes are linked to gene mutations, such as growth-promoting gene up-regulation (cMyc and Hras), pro-apoptotic gene down-regulation (Caspase, p53, Bax), and anti-apoptotic gene (BCl_2) alternation in the level of antioxidant (oxidative imbalance of cell), which may start various pulmonary disorders, including pulmonary fibrosis, chronic obstructive

pulmonary disease (COPD), emphysema and finally lung cancer (Meuwissen and Berns, 2005; Banerjee *et al*., 2006; Manna *et al*., 2009; Hong *et al.*, 2007).

According to experimental data, B(a)P-induced carcinogenesis in mice results in cellular and histological alterations that can be classified as hyperplasia, dysplasia, carcinoma *in situ*, and invasive carcinoma. The present study showed that after 16 weeks of B(a)P administration, the B(a)P-group of animals (Gp.1) displayed progressive histological abnormalities that were identified as papillary progression in the bronchiolar epithelium (Early carcinoma) in only Gp. 1 of mice. BT, OG, St, BTOG, BTSt & BTOGSt administrated animals expressed a reduction in hyperplasia, emphysema, mononuclear cell infiltration (MNC) as compared to Gp.1. The early stage of carcinoma was highest in B(a)P-treated animals (Gp.1) ($p \leq 0.05$) in addition to the widespread proliferation of papillary progression in the alveolar, bronchiolar epithelium in all Gp. 1 animal.

Earlier reports also revealed a regular increase in lung lesions and finished with hyperplasia, dysplasia, and carcinoma *in situ* was regularly seen after B(a)P treatment (Banerjee *et al*., 2006; Manna *et al*., 2009). The tumor formation was noted in this study, due to the route of administration, age of mice, schedule of dose, the concentration of dose & mice strain (Swiss albino mice). Some reports showed that single or multiple intraperitoneal/oral doses of B(a)P with dose ranging from 50-100 mg/kg B.W in A/J, Balb/c or Swiss Albino mice leads the formation of tumor (nodule) after a different period (16-26 weeks) (Yan *et al*., 2005; Yan *et al*., 2006; Yeo *et al*., 2017; Mohan and Koul, 2018 andVelli *et al*., 2019). Some studies demonstrated that lung tumors develop 16 and 28 weeks after the initial dose of the carcinogen when Swiss albino mice are repeatedly administrated oral intubation of B(a)P at 2 to 5 mg/kg B.W (Katiyar *et al*., 1993; Kamaraj *et al*., 2009; Venugopal *et al*., 2014). However, some findings found that a B(a)P of 50 mg/kg, B.W) twice, was orally injected in Swiss albino mice. The alternation of antioxidant enzyme status, oncogenes expression, and suppressor of tumors gene expression may be the cause of this inhibition in dysplasia, hyperplasia, and carcinoma by all aqueous infusions (BT, OG, St, BTOG, BTSt and BTOGSt) extracts. Plant extracts, which often contain a variety of bioactive compounds, have been studied for their ability to modulate multiple mechanisms involved in carcinogenesis, such as inhibiting the activation of procarcinogens, neutralizing ROS, reducing oxidative stress and potential damage to cellular components, including DNA, enhancing the activity of the body's endogenous defense systems, slow down the proliferation of cancer cells by interfering with cell

cycle regulation and cell signaling pathways, mitigate inflammation, reducing the likelihood of tumor initiation as well as progression and promote apoptosis in cancer cells, thus preventing their survival and spread (Kasala *et al*., 2015).

Administering an aqueous extract of black tea and polyphenols can hinder the development of lung tumors caused by B(a)P (Krishnan *et al*., 2005). OG extract (leaves) was shown to have potential for treating lung cancer by suppressing cell viability in A549 cells, breast carcinoma (MCF 57), murine macrophages Raw 264.7 cancer cell line, acute monocytic leukemia (THP-1) and normal fibroblast A1R5 cell line that were stimulated to undergo apoptosis and reduced anti-apoptotic signaling (Chen *et al*., 2010; Nganteng *et al*., 2022). Numerous research studies have indicated that derivatives obtained from *S. rebaudiana*, such as aglycon (isolated from stevia) and steviol glycosides, can lead to the cessation of the cell cycle and PCD in breast cancer cells (Peteliuk *et al*., 2021). Steviol acts as anti-proliferative and its acts to reduce viability in different human cancers such as gastrointestinal tract, prostate, lung, breast and colorectal (Boonkaewwan *et al*., 2008; Chen *et al*., 2018; Peteliuk *et al*., 2021). It is believed that whole foods are more beneficial to health than individual nutrients or supplements. This is because whole foods contain a complex mix of nutrients, phytochemicals, and other compounds that work together synergistically to provide health benefits (Liu *et al*., 2013). In the current study, all infusions were found equally effective against the reduction of B(a)P-induced early carcinoma in the mice as illustrated in the **Table 46** and **47s.** Phytochemicals refer to naturally occurring biologically active compounds possessed by plants, offering several health benefits, like anti-inflammatory, anti-cancer etc. When consumed as a binary/ternary mixture, their interactions (additive, synergistic and antagonistic) can result in unique biological effects that cannot be predicted based on the individual effects of each compound. BT, OG, and St are examples of phytochemicals that may act differently when combined *in vivo*. Catechins are found in high concentration in black tea and TF/TRs (theaflavins/thearubugins), which are potent antioxidants that may help, reduce the risk of cancer and heart disease. OG is a source of rosmarinic acid, rutin, catechins, nepetoidin, and chlorogenic acid which has been shown to have anti-inflammatory properties and may help to prevent cancer. *Stevia* consumption appears to have positive effect on diseases- hyperglycemia, dyslipidemia, and hypertension, while numerous studies describe its antioxidant, anti-inflammatory, and anti-cancer effects. These properties are attributed to the plant's leaf extracts which contain

steviol glycosides and polyphenols, with potentially important bioactive effects (Purkayastha *et al*., 2016; Salehi *et al*., 2019; Wang *et al*., 2020).

In a BTOG and BTOGSt binary/ternary combination, the two/three compounds may interact in such a way that they normalize the activity of each other. e.g. BT catechins, TRs, and TFs may enhance the anti-inflammatory properties of rosmarinic acid, chlorogenic acid in OG, and steviol glycosides in stevia while rosmarinic acid, steviol glycosides may increase the bioavailability of catechins in BT. The phenolic compounds in binary/ternary mixtures may act differently *in vivo* conditions due to their complex interactions and influence from various biological pathways. Therefore, it is important to consider the potential synergistic, additive, or antagonistic effects of phytochemicals in binary/ternary decoction when evaluating their health benefits. The treatment of BT, OG, St, BTOG, BTSt and BTOGSt decreased the occurrence of hyperplastic, dysplastic, and mononuclear cell infiltration as compared to Gp. 1 animal. The widespread proliferation of alveolar, bronchiolar hyperplastic zone along with MNC was reduced in all aqueous infusions (Gp. 2,3,4,5,6,7 and 8) related to B(a)P administrated group (Gp.1). Enhanced levels of BCl_2 might be the reason for reduction of lung lesion which in turn can regulate cell survival and apoptosis. By promoting cell survival and inhibiting apoptosis, BCl_2 can help to reduce the number and size of different stages/zones of cancer (hyperplasia, dysplasia and metastasis-early carcinoma) in the lung.

Histological alternations refer to changes in the tissue structure and identifying the early signs of carcinogenesis or potentially preventing the development of cancer that can be observed under the microscope. Thus, the combination BTOG and BTOGSt showed a shielding effect against B(a)P-induced alternations. In numerous animal studies, black tea has shown its chemopreventive potential. In many reports, black tea displayed its chemopreventive potential effect (Wang *et al*., 1992; Yang *et al*., 1997; Landau *et al*., 1998; Shukla *et al*., 2002; Banerjee *et al*., 2005; Krishan *et al*., 2005; Murugan *et al*., 2007; George *et al*., 2011; Eldeen *et al*., 2015; Hudlikar *et al*., 2019). BT with reversterol and curcumin in combination indicated an increased reduction in the tumors in the lungs and proliferation of cells in the liver (George *et al*., 2011; Eldeen *et al*., 2015) as compared to alone. Thus, *in vitro* studies expressed that black tea, with its phytochemicals, other herbs, and drugs demonstrates greater activity, and enhances its bioavailability effects than individual extracts (Hudlikar *et al*., 2019; Guleria *et al*., 2022). The present work revealed that the administration of BT, OG and

St showed cytotoxicity in A549 (lung cancer cell line) and anti-genotoxicity against H_2O_2-induced DNA damage followed by BT, OG and St alone. The study also found that BT, OG, St, and BTOG & BTOGSt resulted in a reduction in hyperplastic, dysplastic, and metastasis (early stage of carcinoma) zones, indicating that these substances could potentially act as chemopreventive agents against the progression of lung carcinogenesis. Hence, it is possible to think of BTOG and BTOGSt as chemopreventive drugs to stop the development of respiratory tumors.

When people start smoking many harmful chemicals are released like pro-oxidants, free radicals, NNK, B(a)P, etc the cigarette smoke enters the lungs the cells of the lungs start to form an irregular shape after binding these chemical molecules with a lung cell the DNA starts mutated and damaged. The cell signaling pathways genes are overexpressed. The cells start dividing in uncontrolled form and become tumors. After the treatment with herbal teas/plant extracts, there are some bioactive compounds, Polyphenols are present in tea which bind with the cells of lung tumors and block the overexpression of various genes like BCl_2, anti-apoptotic proteins, etc. It activates pro-apoptotic proteins, arrests the cell cycle, induces apoptosis, and treats/recovers the tumor cell/lung cancer as shown in **Fig. 92.**

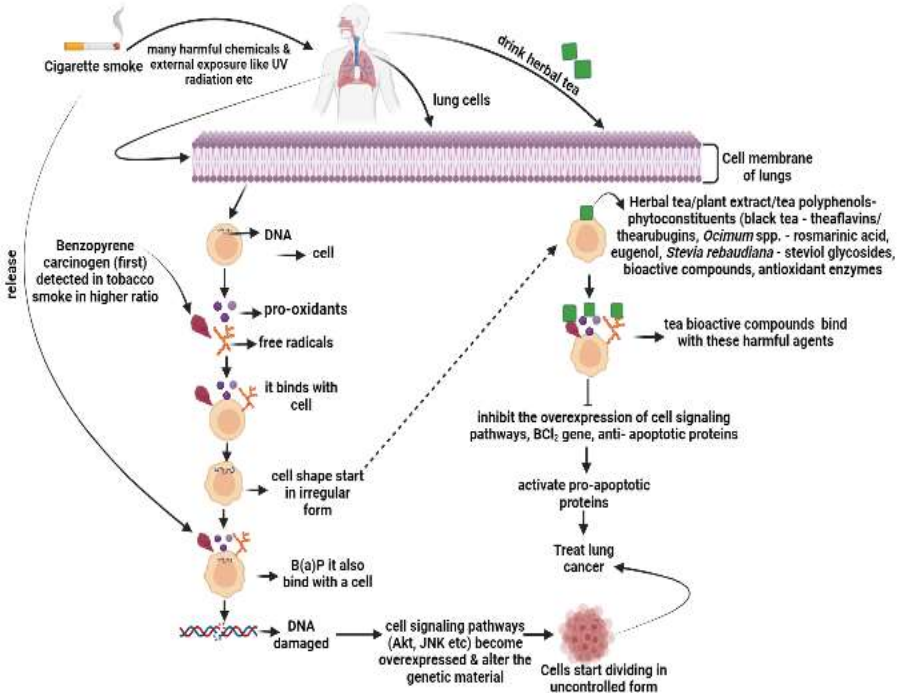

Fig. 92: Hypothetical diagram of lung cancer mechanism

CHAPTER 8

SUMMARY & CONCLUSION

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For several biological activities, black tea, herbal teas (including *O. gratissimum*, *O. sanctum*, *O. canum* and *S. rebaudiana*), and the binary/ternary combination of black tea & herbal teas were all assessed. In chemical (DPPH, ABTS and NO) and *ex vivo* models (LPO and hemolysis) BT and BT mixtures demonstrated greater radical quenching properties than single plant extracts/individual herbal teas. Using Compusyn software, the dose-effect relationship was used to determine the antioxidant interactions. The interactions between BT/herb decoctions ranged from slight antagonism to additive compared to BTCs and other individual infusions. The BT single infusion showed the highest TFC while OG (*O. gratissimum*) expressed maximum TPC in comparison to BTCs and other individual infusions. The phenolic and flavonoid content of BTOG, however, showed no discernible variation. A high negative correlation was observed between TPC/TFC and antioxidant activities (EC_{50}) of alone and binary decoction (BTCs). When evaluating the chemopreventive potential or bioefficacy of an herbal combination, the percentage of each herb can be quite important. This combination (BT and OG) was further examined at various ratios (3:1, 2:1, 1:1, 1:2 & 1:3). The maximum radical quenching ability and strongest additivity were seen in chemical and *ex vivo* models when black tea and *O. gratissimum* mixtures were used in a 3:1 proportion. Further testing was conducted on the BTOG decoction (3:1) to determine its impact on the cytotoxicity of A549 cell lines. The cytotoxicity of all samples (BT, OG, St) and binary (BTOG, BTSt & OGSt) or ternary (BTOGSt) was dose-independent. To check the antigenotoxic potential of a binary and ternary mixture of black tea granules with three different species of *Ocimum*. Black tea expressed maximum antigenotoxicity than other individual samples. While in combination BTOGSt showed the highest effect of antigenotoxic activity as compared to other combinations. Additionally, this mixture was examined *in vivo* for its ability to prevent lung lesions caused by B(a)P from developing into cancer chemopreventive effects. B(a)P-induced animals demonstrated papillary progression (early carcinoma) and the presence of dysplastic, hyperplastic, metastatic zones, emphysema & mononuclear infiltration (MNCs). However, BT, OG, St, BTOG and BTOGSt-treated animals expressed a similar reduction in these regions (Gp. 3, 4, 5, 6 and 8) than group 1. Because of this, chemopreventive agents like BT, OG, St, BTOG, and BTOGSt may be effective in preventing the development of lung tumors. This research gives researchers a solid foundation for developing beverages with BT

based on combinations of antioxidants that may increase the effectiveness of BT as a chemopreventive agent.

CHAPTER 9

BIBLIOGRAPHY

BIBLIOGRAPHY

- Abadi, A. J., Mirzaei, S., Mahabady, M. K., Hashemi, F., Zabolian, A., Hashemi, F., & Sethi, G. (2022). Curcumin and its derivatives in cancer therapy: Potentiating antitumor activity of cisplatin and reducing side effects. *Phytotherapy Research*, *36*(1), 189-213.
- Abbasian, S., Karimi, F., Moghaddam, G., Soroush, A., Moloudian, H., & Ahosseini, M. S. (2013). Antioxidant properties of different black tea samples and some Iranian native plants. *Pharmacie Globale*, *4*(2), 1-5.
- Abeywickrama, K. R. W., Ratnasooriya, W. D., & Amarakoon, A. M. T. (2011). Oral hypoglycaemic, antihyperglycaemic and antidiabetic activities of Sri Lankan Broken Orange Pekoe Fannings (BOPF) grade black tea (*Camellia sinensis* L.) in rats. *Journal of Ethnopharmacology*, *135*(2), 278-286.
- About | Global Adult Tobacco Survey 2, India 2016-17 - Research Project (tiss.edu).
- Abudureheman, B., Yu, X., Fang, D., & Zhang, H. (2022). Enzymatic oxidation of tea catechins and its mechanism. *Molecules*, *27*(3), 942.
- Adjei, A. A. (2019). Lung cancer worldwide. *Journal of Thoracic Oncology*, *14*(6), 956.
- Adnan, M., Ahmad, A., Ahmed, A., Khalid, N., Hayat, I., & Ahmed, I. (2013). Chemical composition and sensory evaluation of tea (*Camellia sinensis*) commercialized in Pakistan. *Pakistan Journal of Botany*, *45*(3), 901-907.
- Adwas, A. A., Elsayed, A., Azab, A. E., & Quwaydir, F. A. (2019). Oxidative stress and antioxidant mechanisms in human body. *Journal of Applied Biotechnology & Bioengineering*, *6*(1), 43-47.
- Ahmad, J., Khan, I., Blundell, R., Azzopardi, J., & Mahomoodally, M. F. (2020). *Stevia rebaudiana* Bertoni: an updated review of its health benefits, industrial applications and safety. *Trends in Food and Science Technol*ogy, *100*, 177-189.
- Ahmad, N., Feyes, D. K., Agarwal, R., Mukhtar, H., & Nieminen, A. L. (1997). Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. *Journal of the National Cancer Institute*, *89*(24), 1881-1886.
- Aiad, M., Tahir, A., Fresco, K., Prenatt, Z., Ramos-Feliciano, K., Walia, J., & Albandar, H. (2022). Does the combined use of aspirin and immunotherapy result in better outcomes in non-small cell lung cancer than immunotherapy alone? *Cureus*, *14*(6).

- Aleesha, R., Augustine, T., Vishnu Priya, P. H., Xavier, S., Tomy, T., & Mishra, B. (2020). Allopolyherbal Formulations: A Novel Symbiotic Approach.
- Alexander, P. (2016). Phytochemical screening and mineral composition of the leaves of *Ocimum gratissimum* (scent leaf). *International Journal of Applied Sciences and Biotechnology*, *4*(2), 161-165.
- Alexandrov, K., Rojas, M., & Satarug, S. (2010). The critical DNA damage by benzo (a)pyrene in lung tissues of smokers and approaches to preventing its formation. *Toxicology letters*, *198*(1), 63-68.
- Alkadi, H. (2020). A review on free radicals and antioxidants. *Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders)*, *20*(1), 16-26.
- Al-Mahdi, Z. K. A., Ewadh, R. M., & Hindi, N. K. K. (2020). Health Benefits of Aqueous Extract of Black and Green Tea Leaves. *Bioactive Compounds in Nutraceutical and Functional Food for Good Human Health*.
- Alm-Eldeen, A. A., Mona, M. H., Shati, A. A., & El-Mekkawy, H. I. (2015). Synergistic effect of black tea and curcumin in improving the hepatotoxicity induced by aflatoxin B1 in rats. *Toxicology and industrial health*, *31*(12), 1269-1280.
- Alotaibi, B. S., Ijaz, M., Buabeid, M., Kharaba, Z. J., Yaseen, H. S., & Murtaza, G. (2021). Therapeutic effects and safe uses of plant-derived polyphenolic compounds in cardiovascular diseases: a review. *Drug design, development and therapy*, 4713-4732.
- Altun, I., & Sonkaya, A. (2018). The most common side effects experienced by patients were receiving first cycle of chemotherapy. *Iranian journal of public health*, *47*(8), 1218-1219.
- Aluko, B. T., Oloyede, O. I., & Afolayan, A. J. (2012). Phytochemical and nutrient compositions of the leaves of *Ocimum canum* Sims. *African Journal of Biotechnology*, *11*(63), 12697-12701.
- Ambekar, S. S., Hattur, S. S., & Bule, P. B. (2017). DNA: Damage and repair mechanisms in humans. *group*, *1*, 7.
- Amoozadeh, A., Mohammadzadeh, M. J., & Motamedzadegan, A. (2016). Investigating the effect of brewing additives on the antioxidant activity of black tea.
- Anastasiou, E., McCarthy, K. J., Gollub, E. L., Ralph, L., van de Wijgert, J. H., & Jones, H. E. (2022). The relationship between hormonal contraception and cervical dysplasia/cancer controlling for human papillomavirus infection: A systematic review. *Contraception*, *107*, 1-9.
- Anderson, R. F., Fisher, L. J., Hara, Y., Harris, T., Mak, W. B., Melton, L. D., & Packer, J. E. (2001). Green tea catechins partially protect DNA from· OH radical-

induced strand breaks and base damage through fast chemical repair of DNA radicals. *Carcinogenesis*, *22*(8), 1189-1193.

- Arias, A., Feijoo, G., & Moreira, M. T. (2022). Exploring the potential of antioxidants from fruits and vegetables and strategies for their recovery. *Innovative Food Science & Emerging Technologies*, 102974.
- Asgary, S., Naderi, G. H., & Askari, N. (2005). Protective effect of flavonoids against red blood cell hemolysis by free radicals. *Experimental & Clinical Cardiology*, *10*(2), 88.
- Asthana, S., Patil, R. S., & Labani, S. (2016). Tobacco-related cancers in India: A review of incidence reported from population-based cancer registries. *Indian Journal of Medical and Paediatric Oncology*, *37*(03), 152-157.
- Aureliano, M., De Sousa-Coelho, A. L., Dolan, C. C., Roess, D. A., & Crans, D. C. (2023). Biological consequences of Vanadium effects on formation of reactive oxygen species and lipid peroxidation. *International Journal of Molecular Sciences*, *24*(6), 5382.
- Aziz, M. A., Diab, A. S., & Mohammed, A. A. (2019). *Antioxidant categories and mode of action* (pp. 3-22). London, UK: IntechOpen.
- Azzam, E. I., Jay-Gerin, J. P., & Pain, D. (2012). Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. *Cancer letters*, *327*(1-2), 48-60.
- Bag, S., Mondal, A., Majumder, A., & Banik, A. (2022). Tea and its phytochemicals: Hidden health benefits & modulation of signaling cascade by phytochemicals. *Food Chemistry*, *371*, 131098.
- Bagherniya, M., Nobili, V., Blesso, C. N., & Sahebkar, A. (2018). Medicinal plants and bioactive natural compounds in the treatment of non-alcoholic fatty liver disease: A clinical review. *Pharmacological Research*, *130*, 213-240.
- Bai, Y., Yuan, H., Li, J., Tang, Y., Pu, C., & Han, P. (2014). Relationship between bladder cancer and total fluid intake: a meta-analysis of epidemiological evidence. *World journal of surgical oncology*, *12*(1), 1-11.
- Baibado, T. J., Mei, Y. A. N. G., Xiaofang, P. E. N. G., & Hon-Yeung, C. (2011). Biological activities and functions of Camellia sinensis (Tea). *Hong Kong Pharmaceutical Journal*, *18*(1), 31-39.
- Baker, J. A., Boakye, K., McCann, S. E., Beehler, G. P., Rodabaugh, K. J., Villella, J. A., & Moysich, K. B. (2007). Consumption of black tea or coffee and risk of ovarian cancer. *International Journal of Gynecologic Cancer*, *17*(1).

- Baker, J. A., McCann, S. E., Reid, M. E., Nowell, S., Beehler, G. P., & Moysich, K. B. (2005). Associations between black tea and coffee consumption and risk of lung cancer among current and former smokers. *Nutrition and cancer*, *52*(1), 15-21.
- Baker, J. T., Borris, R. P., Carté, B., Cordell, G. A., Soejarto, D. D., Cragg, G. M., & Tyler, V. E. (1995). Natural product drug discovery and development: new perspectives on international collaboration. *Journal of natural products*, *58*(9), 1325-1357.
- Ball, A., & Truskewycz, A. (2013). Polyaromatic hydrocarbon exposure: an ecological impact ambiguity. *Environmental Science and Pollution Research*, *20*, 4311-4326.
- Banerjee, S., Manna, S., Mukherjee, S., Pal, D., Panda, C. K., & Das, S. (2006). Black tea polyphenols restrict benzopyrene-induced mouse lung cancer progression through inhibition of Cox-2 and induction of caspase-3 expression. *Asian Pacific Journal of Cancer Prevention*, *7*(4), 661-666.
- Banerjee, S., Manna, S., Saha, P., Panda, C. K., & Das, S. (2005). Black tea polyphenols suppress cell proliferation and induce apoptosis during benzo(a)pyrene-induced lung carcinogenesis. *European Journal of Cancer Prevention*, *14*(3), 215-221.
- Barnes, J. L., Zubair, M., John, K., Poirier, M. C., & Martin, F. L. (2018). Carcinogens and DNA damage. *Biochemical Society Transactions*, *46*(5), 1213-1224.
- Basak, D., Uddin, M. N., & Hancock, J. (2020). The role of oxidative stress and its counteractive utility in colorectal cancer (CRC). *Cancers*, *12*(11), 3336.
- Basu Majumder, A., Bera, B., & Rajan, A. (2010). Tea statistics: global scenario. *Inc J Tea Sci*, *8*(1), 121-4.
- Basumallik, N., & Agarwal, M. (2021). Small cell lung cancer. In *StatPearls [internet]*. StatPearls Publishing.
- Benelli, G., Pavela, R., Maggi, F., Wandjou, J. G. N., Koné-Bamba, D., Sagratini, G., & Caprioli, G. (2019). Insecticidal activity of the essential oil and polar extracts from *Ocimum gratissimum* grown in Ivory Coast: Efficacy on insect pests and vectors and impact on non-target species. *Industrial Crops and Products*, *132*, 377-385.
- Bergerot, C. D., Philip, E. J., Bergerot, P. G., Siddiq, N., Tinianov, S., & Lustberg, M. (2022). Fear of cancer recurrence or progression: What is it and what can we do about it? *American Society of Clinical Oncology Educational Book*, *42*, 18-27.
- Bhardwaj, P., Kumar, M., Dhatwalia, S. K., Garg, M. L., & Dhawan, D. K. (2022). Protective role of AKBA against benzo (a) pyrene-induced lung carcinogenesis by

modulating biotransformation enzymes and oxidative stress. *Journal of Biochemical and Molecular Toxicology*, *36*(7), e23072.

- Bhattacharjya, D., Adhikari, S., Biswas, A., Bhuimali, A., Ghosh, P., & Saha, S. (2019). *Ocimum* phytochemicals and their potential impact on human health. *Phytochemicals in Human Health*.
- Bhattacharya, S., & Sen-Mandi, S. (2011). Variation in antioxidant and aroma compounds at different altitude: A study on tea (*Camellia sinensis* L. Kuntze) clones of Darjeeling and Assam, India. *African Journal of Biochemistry Research*, *5*(5), 148-159.
- Bhattacharyya, P., & Bishayee, A. (2013). *Ocimum sanctum* Linn. (Tulsi): an ethnomedicinal plant for the prevention and treatment of cancer. *Anti-cancer drugs*, *24*(7), 659-666.
- Bhavani, T., Mohan, R. R., Mounica, C., Nyamisha, J., Krishna, A. G., Prabhavathi, P., & Baba, K. H. (2019). Phytochemical screening & antimicrobial activity of *Ocimum gratissimum* review. *Journal of Pharmacognosy and Phytochemistry*, *8*(2), 76-79.
- Bilano, V., Gilmour, S., Moffiet, T., d'Espaignet, E. T., Stevens, G. A., Commar, A., & Shibuya, K. (2015). Global trends and projections for tobacco use, 1990–2025: an analysis of smoking indicators from the WHO Comprehensive Information Systems for Tobacco Control. *The Lancet*, *385*(9972), 966-976.
- Blot, W. J., McLaughlin, J. K., & Chow, W. H. (1997). Cancer rates among drinkers of black tea. *Critical Reviews in Food Science & Nutrition*, *37*(8), 739-760.
- Bode, A. M., & Dong, Z. (2003). Signal transduction pathways: targets for green and black tea polyphenols. *BMB Reports*, *36*(1), 66-77.
- Boffetta, P., & Hashibe, M. (2006). Alcohol and cancer. *The lancet oncology*, *7*(2), 149-156.
- Bond, T. J., & Derbyshire, E. J. (2020). Rooibos tea and Health: A Systematic Review of the Evidence from the Last Two Decades. *Nutrition and Food Science Technology*, *6*, 1-11.
- Bonovas, S., Tsantes, A., Drosos, T., & Sitaras, N. M. (2008). Cancer chemoprevention: a summary of the current evidence. *Anticancer research*, *28*(3B), 1857-1866.
- Boonkaewwan, C., Ao, M., Toskulkao, C., & Rao, M. C. (2008). Specific immunomodulatory and secretory activities of stevioside and steviol in intestinal cells. *Journal of agricultural and food chemistry*, *56*(10), 3777-3784.

- Bostrom, C. E., Gerde, P., Hanberg, A., Jernstrom, B., Johansson, C., Kyrklund, T., & Westerholm, R. (2002). Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. *Environmental health perspectives*, *110*(3), 451-488.
- Botting, K.J, Young, M.M, Pearson, A.E, Harris, P.J & Ferguson, L.R 1999, 'Antimutagens in food plants eaten by Polynesians: micronutrients, phytochemicals and protection against bacterial mutagenicity of the heterocyclic amine 2-amino-3-methylimidazo [4, 5-f] quinoline', *Food & Chemical Toxicology*, *37*(2-3), 95-103
- Bouslamti, M., Loukili, E. H., Elrherabi, A., El Moussaoui, A., Chebaibi, M., Bencheikh, N., & Benjelloun, A. S. (2023). Phenolic Profile, Inhibition of α-Amylase and α-Glucosidase Enzymes, and Antioxidant Properties of Solanum elaeagnifolium Cav. (Solanaceae): In Vitro and In Silico Investigations. *Processes*, *11*(5), 1384.
- Boyer, J., & Liu, R. H. (2004). Apple phytochemicals and their health benefits. *Nutrition journal*, *3*, 1-15.
- Brahmachari, G., Mandal, L. C., Roy, R., Mondal, S., & Brahmachari, A. K. (2011). Stevioside and related compounds–molecules of pharmaceutical promise: a critical overview. *Archive of Pharmacy*, *344*(1), 5-19.
- Brambilla, E., Pugatch, B., Geisinger, K., Gal, A., Sheppard, M. N., Guinee, D. G., & Petersen, I. (2004). Large cell carcinoma. *World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart*, *48*.
- Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, *68*(6), 394-424.
- Bray, F., Jemal, A., Grey, N., Ferlay, J., & Forman, D. (2012). Global cancer transitions according to the Human Development Index (2008–2030): a population-based study. *The lancet oncology*, *13*(8), 790-801.
- Brimson, J. M., Prasanth, M. I., Kumaree, K. K., Thitilertdecha, P., Malar, D. S., Tencomnao, T., & Prasansuklab, A. (2022). Tea Plant (Camellia sinensis): A Current Update on Use in Diabetes, Obesity, and Cardiovascular Disease. *Nutrients*, *15*(1), 37.
- Brodowska, K. M. (2017). Natural flavonoids: classification, potential role, and application of flavonoid analogues. *European Journal of Biological Research*, *7*(2), 108-123.

- Brustle, K., & Heidecker, B. (2017). Checkpoint inhibitor induced cardiotoxicity: managing the drawbacks of our newest agents against cancer. *Oncotarget*, *8*(63), 106165.
- Burczynski, M. E., & Penning, T. M. (2000). Genotoxic polycyclic aromatic hydrocarbon ortho-quinones generated by aldo-keto reductases induce CYP1A1 via nuclear translocation of the aryl hydrocarbon receptor. *Cancer research*, *60*(4), 908-915.
- Burzic, A., O'Dowd, E. L., & Baldwin, D. R. (2022). The future of lung cancer screening: current challenges and research priorities. *Cancer Management and Research*, 637-645.
- Buyukbalci, A., & El, S. N. (2008). Determination of *in vitro* antidiabetic effects, antioxidant activities and phenol contents of some herbal teas. *Plant foods for human nutrition*, *63*, 27-33.
- Bvenura, C., & Sivakumar, D. (2017). The role of wild fruits and vegetables in delivering a balanced and healthy diet. *Food Research International*, *99*, 15-30.
- Cabrera, C., Giménez, R., & Lopez, M. C. (2003). Determination of tea components with antioxidant activity. *Journal of agricultural and food chemistry*, *51*(15), 4427-4435.
- Canale, M., Andrikou, K., Priano, I., Cravero, P., Pasini, L., Urbini, M., & Ulivi, P. (2022). The role of TP53 mutations in EGFR-mutated non-small-cell lung cancer: Clinical significance and implications for therapy. *Cancers*, *14*(5), 1143.
- Cao, J., Xu, Y., Chen, J., & Klaunig, J. E. (1996). Chemopreventive effects of green and black tea on pulmonary and hepatic carcinogenesis. *Fundamental and Applied Toxicology*, *29*(2), 244-250.
- Carloni, P., Tiano, L., Padella, L., Bacchetti, T., Customu, C., Kay, A., & Damiani, E. (2013). Antioxidant activity of white, green and black tea obtained from the same tea cultivar. *Food research international*, *53*(2), 900-908.
- Carreras-Torres, R., Johansson, M., Haycock, P. C., Wade, K. H., Relton, C. L., Martin, R. M., & Brennan, P. (2017). Obesity, metabolic factors and risk of different histological types of lung cancer: a Mendelian randomization study. *PloS one*, *12*(6), e0177875.
- Carroll, J. E., Bower, J. E., & Ganz, P. A. (2022). Cancer-related accelerated ageing and biobehavioural modifiers: a framework for research and clinical care. *Nature Reviews Clinical Oncology*, *19*(3), 173-187.

- Carter, B. W., Glisson, B. S., Truong, M. T., & Erasmus, J. J. (2014). Small cell lung carcinoma: staging, imaging, and treatment considerations. *Radiographics*, *34*(6), 1707-1721.
- Cassidy, A., Huang, T., Rice, M. S., Rimm, E. B., & Tworoger, S. S. (2014). Intake of dietary flavonoids and risk of epithelial ovarian cancer. *The American journal of clinical nutrition*, *100*(5), 1344-1351.
- Chan, E. W. C., Lim, Y. Y., Chong, K. L., Tan, J. B. L., & Wong, S. K. (2010). Antioxidant properties of tropical and temperate herbal teas. *Journal of Food Composition and Analysis*, *23*(2), 185-189.
- Chan, E. W., Soh, E. Y., Tie, P. P., & Law, Y. P. (2011). Antioxidant and antibacterial properties of green, black, and herbal teas of Camellia sinensis. *Pharmacognosy research*, *3*(4), 266.
- Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M. H., ElSohly, M. A., & Khan, I. A. (2014). Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. *Evidence-based complementary and alternative medicine*, *2014*.
- Chandrashekar, N. (2022). Benzo (a) Pyrene-Induced Oxidative Stress during Lung Cancer and Treatment with Baicalein. In *Handbook of Oxidative Stress in Cancer: Mechanistic Aspects* (pp. 787-804). Singapore: Springer Nature Singapore.
- Charlesby, A. (2016). *Atomic radiation and polymers: international series of monographs on radiation effects in materials*. Elsevier.
- Chatterjee, P., Chandra, S., Dey, P., & Bhattacharya, S. (2012). Evaluation of anti-inflammatory effects of green tea and black tea: A comparative in vitro study. *Journal of advanced pharmaceutical technology & research*, *3*(2), 136.
- Chaudhari, S., Gupta, S. K., Yadav, S., & Yogi, B. (2020). A Review on Phytochemical and Pharmacological Activity of *Camellia Sinensis* (Tea Leave).
- Chawla, R., Thakur, P., Chowdhry, A., Jaiswal, S., Sharma, A., Goel, R., & Arora, R. (2013). Evidence based herbal drug standardization approach in coping with challenges of holistic management of diabetes: a dreadful lifestyle disorder of 21st century. *Journal of Diabetes & Metabolic Disorders*, *12*, 1-16.
- Che, C. T., Wang, Z. J., Chow, M. S. S., & Lam, C. W. K. (2013). Herb-herb combination for therapeutic enhancement and advancement: theory, practice and future perspectives. *Molecules*, *18*(5), 5125-5141.

- Chen, C. Y., Kao, C. L., & Liu, C. M. (2018). The cancer prevention, anti-inflammatory and anti-oxidation of bioactive phytochemicals targeting the TLR4 signaling pathway. *International journal of molecular sciences*, *19*(9), 2729.
- Chen, D., Sun, Z., Gao, J., Peng, J., Wang, Z., Zhao, Y., & Dai, W. (2022). Metabolomics combined with proteomics provides a novel interpretation of the compound differences among Chinese tea cultivars (*Camellia sinensis* var. *sinensis*) with different manufacturing suitabilities. *Food Chemistry*, *377*, 131976.
- Chen, H. M., Lee, M. J., Kuo, C. Y., Tsai, P. L., Liu, J. Y., & Kao, S. H. (2010). *Ocimum gratissimum* aqueous extract induces apoptotic signalling in lung adenocarcinoma cell A549. *Evidence-Based Complementary and Alternative Medicine*, *2011*.
- Chen, J., Xia, Y., Sui, X., Peng, Q., Zhang, T., Li, J., & Zhang, J. (2018). Steviol, a natural product inhibits proliferation of the gastrointestinal cancer cells intensively. *Oncotarget*, *9*(41), 26299.
- Chen, Q., Zhu, Y., Liu, Y., Liu, Y., Dong, C., Lin, Z., & Teng, J. (2022). Black tea aroma formation during the fermentation period. *Food Chemistry*, *374*, 131640.
- Chen, X., Li, H., Zhang, B., & Deng, Z. (2022). The synergistic and antagonistic antioxidant interactions of dietary phytochemical combinations. *Critical reviews in food science and nutrition*, *62*(20), 5658-5677.
- Chen, Z. M., & Lin, Z. (2015). Tea and human health: biomedical functions of tea active components and current issues. *Journal of Zhejiang University-Science B*, *16*(2), 87-102.
- Chen, Z., Fillmore, C. M., Hammerman, P. S., Kim, C. F., & Wong, K. K. (2014). Non-small-cell lung cancers: a heterogeneous set of diseases. *Nature Reviews Cancer*, *14*(8), 535-546.
- Chikara, S., Nagaprashantha, L. D., Singhal, J., Horne, D., Awasthi, S., & Singhal, S. S. (2018). Oxidative stress and dietary phytochemicals: Role in cancer chemoprevention and treatment. *Cancer letters*, *413*, 122-134.
- Chistiakov, D. A., Orekhov, A. N., & Bobryshev, Y. V. (2017). Treatment of cardiovascular pathology with epigenetically active agents: focus on natural and synthetic inhibitors of DNA methylation and histone deacetylation. *International Journal of Cardiology*, *227*, 66-82.
- Chou, T. C. (2006). Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. *Pharmacological reviews*, *58*(3), 621-681.

- Chou, T. C. (2010). Drug combination studies and their synergy quantification using the Chou-Talalay methodsynergy quantification method. *Cancer research*, *70*(2), 440-446.
- Chui, P. L. (2019). Cancer-and chemotherapy-related symptoms and the use of complementary and alternative medicine. *Asia-Pacific journal of oncology nursing*, *6*(1), 4.
- Chung, F. L. (1999). The prevention of lung cancer induced by a tobacco-specific carcinogen in rodents by green and black Tea. *Proceedings of the Society for Experimental Biology and Medicine*, *220*(4), 244-248.
- Chung, F. L., Schwartz, J., Herzog, C. R., & Yang, Y. M. (2003). Tea and cancer prevention: studies in animals and humans. *The Journal of nutrition*, *133*(10), 3268S-3274S.
- Chung, J. Y., Huang, C., Meng, X., Dong, Z., & Yang, C. S. (1999). Inhibition of activator protein 1 activity and cell growth by purified green tea and black tea polyphenols in H-ras-transformed cells: structure-activity relationship and mechanisms involved. *Cancer Research*, *59*(18), 4610-4617.
- Claire, R., Chamberlain, C., Davey, M. A., Cooper, S. E., Berlin, I., Leonardi-Bee, J., & Coleman, T. (2020). Pharmacological interventions for promoting smoking cessation during pregnancy. *Cochrane database of systematic reviews*, (3).
- Clancy, R. M., Leszczynska-Piziak, J., & Abramson, S. B. (1992). Nitric oxide, an endothelial cell relaxation factor, inhibits neutrophil superoxide anion production via a direct action on the NADPH oxidase. *The Journal of clinical investigation*, *90*(3), 1116-1121.
- Clark, J., & You, M. (2006). Chemoprevention of lung cancer by tea. *Molecular nutrition & food research*, *50*(2), 144-151.
- Cleverdon, R., Elhalaby, Y., McAlpine, M. D., Gittings, W., & Ward, W. E. (2018). Total polyphenol content and antioxidant capacity of tea bags: comparison of black, green, red rooibos, chamomile and peppermint over different steep times. *Beverages*, *4*(1), 15.
- Cohen, M. M. (2014). Tulsi-*Ocimum sanctum*: A herb for all reasons. *Journal of Ayurveda and integrative medicine*, *5*(4), 251.
- Colalto, C. (2010). Herbal interactions on absorption of drugs: Mechanisms of action and clinical risk assessment. *Pharmacological Research*, *62*(3), 207-227.
- Collins, L. G., Haines, C., Perkel, R., & Enck, R. E. (2007). Lung cancer: diagnosis and management. *American family physician*, *75*(1), 56-63.

- Conde, E., Angulo, B., Izquierdo, E., Paz-Ares, L., Belda-Iniesta, C., Hidalgo, M., & López-Ríos, F. (2013). Lung adenocarcinoma in the era of targeted therapies: histological classification, sample prioritization, and predictive biomarkers. *Clinical and Translational Oncology*, *15*, 503-508.
- Cragg, G. M., & Pezzuto, J. M. (2016). Natural products as a vital source for the discovery of cancer chemotherapeutic and chemopreventive agents. *Medical Principles and Practice*, *25*(2), 41-59.
- Crino, L., Weder, W., Van Meerbeeck, J., & Felip, E. S. M. O. (2010). Early stage and locally advanced (non-metastatic) non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of oncology*, *21*, 103-115.
- Dadashpour, M., Rasooli, I., Sorouri Zanjani, R., Sefidkon, F., Taghizadeh, M., & Darvish Alipour Astaneh, S. (2011). Antimicrobial, nitric oxide radical scavenging and cytotoxic properties of Thymus daenensis essential oil. *Pathobiology Research*, *14*(1), 37-47.
- Damiani, E., Bacchetti, T., Padella, L., Tiano, L., & Carloni, P. (2014). Antioxidant activity of different white teas: Comparison of hot and cold tea infusions. *Journal of Food Composition and Analysis*, *33*(1), 59-66.
- Dar, M. H., & Sehgal, A. (2020). Evaluation of antiangiogenic and antigenotoxic potential of green and black tea extracts by chicken chorioallantoic membrane assay. *Journal of Entomology and Zoology Studies*, *8*(2), 424-430.
- Das, D. N., & Ravi, N. (2022). Influences of polycyclic aromatic hydrocarbon on the epigenome toxicity and its applicability in human health risk assessment. *Environmental Research*, *213*, 113677.
- Davies, M. J., Judd, J. T., Baer, D. J., Clevidence, B. A., Paul, D. R., Edwards, A. J., & Chen, S. C. (2003). Black tea consumption reduces total and LDL cholesterol in mildly hypercholesterolemic adults. *The Journal of nutrition*, *133*(10), 3298S-3302S.
- Davis, J. M., Thomas, L. C., Dirkes, J. E., & Swartzwelder, H. S. (2020). Strategies for referring cancer patients in a smoking cessation program. *International journal of environmental research and public health*, *17*(17), 6089.
- De Flora, S., & Ferguson, L. R. (2005). Overview of mechanisms of cancer chemopreventive agents. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *591*(1-2), 8-15.
- De Wit, S., Glen, C., de Boer, R. A., & Lang, N. (2022). Mechanisms shared between cancer, heart failure and targeted anti-cancer therapies. *Cardiovascular Research*.

- Deb, S., & Jolvis Pou, K. R. (2016). A review of withering in the processing of black tea. *Journal of Biosystems Engineering*, *41*(4), 365-372.
- Deldar Abad Paskeh, M., Asadi, S., Zabolian, A., Saleki, H., Khoshbakht, M. A., Sabet, S., & Sethi, G. (2021). Targeting cancer stem cells by dietary agents: an important therapeutic strategy against human malignancies. *International Journal of Molecular Sciences*, *22*(21), 11669.
- Demedts, I. K., Vermaelen, K. Y., & Van Meerbeeck, J. P. (2010). Treatment of extensive-stage small cell lung carcinoma: current status and future prospects. *European Respiratory Journal*, *35*(1), 202-215.
- Demirci-Çekiç, S., Özkan, G., Avan, A. N., Uzunboy, S., Çapanoğlu, E., & Apak, R. (2022). Biomarkers of oxidative stress and antioxidant defense. *Journal of pharmaceutical and biomedical analysis*, *209*, 114477.
- Deng, X., Shao, Z., & Zhao, Y. (2021). Solutions to the drawbacks of photothermal and photodynamic cancer therapy. *Advanced Science*, *8*(3), 2002504.
- Devarakonda, S., Sankararaman, S., Herzog, B. H., Gold, K. A., Waqar, S. N., Ward, J. P., & Morgensztern, D. (2019). Circulating Tumor DNA Profiling in Small-Cell Lung Cancer Identifies Potentially Targetable AlterationsCirculating Tumor DNA Profiling in SCLC. *Clinical Cancer Research*, *25*(20), 6119-6126.
- Dhawan, A., Anderson, D., de Pascual-Teresa, S., Santos-Buelga, C., Clifford, M. N., & Ioannides, C. (2002). Evaluation of the antigenotoxic potential of monomeric and dimeric flavanols, and black tea polyphenols against heterocyclic amine-induced DNA damage in human lymphocytes using the

  Comet assay. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *515*(1-2), 39-56.
- Didkowska, J., Wojciechowska, U., Mańczuk, M., & Łobaszewski, J. (2016). Lung cancer epidemiology: contemporary and future challenges worldwide. *Annals of translational medicine*, *4*(8).
- Dillard, C. J., & German, J. B. (2000). Phytochemicals: nutraceuticals and human health. *Journal of the Science of Food and Agriculture*, *80*(12), 1744-1756.
- Doll, R., & Hill, A. B. (1950). Smoking and carcinoma of the lung. *British medical journal*, *2*(4682), 739.
- Dutta, S., Mahalanobish, S., Saha, S., Ghosh, S., & Sil, P. C. (2019). Natural products: An upcoming therapeutic approach to cancer. *Food and Chemical Toxicology*, *128*, 240-255.

- Ekinci, M., Santos-Oliveira, R., & Ilem-Ozdemir, D. (2022). Biodistribution of 99mTc-PLA/PVA/Atezolizumab nanoparticles for non-small cell lung cancer diagnosis. *European Journal of Pharmaceutics and Biopharmaceutics*, *176*, 21-31.
- Enko, J., & Gliszczyńska-Świgło, A. (2015). Influence of the interactions between tea (Camellia sinensis) extracts and ascorbic acid on their antioxidant activity: analysis with interaction indexes and isobolograms. *Food Additives & Contaminants: Part A*, *32*(8), 1234-1242.
- Erasmus, L. M., Theron, K. A., Muller, M., Van der Rijst, M., & Joubert, E. (2017). Optimising high-temperature oxidation of Cyclopia species for maximum development of characteristic aroma notes of honeybush herbal tea infusions. *South African Journal of Botany*, *110*, 144-151.
- Esmeeta, A., Adhikary, S., Dharshnaa, V., Swarnamughi, P., Maqsummiya, Z. U., Banerjee, A., & Duttaroy, A. K. (2022). Plant-derived bioactive compounds in colon cancer treatment: An updated review. *Biomedicine & Pharmacotherapy*, *153*, 113384.
- Fakeye, T. O., Adisa, R., & Musa, I. E. (2009). Attitude and use of herbal medicines among pregnant women in Nigeria. *BMC Complementary and alternative medicine*, *9*, 1-7.
- Fantini, M., Benvenuto, M., Masuelli, L., Frajese, G. V., Tresoldi, I., Modesti, A., & Bei, R. (2015). *In vitro* and *in vivo* antitumoral effects of combinations of polyphenols, or polyphenols and anticancer drugs: perspectives on cancer treatment. *International journal of molecular sciences*, *16*(5), 9236-9282.
- Farhan, M., Rizvi, A., Ahmad, A., Aatif, M., Alam, M. W., & Hadi, S. M. (2022). Structure of some green tea catechins and the availability of intracellular copper influence their ability to cause selective oxidative DNA damage in malignant cells. *Biomedicines*, *10*(3), 664.
- Farooq, S., & Sehgal, A. (2018). Antioxidant activity of different forms of green tea: Loose leaf, bagged and matcha. *Current Research in Nutrition and Food Science Journal*, *6*(1), 35-40.
- Farooq, S., & Sehgal, A. (2019). Synergistic antioxidant interactions between green tea and *Ocimum gratissimum*. *Asian Pacific Journal of Tropical Biomedicine*, *9*(8), 333.
- Farooq, S., & Sehgal, A. (2021). Chemopreventive Efficacy of Green Tea, *Ocimum gratissimum* and their Combination Against Benzo [a] pyrene Induced Preneoplastic Lung Lesions in Murine Model. *Journal of Biologically Active Products from Nature*, *11*(1), 22-31.

- Feitelson, M. A., Arzumanyan, A., Kulathinal, R. J., Blain, S. W., Holcombe, R. F., Mahajna, J., & Nowsheen, S. (2015, December). Sustained proliferation in cancer: Mechanisms and novel therapeutic targets. In *Seminars in cancer biology* (Vol. 35, pp. S25-S54). Academic Press.
- Feng, Z., Li, Y., Li, M., Wang, Y., Zhang, L., Wan, X., & Yang, X. (2019). Tea aroma formation from six model manufacturing processes. *Food chemistry*, *285*, 347-354.
- Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., & Bray, F. (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International journal of cancer*, *136*(5), E359-E386.
- Ferrell, B., Sun, V., Hurria, A., Cristea, M., Raz, D. J., Kim, J. Y., & Koczywas, M. (2015). Interdisciplinary palliative care for patients with lung cancer. *Journal of pain and symptom management*, *50*(6), 758-767.
- Finger, A. (1994). In-vitro studies on the effect of polyphenol oxidase and peroxidase on the formation of polyphenolic black tea constituents. *Journal of the Science of Food and Agriculture*, *66*(3), 293-305.
- Fitzgerald, K., & Simone, C. B. (2020). Combining immunotherapy with radiation therapy in non–small cell lung cancer. *Thoracic Surgery Clinics*, *30*(2), 221-239.
- Ganguly, S., Mantha, S., & Panda, K. (2016). Simultaneous determination of black tea-derived catechins and theaflavins in tissues of tea consuming animals using ultra-performance liquid-chromatography tandem mass spectrometry. *PLoS One*, *11*(10), e0163498.
- Gupta, R. K., Chawla, P. R. I. Y. A. N. K. A., Tripathi, M., Shukla, A. K., & Pandey, A. J. I. J. O. P. (2014). Synergistic antioxidant activity of tea with ginger, black pepper and tulsi. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(5), 477-9.
- Gani, A., Rasool, N., Shah, A., Ahmad, M., Gani, A., Wani, T. A., & Masoodi, F. A. (2015). DNA scission inhibition, antioxidant, and antiproliferative activities of water chestnut (Trapa natans) extracted in different solvents. *Cyta-Journal of Food*, *13*(3), 415-419.
- Ganmaa, D., Willett, W. C., Li, T. Y., Feskanich, D., van Dam, R. M., Lopez-Garcia, E., & Holmes, M. D. (2008). Coffee, tea, caffeine and risk of breast cancer: a 22-year follow-up. *International journal of cancer*, *122*(9), 2071-2076.
- Gantait, S., Das, A., & Banerjee, J. (2018). Geographical distribution, botanical description and self-incompatibility mechanism of genus Stevia. *Sugar Tech*, *20*, 1-10.

- García-Perez, P., Barreal, M. E., Rojo-De Dios, L., Cameselle-Teijeiro, J. F., & Gallego, P. P. (2019). Bioactive natural products from the genus Kalanchoe as cancer chemopreventive agents: a review. *Studies in natural products chemistry*, *61*, 49-84.
- Gardner, E. J., Ruxton, C. H. S., & Leeds, A. R. (2007). Black tea–helpful or harmful? A review of the evidence. *European journal of clinical nutrition*, *61*(1), 3-18.
- Garg, P., Garg, R., Horne, D., Awasthi, S., Salgia, R., & Singhal, S. S. (2023). Prognostic significance of natural products against multidrug tumor resistance. *Cancer Letters*, 216079.
- Garg, R., Gupta, S., & Maru, G. B. (2008). Dietary curcumin modulates transcriptional regulators of phase I and phase II enzymes in benzo [a] pyrene-treated mice: mechanism of its anti-initiating action. *Carcinogenesis*, *29*(5), 1022-1032.
- Gavahian, M., Chu, Y. H., Khaneghah, A. M., Barba, F. J., & Misra, N. N. (2018). A critical analysis of the cold plasma induced lipid oxidation in foods. *Trends in Food Science & Technology*, *77*, 32-41.
- Gawlik-Dziki, U. (2012). Dietary spices as a natural effectors of lipoxygenase, xanthine oxidase, peroxidase and antioxidant agents. *LWT-Food Science and Technology*, *47*(1), 138-146.
- Gelboin, H. V. (1980). Benzo[alpha]pyrene metabolism, activation and carcinogenesis: role and regulation of mixed-function oxidases and related enzymes. *Physiological reviews*, *60*(4), 1107-1166.
- Geneste, O., Camus, A. M., Castegnaro, M., Petruzzelli, S., Macchiarini, P., Angeletti, C. A., & Bartsch, H. (1991). Comparison of pulmonary DNA adducts levels, measured by 32P-postlabelling and aryl hydrocarbon hydroxylase activity in lung parenchyma of smokers and ex-smokers. *Carcinogenesis*, *12*(7), 1301-1305.
- George, B. P., Chandran, R., & Abrahamse, H. (2021). Role of phytochemicals in cancer chemoprevention: Insights. *Antioxidants*, *10*(9), 1455.
- George, J., Singh, M., Srivastava, A. K., Bhui, K., Roy, P., Chaturvedi, P. K., & Shukla, Y. (2011). Resveratrol and black tea polyphenol combination synergistically suppress mouse skin tumors growth by inhibition of activated MAPKs and p53. *PloS one*, *6*(8), e23395.
- Gesthalter, Y., & Smyth, R. (2022). Treatment of small cell lung cancer. *American Journal of Respiratory and Critical Care Medicine*, *205*(2), 3-4.
- Gezici, S. (2022). Therapeutic updates and future prospects on anticancer effects of medicinal plants and phytochemicals. In *Herbal Bioactive-Based Drug Delivery Systems* (283-310). Academic Press.

- Gholami, A., & Mehrabi, F. (2022). Cancer and nanotechnology: A mini review. *Advances in Applied NanoBio-Technologies*, *3*(1), 1-6.
- Giovannucci, E. (2022). Molecular biologic and epidemiologic insights for preventability of colorectal cancer. *JNCI: Journal of the National Cancer Institute*, *114*(5), 645-650.
- Glasauer, A., Sena, L. A., Diebold, L. P., Mazar, A. P., & Chandel, N. S. (2014). Targeting SOD1 reduces experimental non–small-cell lung cancer. *The Journal of clinical investigation*, *124*(1), 117-128.
- Globocan.iarc.fr. Redirect [cited 14 January 2016]. Available online: http://globocan.iarc.fr
- Go, R. E., Hwang, K. A., & Choi, K. C. (2015). Cytochrome P450 1 family and cancers. *The Journal of steroid biochemistry and molecular biology*, *147*, 24-30.
- Gomez, D. R., Tang, C., Zhang, J., Blumenschein Jr, G. R., Hernandez, M., Lee, J. J., & Heymach, J. V. (2019). Local consolidative therapy vs. maintenance therapy or observation for patients with oligometastatic non–small-cell lung cancer: long-term results of a multi-institutional, phase II, randomized study. *Journal of Clinical Oncology*, *37*(18), 1558.
- Gonzalez-Vallinas, M., Gonzalez-Castejon, M., Rodríguez-Casado, A., & Ramírez de Molina, A. (2013). Dietary phytochemicals in cancer prevention and therapy: a complementary approach with promising perspectives. *Nutrition reviews*, *71*(9), 585-599.
- Gordaliza, M. (2007). Natural products as leads to anticancer drugs. *Clinical and Translational Oncology*, *9*, 767-776.
- Goyal, P. K., Verma, P., Sharma, P., Parmar, J., & Agarwal, A. (2010). Evaluation of anti-cancer and anti-oxidative potential of *Syzygium cumini* against benzo[a]pyrene (BaP) induced gastric carcinogenesis in mice. *Asian Pac J Cancer Prev*, *11*(3), 753-758.
- Goyal, S. K., Samsher, N., & Goyal, R. K. (2010). Stevia (*Stevia rebaudiana*) a bio-sweetener: a review. *International journal of food sciences and nutrition*, *61*(1), 1-10.
- Granger, M. P., Wright, W. E., & Shay, J. W. (2002). Telomerase in cancer and aging. *Critical reviews in oncology/hematology*, *41*(1), 29-40.
- Grassberger, C., & Paganetti, H. (2016). Methodologies in the modeling of combined chemo-radiation treatments. *Physics in Medicine & Biology*, *61*(21), R344.

- Groelly, F. J., Fawkes, M., Dagg, R. A., Blackford, A. N., & Tarsounas, M. (2023). Targeting DNA damage response pathways in cancer. *Nature Reviews Cancer*, *23*(2), 78-94.
- Gu, K. J., & Li, G. (2020). An overview of cancer prevention: Chemoprevention and immunoprevention. *Journal of Cancer Prevention*, *25*(3), 127.
- Gu, L., Wang, X., Liu, Z., Ju, P., Zhang, L., Zhang, Y., & Chen, X. (2014). A study of Semen Strychni-induced renal injury and herb–herb interaction of *Radix Glycyrrhizae* extract and/or *Rhizoma Ligustici* extract on the comparative toxicokinetics of strychnine and brucine in rats. *Food and Chemical Toxicology*, *68*, 226-233.
- Guleria, K., & Sehgal, A. (2020). Appraisal of antioxidant effect of fresh and dried leaves of lemongrass (*Cymbopogon citratus*). *Plant Archives*, *20*(2), 2554-2557.
- Guleria, K., & Sehgal, A. (2022). Additive to Antagonistic Antioxidant Interaction of Black Tea with Three Different Species of *Ocimum*. *Journal of Culinary Science & Technology*, 1-13.
- Guleria, K., Sehgal, A., Bhat, I. A., Singh, S. K., Vamanu, E., & Singh, M. P. (2022). Impact of Altering the Ratio of Black Tea Granules and Ocimum gratissimum Leaves in a Binary Infusion on Radical Scavenging Potential Employing Cell Free Models and Ex Vivo Assays. *Applied Sciences*, *12*(20), 10632.
- Gullett, N. P., Amin, A. R., Bayraktar, S., Pezzuto, J. M., Shin, D. M., Khuri, F. R., & Kucuk, O. (2010, June). Cancer prevention with natural compounds. In *Seminars in oncology* (Vol. 37, No. 3, pp. 258-281). WB Saunders.
- Gupta, E., Purwar, S., Sundaram, S., & Rai, G. K. (2013). Nutritional and therapeutic values of *Stevia rebaudiana*: A review. *Journal of Medicinal Plants Research*, *7*(46), 3343-3353.
- Gupta, R. C., Chang, D., Nammi, S., Bensoussan, A., Bilinski, K., & Roufogalis, B. D. (2017). Interactions between antidiabetic drugs and herbs: an overview of mechanisms of action and clinical implications. *Diabetology & metabolic syndrome*, *9*(1), 1-12.
- Gupta, S. K., Prakash, J., & Srivastava, S. (2002). Validation of traditional claim of Tulsi, *Ocimum sanctum* Linn. as a medicinal plant.
- Gupta-Elera, G., Garrett, A. R., Robison, R. A., & O'Neill, K. L. (2012). The role of oxidative stress in prostate cancer. *European Journal of Cancer Prevention*, *21*(2), 155-162.

- Gurav, T. P., Dholakia, B. B., & Giri, A. P. (2022). A glance at the chemodiversity of *Ocimum* species: Trends, implications, and strategies for the quality and yield improvement of essential oil. *Phytochemistry Reviews*, *21*(3), 879-913.
- Gurley, B. J., Fifer, E. K., & Gardner, Z. (2012). Pharmacokinetic herb-drug interactions (part 2): drug interactions involving popular botanical dietary supplements and their clinical relevance. *Planta medica*, *78*(13), 1490-1514.
- Gusman, J., Malonne, H., & Atassi, G. (2001). A reappraisal of the potential chemopreventive and chemotherapeutic properties of resveratrol. *Carcinogenesis*, *22*(8), 1111-1117.
- Gutte, R., & Deshmukh, V. (2023). A comprehensive review of the preventive action of Natural Nutraceutical Ingredients in reducing Chemotherapy–Induced Side effects. *Functional Food Science*, *3*(2), 1-14.
- Hadi, S. M., Asad, S. F., Singh, S., & Ahmad, A. (2000). Putative mechanism for anticancer and apoptosis-inducing properties of plant-derived polyphenolic compounds. *IUBMB life*, *50*(3), 167-171.
- Haslam, E. (2003). Thoughts on thearubigins. *Phytochemistry*, *64*(1), 61-73.
- Hakkim, F. L., Arivazhagan, G., & Boopathy, R. (2008). Antioxidant property of selected *Ocimum* species and their secondary metabolite content. *Journal of Medicinal Plants Research*, *2*(9), 250-257.
- Halder, B., Bhattacharya, U., Mukhopadhyay, S., & Giri, A. K. (2008). Molecular mechanism of black tea polyphenols induced apoptosis in human skin cancer cells: involvement of Bax translocation and mitochondria mediated death cascade. *Carcinogenesis*, *29*(1), 129-138.
- Han, R. M., Zhang, J. P., & Skibsted, L. H. (2012). Reaction dynamics of flavonoids and carotenoids as antioxidants. *Molecules*, *17*(2), 2140-2160.
- Hartwig, A., Arand, M., Epe, B., Guth, S., Jahnke, G., Lampen, A., & Eisenbrand, G. (2020). Mode of action-based risk assessment of genotoxic carcinogens. *Archives of toxicology*, *94*, 1787-1877.
- Hasan, A. E. Z., Safithri, M., Huda, A. S., & Kurniasih, R. (2022). In Silico, To Determine The Active Compounds Of Black Tea And Turmeric In Increasing The Activity Of The Enzyme SOD. *Indonesian Journal of Applied Research (IJAR)*, *3*(1), 32-45.
- Hayakawa, K. (2022). Polycyclic aromatic hydrocarbons. In *Handbook of Air Quality and Climate Change* (pp. 1-17). Singapore: Springer Singapore.

- Hayat, K., Iqbal, H., Malik, U., Bilal, U., & Mushtaq, S. (2015). Tea and its consumption: benefits and risks. *Critical reviews in food science and nutrition*, *55*(7), 939-954.
- Hazra, A., Dasgupta, N., Sengupta, C., Bera, B., & Das, S. (2019). Tea: A worthwhile, popular beverage crop since time immemorial. *Agronomic Crops: Volume 1: Production Technologies*, 507-531.
- He, Y., Li, S., Zhang, W., Dai, W., Cui, T., Wang, G., & Li, C. (2017). Dysregulated autophagy increased melanocyte sensitivity to H_2O_2-induced oxidative stress in vitiligo. *Scientific reports*, *7*(1), 42394.
- Hecht, S. S. (2003). Tobacco carcinogens, their biomarkers and tobacco-induced cancer. *Nature Reviews Cancer*, *3*(10), 733-744.
- Hecht, S. S., & Hatsukami, D. K. (2022). Smokeless tobacco and cigarette smoking: chemical mechanisms and cancer prevention. *Nature Reviews Cancer*, *22*(3), 143-155.
- Heist, R. S., & Engelman, J. A. (2012). SnapShot: non-small cell lung cancer. *Cancer cell*, *21*(3), 448-448.
- Henley, S. J., Ellington, T., Stanfill, S., Mills, K., & Thun, M. J. (2022). Carcinogens and Toxicants in Smokeless Tobacco Products. *Tobacco And Cancer: The Science And The Story*, 129-156.
- Hidalgo, M., Sánchez-Moreno, C., & de Pascual-Teresa, S. (2010). Flavonoid–flavonoid interaction and its effect on their antioxidant activity. *Food chemistry*, *121*(3), 691-696.
- Hilal, Y., & Engelhardt, U. (2007). Characterisation of white tea–Comparison to green and black tea. *Journal für Verbraucherschutz und Lebensmittelsicherheit*, *2*, 414-421.
- Ho, J., Chaiswing, L., & St. Clair, D. K. (2022). Extracellular vesicles and cancer therapy: Insights into the role of oxidative stress. *Antioxidants*, *11*(6), 1194.
- Hochhegger, B., Camargo, S., da Silva Teles, G. B., Chate, R. C., Szarf, G., Guimaraes, M. D.,& Dos Santos, R. S. (2022). Challenges of implementing lung cancer screening in a developing Country: Results of the Second Brazilian Early Lung Cancer Screening Trial (BRELT2). *JCO Global Oncology*, *8*, e2100257.
- Hodgson, J. M., Puddey, I. B., Woodman, R. J., Mulder, T. P., Fuchs, D., Scott, K., & Croft, K. D. (2012). Effects of black tea on blood pressure: a randomized controlled trial. *Archives of internal medicine*, *172*(2), 186-188.
- Hoffmann, D., Hoffmann, I., & El-Bayoumy, K. (2001). The less harmful cigarette: a controversial issue. A tribute to Ernst L. Wynder. *Chemical research in toxicology*, *14*(7), 767-790.

- Hong, T. M., Chen, Y. L., Wu, Y. Y., Yuan, A., Chao, Y. C., Chung, Y. C., & Yang, P. C. (2007). Targeting neuropilin 1 as an antitumor strategy in lung cancer. *Clinical Cancer Research*, *13*(16), 4759-4768.
- Hong, W. K., & Sporn, M. B. (1997). Recent advances in chemoprevention of cancer. *Science*, *278*(5340), 1073-1077.
- Hosseini, A., & Ghorbani, A. (2015). Cancer therapy with phytochemicals: evidence from clinical studies. *Avicenna journal of phytomedicine*, *5*(2), 84.
- https://gco.iarc.fr/today/data/factsheets/populations/900-india-fact-sheets.pdf
- https://gco.iarc.fr/today/data/factsheets/populations/900-world-fact-sheets.pdf
- https://www.wcrf.org/wp-content/uploads/2021/02/Summary-of-Third-Expert-Report--2018.pdf
- Huang, W. Y., Cai, Y. Z., & Zhang, Y. (2009). Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. *Nutrition and cancer*, *62*(1), 1-20.
- Hudlikar, R. R., Pai, V., Kumar, R., Thorat, R. A., Kannan, S., Ingle, A. D., & Mahimkar, M. B. (2019). Dose-related modulatory effects of polymeric black tea polyphenols (PBPs) on initiation and promotion events in B(a)P and NNK-induced lung carcinogenesis. *Nutrition and cancer*, *71*(3), 508-523.
- Hulbert, A., Hooker, C. M., Keruly, J. C., Brown, T., Horton, K., Fishman, E., & Brock, M. V. (2014). Prospective CT screening for lung cancer in a high-risk population: HIV-positive smokers. *Journal of Thoracic Oncology*, *9*(6), 752-759.
- ICMR (Indian Council of Medical Research) (2006). Report. Assessment of Burden of Non-Communicable Diseases, New Delhi
- Ihsan, A. U., Khan, F. U., Khongorzul, P., Ahmad, K. A., Naveed, M., Yasmeen, S., & Zhou, X. (2018). Role of oxidative stress in pathology of chronic prostatitis/chronic pelvic pain syndrome and male infertility and antioxidants function in ameliorating oxidative stress. *Biomedicine & Pharmacotherapy*, *106*, 714-723.
- Imran, A., Butt, M. S., Xiao, H., Imran, M., Rauf, A., Mubarak, M. S., & Ramadan, M. F. (2019). Inhibitory effect of black tea (*Camellia sinensis*) theaflavins and thearubigins against HCT 116 colon cancer cells and HT 460 lung cancer cells. *Journal of food biochemistry*, *43*(5), e12822.
- Indian Council of Medical Research – National Centre for Disease Informatics and Research. Report of National Cancer Registry Programme (2012–16).

- Islam, S. N., Farooq, S., & Sehgal, A. (2018). Effect of consecutive steeping on antioxidant potential of green, oolong and black tea. *International Journal of Food Science & Technology*, *53*(1), 182-187.
- https://www.ncdirindia.org/All_Reports/Report_2020/resources/NCRP_2020_2012_16.pdf. Accessed 19 Aug 2020
- Islami, F., Guerra, C. E., Minihan, A., Yabroff, K. R., Fedewa, S. A., Sloan, K., & Jemal, A. (2022). American Cancer Society's report on the status of cancer disparities in the United States, 2021. *CA: a cancer journal for clinicians*, *72*(2), 112-143.
- Ivanova, D., Gerova, D., Chervenkov, T., & Yankova, T. (2005). Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *Journal of ethnopharmacology*, *96*(1-2), 145-150.
- Jahangir, T., Safhi, M. M., Sultana, S., & Ahmad, S. (2013). Pluchea lanceolata protects against Benzo (a) pyrene induced renal toxicity and loss of DNA integrity. *Interdisciplinary Toxicology*, *6*(1), 47-54.
- Jayaraman, S., Manoharan, M. S., & Illanchezian, S. (2008). In-vitro antimicrobial and antitumor activities of *Stevia rebaudiana* (Asteraceae) leaf extracts. *Tropical Journal of Pharmaceutical Research*, *7*(4), 1143-1149.
- Jiang, T., Luo, Z., & Ying, T. (2015). Fumigation with essential oils improves sensory quality and enhanced antioxidant ability of shiitake mushroom (*Lentinus edodes*). *Food chemistry*, *172*, 692-698.
- Johnson, T. O., Abolaji, A. O., Omale, S., Longdet, I. Y., Kutshik, R. J., Oyetayo, B. O., & Sagay, A. (2021). Benzo [a] pyrene and Benzo [a] pyrene-7, 8-dihydrodiol-9, 10-epoxide induced locomotor and reproductive senescence and altered biochemical parameters of oxidative damage in Canton-S Drosophila melanogaster. *Toxicology reports*, *8*, 571-580.
- Jordan, S. A., Cunningham, D. G., & Marles, R. J. (2010). Assessment of herbal medicinal products: challenges, and opportunities to increase the knowledge base for safety assessment. *Toxicology and applied pharmacology*, *243*(2), 198-216.
- Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of agricultural and food chemistry*, *47*(10), 3954-3962.
- Kamaraj, S., Ramakrishnan, G., Anandakumar, P., Jagan, S., & Devaki, T. (2009). Antioxidant and anticancer efficacy of hesperidin in benzo (a) pyrene induced lung carcinogenesis in mice. *Investigational New Drugs*, *27*, 214-222.

- Kanwal, M., Ding, X. J., & Cao, Y. (2017). Familial risk for lung cancer. *Oncology letters*, *13*(2), 535-542.
- Kapinova, A., Kubatka, P., Golubnitschaja, O., Kello, M., Zubor, P., Solar, P., & Pec, M. (2018). Dietary phytochemicals in breast cancer research: anticancer effects and potential utility for effective chemoprevention. *Environmental health and preventive medicine*, *23*, 1-18.
- Karagoz, Ş. (2022). What is *Stevia rebaudiana*: Only. *Evaluating the Agricultural Status of Some Unions of Kalapara Upazila, Bangladesh: Preliminary Investigation*, 61.
- Karak, P. (2019). Biological activities of flavonoids: an overview. *Int. J. Pharm. Sci. Res*, *10*(4), 1567-1574.
- Karikas, G. A. (2010). Anticancer and chemopreventing natural products: some biochemical and therapeutic aspects. *J BUON*, *15*(4), 627-638.
- Karimi, E., Oskoueian, E., Hendra, R., & Jaafar, H. Z. (2010). Evaluation of Crocus sativus L. stigma phenolic and flavonoid compounds and its antioxidant activity. *Molecules*, *15*(9), 6244-6256.
- Karlsson, B. G. (2022). The imperial weight of tea: On the politics of plants, plantations and science. *Geoforum*, *130*, 105-114.
- Kasala, E. R., Bodduluru, L. N., Barua, C. C., Sriram, C. S., & Gogoi, R. (2015). Benzo(a)pyrene induced lung cancer: Role of dietary phytochemicals in chemoprevention. *Pharmacological Reports*, *67*(5), 996-1009.
- Katiyar, S. K., Agarwal, R., Zaim, M. T., & Mukhtar, H. (1993). Protection against N-nitrosodiethylamine and benzo [a] pyrene-induced forestomach and lung tumorigenesis in A/J mice by green tea. *Carcinogenesis*, *14*(5), 849-855.
- Katiyar, S., & Mukhtar, H. A. S. A. N. (1996). Tea in chemoprevention of cancer. *International journal of oncology*, *8*(2), 221-238.
- Khan, N., & Mukhtar, H. (2013). Tea and health: studies in humans. *Current pharmaceutical design*, *19*(34), 6141-6147.
- Khan, N., & Mukhtar, H. (2015). Dietary agents for prevention and treatment of lung cancer. *Cancer letters*, *359*(2), 155-164.
- Khan, N., & Mukhtar, H. (2018). Tea polyphenols in promotion of human health. *Nutrients*, *11*(1), 39.
- Khan, N., Siddiqui, I. A., Adhami, V. A., & Mukhtar, H. (2013). Green tea polyphenols for cancer risk reduction. *Green Tea Polyphenols: Nutraceuticals of Modern Life*, 57.

- Khatoon, S., Kalam, N., Balasubramaniam, V. R., Shaikh, M., & Ansari, M. T. (2022). Chemotherapeutic Role of Polyphenols Present in *Ocimum sanctum*. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, *22*(20), 3325-3342.
- Khilar, S., Singh, A. P., Biagi, M., & Sharma, A. (2022). An Insight into attributes of *Stevia rebaudiana* Bertoni: Recent advances in extraction techniques, phytochemistry, food applications and health benefits. *Journal of Agriculture and Food Research*, *10*, 100458.
- Khuder, S. A. (2001). Effect of cigarette smoking on major histological types of lung cancer: a meta-analysis. *Lung cancer*, *31*(2-3), 139-148.
- Killedar, S. G., Pawar, A. V., & Suresh Killedar, C. (2017). Preparation of herbal tea from mulberry leaves. *Journal of Medicinal Plants Studies*, *5*(2), 325-328.
- Kim, D., Lee, Y. S., Kim, D. H., & Bae, S. C. (2020). Lung cancer staging and associated genetic and epigenetic events. *Molecules and Cells*, *43*(1), 1.
- Kim, S. H., Cho, S. K., Hyun, S. H., Park, H. E., Kim, Y. S., & Choi, H. K. (2011). Metabolic profiling and predicting the free radical scavenging activity of guava (*Psidium guajava* L.) leaves according to harvest time by 1H-nuclear magnetic resonance spectroscopy. *Bioscience, biotechnology, and biochemistry*, *75*(6), 1090-1097.
- Kim, Y. K., Guo, Q., & Packer, L. (2002). Free radical scavenging activity of red ginseng aqueous extracts. *Toxicology*, *172*(2), 149-156.
- Kohlmeier, L., Weterings, K. G., Steck, S., & Kok, F. J. (1997). Tea and cancer prevention: an evaluation of the epidemiologic literature. *Nutrition and cancer*, *27*(1), 1-13.
- Kokubo, Y., Iso, H., Saito, I., Yamagishi, K., Yatsuya, H., Ishihara, J., & Tsugane, S. (2013). The impact of green tea and coffee consumption on the reduced risk of stroke incidence in Japanese population: the Japan public health center-based study cohort. *Stroke*, *44*(5), 1369-1374.
- Konarikova, K., Jezovicova, M., Kerestes, J., Gbelcova, H., Ďurackova, Z., & Zitnanova, I. (2015). Anticancer effect of black tea extract in human cancer cell lines. *Springerplus*, *4*, 1-6.
- Korir, M. W., Wachira, F. N., Wanyoko, J. K., Ngure, R. M., & Khalid, R. (2014). The fortification of tea with sweeteners and milk and its effect on in vitro antioxidant potential of tea product and glutathione levels in an animal model. *Food Chemistry*, *145*, 145-153.

- Kotecha, R., Takami, A., & Espinoza, J. L. (2016). Dietary phytochemicals and cancer chemoprevention: a review of the clinical evidence. *Oncotarget*, *7*(32), 52517.
- Krishnan, R., & Maru, G. (2005). Inhibitory effect (s) of polymeric black tea polyphenols on the formation of B (a) P-derived DNA adducts in mouse skin. *Journal of environmental pathology, toxicology and oncology*, *24*(2).
- Krishnan, R., & Maru, G. B. (2006). Isolation and analyses of polymeric polyphenol fractions from black tea. *Food Chemistry*, *94*(3), 331-340.
- Krishnan, R., Raghunathan, R., & Maru, G. B. (2005). Effect of polymeric black tea polyphenols on benzo (a) pyrene [B (a) P]-induced cytochrome P4501A1 and 1A2 in mice. *Xenobiotica*, *35*(7), 671-682.
- Kubik, A. K., Zatloukal, P., Tomášek, L., Pauk, N., Havel, L., Křepela, E., & Petruželka, L. (2004). Dietary habits and lung cancer risk among non-smoking women. *European Journal of Cancer Prevention*, 471-480.
- Kuderer, N. M., Desai, A., Lustberg, M. B., & Lyman, G. H. (2022). Mitigating acute chemotherapy-associated adverse events in patients with cancer. *Nature Reviews Clinical Oncology*, *19*(11), 681-697.
- Kuhnert, N., Drynan, J. W., Obuchowicz, J., Clifford, M. N., & Witt, M. (2010). Mass spectrometric characterization of black tea thearubigins leading to an oxidative cascade hypothesis for thearubigin formation. *Rapid Communications in Mass Spectrometry*, *24*(23), 3387-3404.
- Kulkarni, K. V., & Adavirao, B. V. (2018). A review on: Indian traditional shrub Tulsi (Ocimum sanctum): the unique medicinal plant. *Journal of Medicinal Plants Studies*, *6*(2), 106-110.
- Kulothungan, V., Sathishkumar, K., Leburu, S., Ramamoorthy, T., Stephen, S., Basavarajappa, D., & Mathur, P. (2022). Burden of cancers in India-estimates of cancer crude incidence, YLLs, YLDs and DALYs for 2021 and 2025 based on National Cancer Registry Program. *BMC cancer*, *22*(1), 527.
- Kumar, G., Pillare, S. P., & Maru, G. B. (2010). Black tea polyphenols-mediated in vivo cellular responses during carcinogenesis. *Mini reviews in medicinal chemistry*, *10*(6), 492-505.
- Kumar, R., Collins, D., Dolly, S., McDonald, F., O'Brien, M. E., & Yap, T. A. (2017). Targeting the PD-1/PD-L1 axis in non–small cell lung cancer. *Current problems in cancer*, *41*(2), 111-124.
- Kumar, R., Saha, P., Lokare, P., Datta, K., Selvakumar, P., & Chourasia, A. (2022). A Systemic Review of *Ocimum sanctum* (Tulsi): Morphological Characteristics,

Phytoconstituents and Therapeutic Applications. *International Journal for Research in Applied Sciences and Biotechnology*, *9*(2), 221-226.

- Kumar, V., Kaur, J., Tanwar, B., Goyal, A., Gat, Y., Kumar, A., & Kaur, P. (2018). Tea processing. *Beverages: Processing and technology*, 190-209.
- Kumari, S., Sharma, S., Advani, D., Khosla, A., Kumar, P., & Ambasta, R. K. (2021). Unboxing the molecular modalities of mutagens in cancer. *Environmental Science and Pollution Research*, 1-49.
- Kurek, J. M., & Krejpcio, Z. (2019). The functional and health-promoting properties of *Stevia rebaudiana* Bertoni and its glycosides with special focus on the antidiabetic potential–A review. *Journal of Functional Foods*, *61*, 103465.
- Kuroda, Y., & Hara, Y. (1999). Antimutagenic and anticarcinogenic activity of tea polyphenols. *Mutation Research/Reviews in Mutation Research*, *436*(1), 69-97.
- Kusano, R., Andou, H., Fujieda, M., Tanaka, T., Matsuo, Y., & Kouno, I. (2008). Polymer-like polyphenols of black tea and their lipase and amylase inhibitory activities. *Chemical and Pharmaceutical Bulletin*, *56*(3), 266-272.
- Lutz, W. K. (1990). Endogenous genotoxic agents and processes as a basis of spontaneous carcinogenesis. *Mutation Research/Reviews in Genetic Toxicology*, *238*(3), 287-295.
- Labbe, D., Tremblay, A., & Bazinet, L. (2006). Effect of brewing temperature and duration on green tea catechin solubilization: Basis for production of EGC and EGCG-enriched fractions. *Separation and Purification Technology*, *49*(1), 1-9.
- Lahiri, A., Maji, A., Potdar, P. D., Singh, N., Parikh, P., Bisht, B., & Paul, M. K. (2023). Lung cancer immunotherapy: progress, pitfalls, and promises. *Molecular Cancer*, *22*(1), 1-37.
- Landau, J. M., Wang, Z. Y., Yang, G. Y., Ding, W., & Yang, C. S. (1998). Inhibition of spontaneous formation of lung tumors and rhabdomyosarcomas in A/J mice by black and green tea. *Carcinogenesis*, *19*(3), 501-507.
- Lando, H. A., Hipple, B. J., Muramoto, M., Klein, J. D., Prokhorov, A. V., Ossip, D. J., & Winickoff, J. P. (2010). Tobacco is a global paediatric concern. *Bulletin of the World Health Organization*, *88*(1), 2-2A.
- Le, Q. U., Lay, H. L., Wu, M. C., & Do, T. N. (2019). Camellia sinensis Tea and Cancer Risk: A Systematic Review. *Journal of Ayurvedic and Herbal Medicine*, *5*(3), 110-116.

- Lee, B. M., & Shim, G. A. (2007). Dietary exposure estimation of benzo [a] pyrene and cancer risk assessment. *Journal of Toxicology and Environmental Health, Part A*, *70*(15-16), 1391-1394.
- Lee, J. Y., Hwang, W. I., & Lim, S. T. (2004). Antioxidant and anticancer activities of organic extracts from Platycodon grandiflorum A. De Candolle roots. *Journal of ethnopharmacology*, *93*(2-3), 409-415.
- Lehr, R. E., & Jerina, D. M. (1977). Relationships of quantum mechanical calculations, relative mutagenicity of benzom anthracene diol epoxides, and "bay region" concept of aromatic hydrocarbon carcinogenicity. *Journal of Toxicology and Environmental Health, Part A Current Issues*, *2*(6), 1259-1265.
- Lewis Jr, J. S., Ali, S., Luo, J., Thorstad, W. L., & Madabhushi, A. (2014). A quantitative histomorphometric classifier (QuHbIC) identifies aggressive versus indolent p16-positive oropharyngeal squamous cell carcinoma. *The American journal of surgical pathology*, *38*(1), 128-137.
- Li, S., Li, S. K., Gan, R. Y., Song, F. L., Kuang, L., & Li, H. B. (2013). Antioxidant capacities and total phenolic contents of infusions from 223 medicinal plants. *Industrial Crops and Products*, *51*, 289-298.
- Li, S., Yin, S., Ding, H., Shao, Y., Zhou, S., Pu, W., & Yu, H. (2023). Polyphenols as potential metabolism mechanisms regulators in liver protection and liver cancer prevention. *Cell Proliferation*, *56*(1), e13346.
- Li, W., Liu, J. B., Hou, L. K., Yu, F., Zhang, J., Wu, W., & Fu, D. (2022). Liquid biopsy in lung cancer: significance in diagnostics, prediction, and treatment monitoring. *Molecular Cancer*, *21*(1), 25.
- Li, X., Han, L., Li, Y., Zhang, J., Chen, J., Lu, W., & Wei, G. (2016). Protective effect of sinapine against hydroxyl radical-induced damage to mesenchymal stem cells and possible mechanisms. *Chemical and Pharmaceutical Bulletin*, *64*(4), 319-325.
- Li, Y., & Hecht, S. S. (2022). Carcinogenic components of tobacco and tobacco smoke: A 2022 update. *Food and Chemical Toxicology*, 113179.
- Li, Y., Shibahara, A., Matsuo, Y., Tanaka, T., & Kouno, I. (2010). Reaction of the black tea pigment theaflavin during enzymatic oxidation of tea catechins. *Journal of natural products*, *73*(1), 33-39.
- Liao, J., Yang, G. Y., Park, E. S., Meng, X., Sun, Y., Jia, D., & Yang, C. S. (2004). Inhibition of lung carcinogenesis and effects on angiogenesis and apoptosis in A/J mice by oral administration of green tea. *Nutrition and cancer*, *48*(1), 44-53.

- Lichtenstein, P., Holm, N. V., Verkasalo, P. K., Iliadou, A., Kaprio, J., Koskenvuo, M., & Hemminki, K. (2000). Environmental and heritable factors in the causation of cancer—analyses of cohorts of twins from Sweden, Denmark, and Finland. *New England journal of medicine*, *343*(2), 78-85.
- Lila, M. A., & Raskin, I. (2005). Health-related interactions of phytochemicals. *Journal of Food Science*, *70*(1), 20-27.
- Lin, F., Han, B., Ding, Y., Li, Q., Gao, W., & Zheng, L. (2018). Distribution characteristics, sources, and ecological risk assessment of polycyclic aromatic hydrocarbons in sediments from the Qinhuangdao coastal wetland, China. *Marine Pollution Bulletin*, *127*, 788-793.
- Lin, J., Li, X., Chen, L., Lu, W., Chen, X., Han, L., & Chen, D. (2014). Protective effect against hydroxyl radical-induced DNA damage and antioxidant mechanism of [6]-gingerol: A Chemical Study. *Bulletin of the Korean Chemical Society*, *35*(6), 1633-1638.
- Lin, J., Zhang, P., Pan, Z., Xu, H., Luo, Y., & Wang, X. (2013). Discrimination of oolong tea (Camellia sinensis) varieties based on feature extraction and selection from aromatic profiles analysed by HS-SPME/GC–MS. *Food chemistry*, *141*(1), 259-265.
- Lin, Y. S., Tsai, Y. J., Tsay, J. S., & Lin, J. K. (2003). Factors affecting the levels of tea polyphenols and caffeine in tea leave. *Journal of agricultural and food chemistry*, *51*(7), 1864-1873.
- Liu, G., Zhu, W., Li, S., Zhou, W., Zhang, H., Wang, J., & Xu, X. (2022). Antioxidant capacity and interaction of endogenous phenolic compounds from tea seed oil. *Food Chemistry*, *376*, 131940.
- Liu, K., Gao, X., Kang, B., Liu, Y., Wang, D., & Wang, Y. (2022). The role of tumor stem cell exosomes in cancer invasion and metastasis. *Frontiers in Oncology*, *12*.
- Liu, P., Kong, M., Yuan, S., Liu, J., & Wang, P. (2014). History and experience: a survey of traditional chinese medicine treatment for Alzheimer's disease. *Evidence-Based Complementary and Alternative Medicine*, *2014*.
- Liu, R., Lin, Y., Ruan, T., & Jiang, G. (2017). Occurrence of synthetic phenolic antioxidants and transformation products in urban and rural indoor dust. *Environmental Pollution*, *221*, 227-233.
- Liu, S., & Huang, H. (2015). Assessments of antioxidant effect of black tea extract and its rationals by erythrocyte haemolysis assay, plasma oxidation assay and cellular antioxidant activity (CAA) assay. *Journal of Functional Foods*, *18*, 1095-1105.

- Liu, Y., Chen, Q., Liu, D., Yang, L., Hu, W., Kuang, L., & Liu, Y. (2023). Multi-omics and enzyme activity analysis of flavour substances formation: Major metabolic pathways alteration during Congou black tea processing. *Food Chemistry*, *403*, 134263.
- Liu, Y., Flynn, T. J., Ferguson, M. S., & Hoagland, E. M. (2013). Use of the Combination Index to determine interactions between plant-derived phenolic acids on hepatotoxicity endpoints in human and rat hepatoma cells. *Phytomedicine*, *20*(5), 461-468.
- Liu, Y., Zhang, Q., Xing, B., Luo, N., Gao, R., Yu, K., & Zhang, Z. (2022). Immune phenotypic linkage between colorectal cancer and liver metastasis. *Cancer Cell*, *40*(4), 424-437.
- Loganathan, V., & Mohan, L. (2023). Dietary Antioxidants and Bioactive Compounds in Food Processing. In *Recent Developments in Antioxidants from Natural Sources*. IntechOpen.
- Long, P., Rakariyatham, K., Ho, C. T., & Zhang, L. (2023). Thearubigins: Formation, structure, health benefit and sensory property. *Trends in Food Science & Technology*.
- Lopez, A. D., Collishaw, N. E., & Piha, T. (1994). A descriptive model of the cigarette epidemic in developed countries. *Tobacco control*, *3*(3), 242.
- Loughran, P. A., Roman, L. J., Miller, R. T., & Masters, B. S. S. (2001). The kinetic and spectral characterization of the E. coli-expressed mammalian CYP4A7: cytochrome b5 effects vary with substrate. *Archives of Biochemistry and Biophysics*, *385*(2), 311-321.
- Lu, G., Xiao, H., You, H., Lin, Y., Jin, H., Snagaski, B., & Yang, C. S. (2008). Synergistic inhibition of lung tumorigenesis by a combination of green tea polyphenols and atorvastatin. *Clinical cancer research*, *14*(15), 4981-4988.
- Lu, Y. P., Lou, Y. R., Xie, J. G., Yen, P., Huang, M. T., & Conney, A. H. (1997). Inhibitory effect of black tea on the growth of established skin tumors in mice: effects on tumor size, apoptosis, mitosis and bromodeoxyuridine incorporation into DNA. *Carcinogenesis*, *18*(11), 2163-2169.
- Ma, L., Zhang, M., Zhao, R., Wang, D., Ma, Y., & Ai, L. (2021). Plant natural products: promising resources for cancer chemoprevention. *Molecules*, *26*(4), 933.
- Ma, Q., Wang, C. Z., Sawadogo, W. R., Bian, Z. X., & Yuan, C. S. (2022). Herbal medicines for constipation and phytochemical comparison of active components. *The American Journal of Chinese Medicine*, *50*(03), 723-732.

- Malongane, F., McGAW, L. J., & Mudau, F. N. (2017). The synergistic potential of various teas, herbs and therapeutic drugs in health improvement: a review. *Journal of the Science of Food and Agriculture*, *97*(14), 4679-4689.
- Manjudevi, M., Thirugnanasampandan, R., Vishnupriya, B., & Ramnath, M. G. (2022). In vitro propagation of *Ocimum sanctum* Linn., *Ocimum canum* Sims., and *Ocimum tenuiflorum* Linn., and evaluation of antioxidant, MMP-9 down regulation of eugenol and camphor. *S. Afr. J. Bot.*
- Manna, S., Mukherjee, S., Roy, A., Das, S., & Panda, C. K. (2009). Tea polyphenols can restrict benzo(a)pyrene-induced lung carcinogenesis by altered expression of p53-associated genes and H-ras, c-myc and cyclin D1. *The Journal of Nutritional Biochemistry*, *20*(5), 337-349.
- Mansouri, I., Laaroussi, H., El Ghouizi, A., Lyoussi, B., & El Arabi, I. (2020). Phytochemical content and antioxidant activity of flesh fruits Rosa canina extracts collected from ait ayach midelt. *Indian Journal of Agricultural Research*, *54*(3), 373-377.
- Marcinek, K., & Krejpcio, Z. (2016). *Stevia rebaudiana* Bertoni: health promoting properties and therapeutic applications. *Journal für Verbraucherschutz und Lebensmittelsicherheit*, *11*, 3-8.
- Marnewick, J., Joubert, E., Joseph, S., Swanevelder, S., Swart, P., & Gelderblom, W. (2005). Inhibition of tumour promotion in mouse skin by extracts of rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*), unique South African herbal teas. *Cancer letters*, *224*(2), 193-202.
- Mates, J. M., & Sanchez-Jimenez, F. M. (2000). Role of reactive oxygen species in apoptosis: implications for cancer therapy. *The international journal of biochemistry & cell biology*, *32*(2), 157-170.
- Mathew, S., & Abraham, T. E. (2006). Studies on the antioxidant activities of cinnamon (Cinnamomum verum) bark extracts, through various in vitro models. *Food chemistry*, *94*(4), 520-528.
- McKeage, M. J., Kotasek, D., Markman, B., Hidalgo, M., Millward, M. J., Jameson, M. B., & Hughes, B. G. (2018). Phase IB trial of the anti-cancer stem cell DLL4-binding agent demcizumab with pemetrexed and carboplatin as first-line treatment of metastatic non-squamous NSCLC. *Targeted Oncology*, *13*, 89-98.
- McMacken, M., & Shah, S. (2017). A plant-based diet for the prevention and treatment of type 2 diabetes. *Journal of geriatric cardiology: JGC*, *14*(5), 342.

- Mehta, R. G., Murillo, G., Naithani, R., & Peng, X. (2010). Cancer chemoprevention by natural products: how far have we come?. *Pharmaceutical research*, *27*, 950-961.
- Mensor, L. L., Menezes, F. S., Leitão, G. G., Reis, A. S., Santos, T. C. D., Coube, C. S., & Leitão, S. G. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy research*, *15*(2), 127-130.
- Menter, D. G., & Bresalier, R. S. (2023). An aspirin a day: new pharmacological developments and cancer chemoprevention. *Annual Review of Pharmacology and Toxicology*, *63*.
- Meuwissen, R., & Berns, A. (2005). Mouse models for human lung cancer. *Genes & development*, *19*(6), 643-664.
- Miekus, N., Marszałek, K., Podlacha, M., Iqbal, A., Puchalski, C., & Swiergiel, A. H. (2020). Health benefits of plant-derived sulfur compounds, glucosinolates, and organosulfur compounds. *Molecules*, *25*(17), 3804.
- Milani, G., Curci, F., Cavalluzzi, M. M., Crupi, P., Pisano, I., Lentini, G., & Corbo, F. (2020). Optimization of microwave-assisted extraction of antioxidants from bamboo shoots of *Phyllostachys pubescens*. *Molecules*, *25*(1), 215.
- Milliron, B. J., Packel, L., Dychtwald, D., Klobodu, C., Pontiggia, L., Ogbogu, O., & Deutsch, J. (2022). When eating becomes torturous: understanding nutrition-related cancer treatment side effects among individuals with cancer and their caregivers. *Nutrients*, *14*(2), 356.
- Mimoto, J., Kiura, K., Matsuo, K., Yoshino, T., Takata, I., Ueoka, H., & Harada, M. (2000). (–)-Epigallocatechin gallate can prevent cisplatin-induced lung tumorigenesis in A/J mice. *Carcinogenesis*, *21*(5), 915-919.
- Mohammed, A., Tanko, Y., Okasha, M. A., Magaji, R. A., & Yaro, A. H. (2007). Effects of aqueous leaves extract of *Ocimum gratissimum* on blood glucose levels of streptozocin induced diabetic wistar rats. *African Journal of Biotechnology*, *6*(18).
- Mohammed, H. H., & Abdullah, F. O. (2022). Microwave-assisted extraction and phytochemical profile of Nonea pulmonarioides and its antifungal, antibacterial, and antioxidant activities. *Journal of Food Quality*, *2022*.
- Mohan, K. C., Hara, Y., Abraham, S. K., & Nagini, S. (2005). Comparative evaluation of the chemopreventive efficacy of green and black tea polyphenols in the hamster buccal pouch carcinogenesis model. *Clinical biochemistry*, *38*(10), 879-886.

- Mohan, V., & Koul, A. (2018). Anticancer potential of *Tinospora cordifolia* and arabinogalactan against benzo (a) pyrene induced pulmonary tumorigenesis: a study in relevance to various biomarkers. *Journal of Herbmed Pharmacology*, *7*(4), 225-235.
- Molina, J. R., Yang, P., Cassivi, S. D., Schild, S. E., & Adjei, A. A. (2008, May). Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. In *Mayo clinic proceedings* (Vol. 83, No. 5, pp. 584-594). Elsevier.
- Mondal, S., Mirdha, B. R., & Mahapatra, S. C. (2009). The science behind sacredness of Tulsi (*Ocimum sanctum* Linn.). *Indian Journal of Physiology and Pharmacology*, *53*(4), 291-306.
- Monga, S., Dhanwal, P., Kumar, R., Kumar, A., & Chhokar, V. (2017). Pharmacological and physico-chemical properties of Tulsi (Ocimum gratissimum L.): An updated review. *Pharma. Innovation*, *6*, 181-186.
- Moraes, M. C., Neto, J. B., & Menck, C. F. (2012). DNA repair mechanisms protect our genome from carcinogenesis. *Frontier in Bioscience*, *17*, 1362-1388.
- Motofei, I. G. (2022, November). Biology of cancer; from cellular and molecular mechanisms to developmental processes and adaptation. In *Seminars in Cancer Biology* (Vol. 86, pp. 600-615). Academic Press.
- Muhammad, A. R., Ahmad, M. T., Zakaria, R., Rahim, H. R. A., Yusoff, S. F. A. Z., Hamdan, K. S., & Harun, S. W. (2017). Q-switching pulse operation in 1.5-μm region using copper nanoparticles as saturable absorber. *Chinese Physics Letters*, *34*(3), 034205.
- Muhammad, D. R. A., Praseptiangga, D., Van de Walle, D., & Dewettinck, K. (2017). Interaction between natural antioxidants derived from cinnamon and cocoa in binary and complex mixtures. *Food Chemistry*, *231*, 356-364.
- Murugan, R. S., Mohan, K. V. P. C., Uchida, K., Hara, Y., Prathiba, D., & Nagini, S. (2007). Modulatory effects of black tea polyphenols on oxidant–antioxidant profile and expression of proliferation, apoptosis, and angiogenesis-associated proteins in the rat forestomach carcinogenesis model. *Journal of gastroenterology*, *42*, 352-361.
- Muthuiah, M. J., Thomas, J., Kumar, R. R., & Azad Mandal, A. K. (2009). Studies on radical scavenging activity of tea leaves and effect of additives on activities of black tea liquor. *International journal of food science & technology*, *44*(10), 2070-2074.
- Muto, S., Yokoi, T., Gondo, Y., Katsuki, M., Shioyama, Y., Fujita, K. I., & Kamataki, T. (1999). Inhibition of benzo [a] pyrene-induced mutagenesis by (–)-epigallocatechin gallate in the lung of rpsL transgenic mice. *Carcinogenesis*, *20*(3), 421-424.

- Nabavi, S. M., Ebrahimzadeh, M. A., Nabavi, S. F., Hamidinia, A., & Bekhradnia, A. R. (2008). Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica* Mey. *Pharmacologyonline*, *2*(9), 560-567.
- Nascimento, J. C., Barbosa, L. C., Paula, V. F., David, J. M., Fontana, R., Silva, L. A., & Franca, R. S. (2011). Chemical composition and antimicrobial activity of essential oils of *Ocimum canum* Sims. and *Ocimum selloi* Benth. *Anais da Academia Brasileira de Ciências*, *83*, 787-800.
- Nath, A., Sathishkumar, K., Das, P., Sudarshan, K. L., & Mathur, P. (2022). A clinicoepidemiological profile of lung cancers in India–Results from the National Cancer Registry Programme. *Indian Journal of Medical Research*, *155*(2), 264-272.
- Neunert, G., Górnaś, P., Dwiecki, K., Siger, A., & Polewski, K. (2015). Synergistic and antagonistic effects between alpha-tocopherol and phenolic acids in liposome system: spectroscopic study. *European Food Research and Technology*, *241*, 749-757.
- Nganteng, D. N. D., Melong, R., Mbiekop, E. P., Maffo, T., Allémann, É., Delie, F., & Dzoyem, J. P. (2022). Chemical constituents and cytotoxic activity of *Ocimum gratissimum* L. *South African Journal of Botany*, *150*, 330-333.
- Ngassoum, M. B., Ousmaila, H., Ngamo, L. T., Maponmetsem, P. M., Jirovetz, L., & Buchbauer, G. (2004). Aroma compounds of essential oils of two varieties of the spice plant *Ocimum canum* Sims from northern Cameroon. *Journal of Food Composition and Analysis*, *17*(2), 197-204.
- Nibir, Y. M., Sumit, A. F., Akhand, A. A., Ahsan, N., & Hossain, M. S. (2017). Comparative assessment of total polyphenols, antioxidant and antimicrobial activity of different tea varieties of Bangladesh. *Asian Pacific Journal of Tropical Biomedicine*, *7*(4), 352-357.
- Nicholson, S. A., Beasley, M. B., Brambilla, E., Hasleton, P. S., Colby, T. V., Sheppard, M. N., & Travis, W. D. (2002). Small cell lung carcinoma (SCLC): a clinicopathologic study of 100 cases with surgical specimens. *The American journal of surgical pathology*, *26*(9), 1184-1197.
- Nicol, S., Dugay, J., & Hennion, M. C. (2001). Simultaneous determination of polycyclic aromatic hydrocarbons and their nitrated derivatives in airborne particulate matter using gas chromatography-tandem mass spectrometry. *Journal of separation science*, *24*(6), 451-458.
- Nie, X. C., Dong, D. S., Bai, Y., & Xia, P. (2014). Meta-analysis of black tea consumption and breast cancer risk: update 2013. *Nutrition and cancer*, *66*(6), 1009-1014.

- Nikmaram, N., Leong, S. Y., Koubaa, M., Zhu, Z., Barba, F. J., Greiner, R., & Roohinejad, S. (2017). Effect of extrusion on the anti-nutritional factors of food products: An overview. *Food control*, *79*, 62-73.
- Nimse, S. B., & Pal, D. (2015). Free radicals, natural antioxidants, and their reaction mechanisms. *RSC advances*, *5*(35), 27986-28006.
- Nonaka, G. I., Sakai, R., & Nishioka, I. (1984). Hydrolysable tannins and proanthocyanidins from green tea. *Phytochemistry*, *23*(8), 1753-1755.
- Nowak, J. Z. (2013). Oxidative stress, polyunsaturated fatty acids-derived oxidation products and bisretinoids as potential inducers of CNS diseases: focus on age-related macular degeneration. *Pharmacological reports*, *65*(2), 288-304.
- Nurgali, K., Jagoe, R. T., & Abalo, R. (2018). Adverse effects of cancer chemotherapy: Anything new to improve tolerance and reduce sequelae?. *Frontiers in pharmacology*, *9*, 245.
- Obanda, M., Owuor, P. O., & Bore, J. K. (1997). Effects of moisture loss and temperature of leaf during withering on black tea quality parameters. *Tea-Tea Board of Kenya (Kenya)*.
- Oh, J., Jo, H., Cho, A. R., Kim, S. J., & Han, J. (2013). Antioxidant and antimicrobial activities of various leafy herbal teas. *Food control*, *31*(2), 403-409.
- Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*, *95*(2), 351-358.
- Okeke, L. A., Anie, C. O., Nwobodo, D. C., & Okolo, V. K. (2023). Phytochemical analysis and antibacterial activities of various leaf extracts of *Ocimum gratissimum* and *Newbouldia laevis*. *GSC Biological and Pharmaceutical Sciences*, *22*(3), 144-152.
- Okoko, T., & Ere, D. (2012). Reduction of hydrogen peroxide–induced erythrocyte damage by Carica papaya leaf extract. *Asian Pacific journal of tropical biomedicine*, *2*(6), 449-453.
- Ola, P. (2022). The Nature of Malignant Cancer and Its Hitherto Elusive Cure.
- Ortiz-Viedma, J., Romero, N., Puente, L., Burgos, K., Toro, M., Ramirez, L., & Aubourg, S. P. (2017). Antioxidant and antimicrobial effects of stevia (*Stevia rebaudiana* Bert.) extracts during preservation of refrigerated salmon paste. *European Journal of Lipid Science and Technology*, *119*(10), 1600467.
- Ouellette, M. M., Zhou, S., & Yan, Y. (2022). Cell signaling pathways that promote radioresistance of cancer cells. *Diagnostics*, *12*(3), 656.

- Palii, S. S., Van Emburgh, B. O., Sankpal, U. T., Brown, K. D., & Robertson, K. D. (2008). DNA methylation inhibitor 5-Aza-2′-deoxycytidine induces reversible genome-wide DNA damage that is distinctly influenced by DNA methyltransferases 1 and 3B. *Molecular and cellular biology*, *28*(2), 752-771.
- Pan, M. H., & Ho, C. T. (2008). Chemopreventive effects of natural dietary compounds on cancer development. *Chemical Society Reviews*, *37*(11), 2558-2574.
- Pan, M. H., Ghai, G., & Ho, C. T. (2008). Food bioactives, apoptosis, and cancer. *Molecular nutrition & food research*, *52*(1), 43-52.
- Pan, M. H., Kang, H. J., Chen, W. J., Lo, C. Y., Li, S., Sang, S., & Ho, C. T. (2008). Induction of apoptosis by acetylated black tea polyphenol through reactive oxygen species production, cytochrome c release, and caspases activation in human leukemia HL-60 cells.
- Pan, M. H., Lai, C. S., Wang, H., Lo, C. Y., Ho, C. T., & Li, S. (2013). Black tea in chemo-prevention of cancer and other human diseases. *Food Science and Human Wellness*, *2*(1), 12-21.
- Pan, S. Y., Nie, Q., Tai, H. C., Song, X. L., Tong, Y. F., Zhang, L. J. F., & Liang, C. (2022). Tea and tea drinking: China's outstanding contributions to the mankind. *Chinese Medicine*, *17*(1), 1-40.
- Pan, Y., Li, H., Shahidi, F., Luo, T., & Deng, Z. (2022). Interactions among dietary phytochemicals and nutrients: Role of cell membranes. *Trends in Food Science & Technology*.
- Pandey, A. K., Singh, P., & Tripathi, N. N. (2014). Chemistry and bioactivities of essential oils of some *Ocimum* species: an overview. *Asian Pacific Journal of Tropical Biomedicine*, *4*(9), 682-694.
- Pandey, P., Garg, A., Singh, V., & Shukla, A. (2016). Evaluation of anthelmintic and antimicrobial activity of Ursolic acid obtained from Tulsi *Ocimum sanctum*. *Asian Journal of Pharmacy and Pharmacology*, *2*(3), 67-71.
- Parasuraman, S., Thing, G. S., & Dhanaraj, S. A. (2014). Polyherbal formulation: Concept of ayurveda: Subramani Parasuraman, http://www. phcogrev. com/printarticle. asp? issn= 0973-7847; year= 2014; v...
- Parcheta, M., Swisłocka, R., Orzechowska, S., Akimowicz, M., Choinska, R., & Lewandowski, W. (2021). Recent developments in effective antioxidants: The structure and antioxidant properties. *Materials*, *14*(8), 1984.
- Pasquet, R. (2013). Consumption of black tea and coffee and the risk of lung cancer.

- Patil, P. S., & Mahajan, H. S. (2022). Lung Cancer: How Well We Have Fared?. *Indo Global Journal of Pharmaceutical Sciences*, *12*, 82-91.
- Pattanayak, P., Behera, P., Das, D., & Panda, S. K. (2010). Ocimum sanctum Linn. A reservoir plant for therapeutic applications: An overview. *Pharmacognosy reviews*, *4*(7), 95.
- Pekal, A., Drozdz, P., Biesaga, M., & Pyrzynska, K. (2011). Evaluation of the antioxidant properties of fruit and flavoured black teas. *European journal of nutrition*, *50*, 681-688.
- Penning, T. M., Burczynski, M. E., Hung, C. F., McCoull, K. D., Palackal, N. T., & Tsuruda, L. S. (1999). Dihydrodiol dehydrogenases and polycyclic aromatic hydrocarbon activation: generation of reactive and redox active o-quinones. *Chemical research in toxicology*, *12*(1), 1-18.
- Pereira, V. P., Knor, F. J., Vellosa, J. C. R., & Beltrame, F. L. (2014). Determination of phenolic compounds and antioxidant activity of green, black and white teas of *Camellia sinensis* (L.) Kuntze, Theaceae. *Revista Brasileira de Plantas Medicinais*, *16*, 490-498.
- Peteliuk, V., Rybchuk, L., Bayliak, M., Storey, K. B., & Lushchak, O. (2021). Natural sweetener *Stevia rebaudiana*: Functionalities, health benefits and potential risks. *EXCLI journal*, *20*, 1412.
- Peterson, J., Dwyer, J., Bhagwat, S., Haytowitz, D., Holden, J., Eldridge, A. L., & Aladesanmi, J. (2005). Major flavonoids in dry tea. *Journal of Food Composition and Analysis*, *18*(6), 487-501.
- Peto, R., Darby, S., Deo, H., Silcocks, P., Whitley, E., & Doll, R. (2000). Smoking, smoking cessation, and lung cancer in the UK since 1950: combination of national statistics with two case-control studies. *British medical journal*, *321*(7257), 323-329.
- Pfeifer, G. P., Denissenko, M. F., Olivier, M., Tretyakova, N., Hecht, S. S., & Hainaut, P. (2002). Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. *Oncogene*, *21*(48), 7435-7451.
- Phan, M. A. T., Paterson, J., Bucknall, M., & Arcot, J. (2018). Interactions between phytochemicals from fruits and vegetables: Effects on bioactivities and bioavailability. *Critical reviews in food science and nutrition*, *58*(8), 1310-1329.
- Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian journal of clinical biochemistry*, *30*, 11-26.

- Pincemaille, J., Schummer, C., Heinen, E., & Moris, G. (2014). Determination of polycyclic aromatic hydrocarbons in smoked and non-smoked black teas and tea infusions. *Food chemistry*, *145*, 807-813.
- Pinto, J. A., Vallejos, C. S., Raez, L. E., Mas, L. A., Ruiz, R., Torres-Roman, J. S., & Rolfo, C. (2018). Gender and outcomes in non-small cell lung cancer: an old prognostic variable comes back for targeted therapy and immunotherapy? *ESMO open*, *3*(3), e000344.
- Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European journal of medicinal chemistry*, *97*, 55-74.
- Poljsak, B., Šuput, D., & Milisav, I. (2013). Achieving the balance between ROS and antioxidants: when to use the synthetic antioxidants. *Oxidative medicine and cellular longevity*, *2013*.
- Poon, S. L., McPherson, J. R., Tan, P., Teh, B. T., & Rozen, S. G. (2014). Mutation signatures of carcinogen exposure: genome-wide detection and new opportunities for cancer prevention. *Genome medicine*, *6*, 1-14.
- Prabhu, K. S., Lobo, R., Shirwaikar, A. A., & Shirwaikar, A. (2009). Ocimum gratissimum: A review of its chemical, pharmacological and ethnomedicinal properties. *The open Complementary medicine journal*, *1*(1).
- Prasad, J. B., & Dhar, M. (2018). Projections of burden of cancers: A new approach for measuring incidence cases for India and its States–till 2025. *Journal of cancer policy*, *16*, 57-62.
- Prasanth, M. I., Sivamaruthi, B. S., Chaiyasut, C., & Tencomnao, T. (2019). A review of the role of green tea (Camellia sinensis) in antiphotoaging, stress resistance, neuroprotection, and autophagy. *Nutrients*, *11*(2), 474.
- Probst, Y. C., Guan, V. X., & Kent, K. (2017). Dietary phytochemical intake from foods and health outcomes: A systematic review protocol and preliminary scoping. *BMJ open*, *7*(2), e013337.
- Qasim, M., Abideen, Z., Adnan, M. Y., Gulzar, S., Gul, B., Rasheed, M., & Khan, M. A. (2017). Antioxidant properties, phenolic composition, bioactive compounds and nutritive value of medicinal halophytes commonly used as herbal teas. *South African Journal of Botany*, *110*, 240-250.
- Qi, D., Miao, A., Chen, W., Wang, W., He, X., & Ma, C. (2021). Characterization of the volatile compounds profile of the innovative broken oolong-black tea in comparison with broken oolong and broken black tea. *Food Control*, *129*, 108197.

- Rababah, T. M., Ereifej, K. I., Esoh, R. B., Al-u'datt, M. H., Alrababah, M. A., & Yang, W. (2011). Antioxidant activities, total phenolics and HPLC analyses of the phenolic compounds of extracts from common Mediterranean plants. *Natural product research*, *25*(6), 596-605.
- Rana, S., & Suttee, A. (2012). Phytochemical investigation and evaluation of free radical scavenging potential of *Benincasa hispida* peel extracts. *International Journal of Current Pharmaceutical Review and Research*, *3*(3), 43-46.
- Rahim, N. F. A., Muhammad, N., Putra, T. N. M. T., Marzuki, W. N. A. S. W., Abdullah, N., Talip, B. A., & Gul, M. T. (2019). Antioxidant Activity and its Interaction Effect on Polyherbal Formulations of *Nephrodium inophyllum, Polygonum minus Annona squamosal* L. and *Stevia rebaudiana*. *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences*, *61*(1), 1-9.
- Rahman, M. M., Behl, T., Islam, M. R., Alam, M. N., Islam, M. M., Albarrati, A., & Bungau, S. G. (2022). Emerging management approach for the adverse events of immunotherapy of cancer. *Molecules*, *27*(12), 3798.
- Rahman, M. M., Islam, M. R., Shohag, S., Hossain, M. E., Rahaman, M. S., Islam, F., & Cavalu, S. (2022). The multifunctional role of herbal products in the management of diabetes and obesity: a comprehensive review. *Molecules*, *27*(5), 1713.
- Rajendran, P., Ekambaram, G., & Sakthisekaran, D. (2008). Protective role of mangiferin against Benzo (a) pyrene induced lung carcinogenesis in experimental animals. *Biological and Pharmaceutical Bulletin*, *31*(6), 1053-1058.
- Rana, A., Rana, S., & Kumar, S. (2022). Green and Sustainable Processing of Tea Constituents and Applications. In *Tea as a Food Ingredient* (pp. 193-206). CRC Press.
- Rana, A., Samtiya, M., Dhewa, T., Mishra, V., & Aluko, R. E. (2022). Health benefits of polyphenols: A concise review. *Journal of Food Biochemistry*, *46*(10), e14264.
- Ranjbar Nedamani, E., Sadeghi Mahoonak, A., Ghorbani, M., & Kashaninejad, M. (2015). Evaluation of antioxidant interactions in combined extracts of green tea (*Camellia sinensis*), rosemary (*Rosmarinus officinalis*) and oak fruit (*Quercus branti*). *Journal of food science and technology*, *52*, 4565-4571.
- Rasheed, Z. (2019). Molecular evidences of health benefits of drinking black tea. *International journal of health sciences*, *13*(3), 1.
- Ravichandran, N., Suresh, G., Ramesh, B., & Siva, G. V. (2011). Fisetin, a novel flavonol attenuates benzo (a) pyrene-induced lung carcinogenesis in Swiss albino mice. *Food and Chemical Toxicology*, *49*(5), 1141-1147.

- Ravikumar, C. (2014). Review on herbal teas. *Journal of Pharmaceutical Sciences and Research*, *6*(5), 236.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, *26*(9-10), 1231-1237.
- Reddy, B. S., Hirose, Y., Lubet, R., Steele, V., Kelloff, G., Paulson, S., & Rao, C. V. (2000). Chemoprevention of colon cancer by specific cyclooxygenase-2 inhibitor, celecoxib, administered during different stages of carcinogenesis. *Cancer research*, *60*(2), 293-297.
- Reddy, M. N., Adnan, M., Alreshidi, M. M., Saeed, M., & Patel, M. (2020). Evaluation of anticancer, antibacterial and antioxidant properties of a medicinally treasured fern tectaria coadunata with its phytoconstituents analysis by HR-LCMS. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, *20*(15), 1845-1856.
- Reinhardt, H. C., Aslanian, A. S., Lees, J. A., & Yaffe, M. B. (2007). p53-deficient cells rely on ATM-and ATR-mediated checkpoint signaling through the p38MAPK/MK2 pathway for survival after DNA damage. *Cancer cell*, *11*(2), 175-189.
- Relli, V., Trerotola, M., Guerra, E., & Alberti, S. (2019). Abandoning the notion of non-small cell lung cancer. *Trends in Molecular Medicine*, *25*(7), 585-594.
- Rietjens, I. M., Michael, A., Bolt, H. M., Siméon, B., Andrea, H., Nils, H., & Gerhard, E. (2022). The role of endogenous versus exogenous sources in the exposome of putative genotoxins and consequences for risk assessment. *Archives of toxicology*, *96*(5), 1297-1352.
- Rizeq, B., Gupta, I., Ilesanmi, J., AlSafran, M., Rahman, M. D., & Ouhtit, A. (2020). The power of phytochemicals combination in cancer chemoprevention. *Journal of Cancer*, *11*(15), 4521-4533.
- Romero-Díez, R., Rodríguez-Rojo, S., Cocero, M. J., Duarte, C. M., Matias, A. A., & Bronze, M. R. (2018). Phenolic characterization of aging wine lees: Correlation with antioxidant activities. *Food chemistry*, *259*, 188-195.
- Roshanak, S., Rahimmalek, M., & Goli, S. A. H. (2016). Evaluation of seven different drying treatments in respect to total flavonoid, phenolic, vitamin C content, chlorophyll, antioxidant activity and color of green tea (*Camellia sinensis* or *C. assamica*) leaves. *Journal of food science and technology*, *53*, 721-729.

- Roy, A., Jauhari, N., & Bharadvaja, N. (2018). Medicinal plants as a potential source of chemopreventive agents. *Anticancer Plants: Natural Products and Biotechnological Implements: Volume 2*, 109-139.
- Royani, A., Hanafi, M., Julistiono, H., & Manaf, A. (2023). The total phenolic and flavonoid contents of Aloe vera and Morinda citrifolia extracts as antibacterial material against Pseudomonas aeruginosa. *Materials Today: Proceedings*, *72*, 2796-2802.
- Rudrapal, M., Khairnar, S. J., Khan, J., Dukhyil, A. B., Ansari, M. A., Alomary, M. N., & Devi, R. (2022). Dietary polyphenols and their role in oxidative stress-induced human diseases: insights into protective effects, antioxidant potentials and mechanism (s) of action. *Frontiers in pharmacology*, *13*, 283.
- Ruxton, C. H. S. (2008). Black tea and health. *Nutrition Bulletin*, *33*(2), 91-101.
- Rybarczyk-Kasiuchnicz, A., Ramlau, R., & Stencel, K. (2021). Treatment of brain metastases of non-small cell lung carcinoma. *International Journal of Molecular Sciences*, *22*(2), 593.
- Saberi, H. (2010). *Tea: a global history*. Reaktion Books.
- Sakamoto, K. (2000). Synergistic effects of thearubigin and genistein on human prostate tumor cell (PC-3) growth via cell cycle arrest. *Cancer letters*, *151*(1), 103-109.
- Saltos, A., Shafique, M., & Chiappori, A. (2020). Update on the biology, management, and treatment of small cell lung cancer (SCLC). *Frontiers in oncology*, *10*, 1074.
- Samanta, S. (2022). Potential bioactive components and health promotional benefits of tea (Camellia sinensis). *Journal of the American Nutrition Association*, *41*(1), 65-93.
- Samarina, L. S., Matskiv, A. O., Shkhalakhova, R. M., Koninskaya, N. G., Hanke, M. V., Flachowsky, H., & Reim, S. (2022). Genetic diversity and genome size variability in the Russian genebank collection of tea plant [Camellia sinensis (L). O. Kuntze]. *Frontiers in Plant Science*, *12*, 800141.
- Sanchez, M., Gonzalez-Burgos, E., Iglesias, I., Lozano, R., & Gomez-Serranillos, M. P. (2020). The pharmacological activity of *Camellia sinensis* (L.) Kuntze on metabolic and endocrine disorders: a systematic review. *Biomolecules*, *10*(4), 603.
- Sarkar, J., Nandy, S. K., Chowdhury, A., Chakraborti, T., & Chakraborti, S. (2016). Inhibition of MMP-9 by green tea catechins and prediction of their interaction by molecular docking analysis. *Biomedicine & pharmacotherapy*, *84*, 340-347.

- Sasco, A. J., Secretan, M. B., & Straif, K. (2004). Tobacco smoking and cancer: a brief review of recent epidemiological evidence. *Lung cancer*, *45*, 3-9.
- Satoh, E., Tohyama, N., & Nishimura, M. (2005). Comparison of the antioxidant activity of roasted tea with green, oolong, and black teas. *International journal of food sciences and nutrition*, *56*(8), 551-559.
- Savita, S. M., Sheela, K., Sunanda, S., Shankar, A. G., & Ramakrishna, P. (2004). *Stevia rebaudiana*–A functional component for food industry. *Journal of Human Ecology*, *15*(4), 261-264.
- Schieber, M., & Chandel, N. S. (2014). ROS function in redox signaling and oxidative stress. *Current biology*, *24*(10), 453-462.
- Schuck, A. G., Ausubel, M. B., Zuckerbraun, H. L., & Babich, H. (2008). Theaflavin-3, 3′-digallate, a component of black tea: An inducer of oxidative stress and apoptosis. *Toxicology in vitro*, *22*(3), 598-609.
- Schuller, H. M., Porter, B., Riechert, A., Walker, K., & Schmoyer, R. (2004). Neuroendocrine lung carcinogenesis in hamsters is inhibited by green tea or theophylline while the development of adenocarcinomas is promoted: implications for chemoprevention in smokers. *Lung Cancer*, *45*(1), 11-18.
- Sehgal, A. (2022). An update on disease preventing potential of green tea in comparison with some tisanes. *South African Journal of Botany*, *144*, 92-96.
- Selvi, M. T., Thirugnanasampandan, R., & Sundarammal, S. (2015). Antioxidant and cytotoxic activities of essential oil of *Ocimum canum* Sims. from India. *Journal of Saudi Chemical Society*, *19*(1), 97-100.
- Shannon, E., Jaiswal, A. K., & Abu-Ghannam, N. (2017). Polyphenolic content and antioxidant capacity of white, green, black, and herbal teas: a kinetic study.
- Shareena, G., & Kumar, D. (2022). Traversing through half a century research timeline on Ginkgo biloba, in transforming a botanical rarity into an active functional food ingredient. *Biomedicine & Pharmacotherapy*, *153*, 113299.
- Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., & Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. *Frontiers in physiology*, *11*, 694.
- Sharma, A., & Dutta, P. P. (2018). Scientific and technological aspects of tea drying and withering: A review. *Agricultural Engineering International: CIGR Journal*, *20*(4), 210-220.
- Sharma, C., Sadek, B., Goyal, S. N., Sinha, S., Kamal, M. A., & Ojha, S. (2015). Small molecules from nature targeting G-protein coupled cannabinoid receptors:

potential leads for drug discovery and development. *Evidence-Based Complementary and Alternative Medicine*, *2015*.

- Sharma, N., Kumari, R. M., Gupta, N., & Nimesh, S. (2019). Plant based bioactive compounds as an alternative for cancer therapy. *Cancer Ther.*, *3*, 215.
- Sher, T., Dy, G. K., & Adjei, A. A. (2008, March). Small cell lung cancer. In *Mayo Clinic Proceedings*, *83*(3), 355-367. Elsevier.
- Shi, Q., Fijten, R. R., Spina, D., Vasquez, Y. R., Arlt, V. M., Godschalk, R. W., & Van Schooten, F. J. (2017). Altered gene expression profiles in the lungs of benzo [a] pyrene-exposed mice in the presence of lipopolysaccharide-induced pulmonary inflammation. *Toxicology and applied pharmacology*, *336*, 8-19.
- Shirwaikar, A., Ram, H. N., & Mohapatra, P. (2006). Antioxidant and antiulcer activity of aqueous extract of a polyherbal formulation.
- Shukla, S., Mehta, A., Bajpai, V. K., & Shukla, S. (2009). In vitro antioxidant activity and total phenolic content of ethanolic leaf extract of Stevia rebaudiana Bert. *Food and Chemical Toxicology*, *47*(9), 2338-2343.
- Shukla, Y., & Pal, S. K. (2004). Dietary cancer chemoprevention: An overview. *International Journal of Human Genetics*, *4*(4), 265-276.
- Shukla, Y., & Taneja, P. (2002). Anticarcinogenic effect of black tea on pulmonary tumors in Swiss albino mice. *Cancer letters*, *176*(2), 137-141.
- Siddique, S., & Chow, J. C. (2022). Recent advances in functionalized nanoparticles in cancer theranostics. *Nanomaterials*, *12*(16), 2826.
- Sierra-Vargas, M. P., Montero-Vargas, J. M., Debray-García, Y., Vizuet-de-Rueda, J. C., Loaeza-Román, A., & Terán, L. M. (2023). Oxidative Stress and Air Pollution: Its Impact on Chronic Respiratory Diseases. *International Journal of Molecular Sciences*, *24*(1), 853.
- Sil, H., Sen, T., Moulik, S., & Chatterjee, A. (2010). Black tea polyphenol (theaflavin) downregulates MMP-2 in human melanoma cell line A375 by involving multiple regulatory molecules. *Journal of Environmental Pathology, Toxicology and Oncology*, *29*(1).
- Simon, M., Mal, F., Perniceni, T., Ferraz, J. M., Strauss, C., Levard, H., & Gayet, B. (2016). Accuracy of staging laparoscopy in detecting peritoneal dissemination in patients with gastroesophageal adenocarcinoma. *Diseases of the Esophagus*, *29*(3), 236-240.
- Singh, A. K., Kashyap, M. P., Tripathi, V. K., Singh, S., Garg, G., & Rizvi, S. I. (2017). Neuroprotection through rapamycin-induced activation of autophagy and

PI3K/Akt1/mTOR/CREB signaling against amyloid-β-induced oxidative stress, synaptic/neurotransmission dysfunction, and neurodegeneration in adult rats. *Molecular neurobiology*, *54*, 5815-5828.

- Singh, B. N., Prateeksha, Rawat, A. K. S., Bhagat, R. M., & Singh, B. R. (2017). Black tea: Phytochemicals, cancer chemoprevention, and clinical studies. *Critical reviews in food science and nutrition*, *57*(7), 1394-1410.
- Singh, B. P., Zughaibi, T. A., Alharthy, S. A., Al-Asmari, A. I., & Rahman, S. (2022). Statistical analysis, source apportionment, and toxicity of particulate-and gaseous-phase PAHs in the urban atmosphere. *Frontiers in Public Health*, *10*.
- Singh, G., Thaker, R., Sharma, A., & Parmar, D. (2021). Therapeutic effects of biochanin A, phloretin, and epigallocatechin-3-gallate in reducing oxidative stress in arsenic-intoxicated mice. *Environmental Science and Pollution Research*, *28*, 20517-20536.
- Singh, M., Saharan, V., Dayma, J., Rajpurohit, D., Sen, Y., & Sharma, A. (2017). In vitro propagation of *Stevia rebaudiana* (Bertoni): An overview. *International Journal of Current Microbiology and Applied Sciences*, *6*(7), 1010-1022.
- Singh, S., Taneja, M., & Majumdar, D. K. (2007). Biological activities of *Ocimum sanctum* L. fixed oil— An overview.
- Singh, V., Verma, D. K., & Mahato, D. K. (2017). Biochemical composition, processing technology and health benefits of green tea: A review. *Engineering Interventions in Foods and Plants*, 119-155.
- Sinha, D., Roy, S., & Roy, M. (2010). Antioxidant potential of tea reduces arsenite induced oxidative stress in Swiss albino mice. *Food and chemical toxicology*, *48*(4), 1032-1039.
- Sinija, V. R., & Mishra, H. N. (2008). Green tea: Health benefits. *Journal of Nutritional & Environmental Medicine*, *17*(4), 232-242.
- Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., & Boyd, M. R. (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. *JNCI: Journal of the National Cancer Institute*, *82*(13), 1107-1112.
- Skroza, D., Šimat, V., Vrdoljak, L., Jolić, N., Skelin, A., Čagalj, M., & Generalić Mekinić, I. (2022). Investigation of Antioxidant Synergisms and Antagonisms among Phenolic Acids in the Model Matrices Using FRAP and ORAC Methods. *Antioxidants*, *11*(9), 1784.
- Smith, C. J., & Hansch, C. (2000). The relative toxicity of compounds in mainstream cigarette smoke condensate. *Food and Chemical Toxicology*, *38*(7), 637-646.

- Smith, C. J., Livingston, S. D., & Doolittle, D. J. (1997). An international literature survey of "IARC Group I carcinogens" reported in mainstream cigarette smoke. *Food and Chemical Toxicology*, *35*(10-11), 1107-1130.
- Smith, K. (2016). *World Atlas of Tea: From the leaf to the cup, the world's teas explored and enjoyed*. Hachette UK.
- Smith, P., Lavery, A., & Turkington, R. C. (2020). An overview of acute gastrointestinal side effects of systemic anti-cancer therapy and their management. *Best Practice & Research Clinical Gastroenterology*, *48*, 101691.
- Smolarz, B., Nowak, A. Z., & Romanowicz, H. (2022). Breast Cancer—Epidemiology, Classification, Pathogenesis and Treatment (Review of Literature). *Cancers*, *14*(10), 2569.
- Sonam, K. S., & Guleria, S. (2017). Synergistic antioxidant activity of natural products. *Ann. Pharmacol. Pharm*, *2*(8), 1-6.
- Soni, R. P., Katoch, M., Kumar, A., Ladohiya, R., & Verma, P. (2015). Tea: Production, composition, consumption and its potential as an antioxidant and antimicrobial agent. *International Journal of Food and Fermentation Technology*, *5*(2), 95-106.
- Souri, E., Amin, G., Farsam, H., Jalalizadeh, H., & Barezi, S. (2022). Screening of thirteen medicinal plant extracts for antioxidant activity. *Iranian Journal of Pharmaceutical Research*, *7*(2), 149-154.
- Sporn, M. B. (1980). Combination chemoprevention of cancer. *Nature*, *287*, 107-108.
- Steele, V. E., & Lubet, R. A. (2010, August). The use of animal models for cancer chemoprevention drug development. In *Seminars in oncology* (Vol. 37, No. 4, pp. 327-338). WB Saunders.
- Steele, V. E., Bagheri, D., Balentine, D. A., Boone, C. W., Mehta, R., Morse, M. A., & Kelloff, G. J. (1999). Preclinical efficacy studies of green and black tea extracts. *Proceedings of the Society for Experimental Biology and Medicine*, *220*(4), 210-212.
- Steele, V. E., Kelloff, G. J., Balentine, D., Boone, C. W., Mehta, R., Bagheri, D., & Sharma, S. (2000). Comparative chemopreventive mechanisms of green tea, black tea and selected polyphenol extracts measured by in vitro bioassays. *Carcinogenesis*, *21*(1), 63-67.
- Stepanov, I., Villalta, P. W., Knezevich, A., Jensen, J., Hatsukami, D., & Hecht, S. S. (2010). Analysis of 23 polycyclic aromatic hydrocarbons in smokeless tobacco by gas chromatography− mass spectrometry. *Chemical research in toxicology*, *23*(1), 66-73.

- Stockert, A. L., & Hill, M. (2018). Anticancer potential of dietary polyphenols. *Bioactive components, diet and medical treatment in cancer prevention*, 25-50.
- Stojic, A., Jovanovic, G., Stanisic, S., Romanic, S. H., Sostaric, A., Udovicic, V., & Milicevic, T. (2022). The PM2. 5-bound polycyclic aromatic hydrocarbon behavior in indoor and outdoor environments, part II: Explainable prediction of benzo [a] pyrene levels. *Chemosphere*, *289*, 133154.
- Subbaramaiah, K., & Dannenberg, A. J. (2003). Cyclooxygenase 2: a molecular target for cancer prevention and treatment. *Trends in pharmacological sciences*, *24*(2), 96-102.
- Sukhmani, G., Yogesh, G., Shalini, A., Vikas, K., Anil, P., & Ashwani, K. (2018). Natural sweeteners: health benefits of stevia. *Foods and raw materials*, *6*(2), 392-402.
- Sun, H., Chen, Y., Cheng, M., Zhang, X., Zheng, X., & Zhang, Z. (2018). The modulatory effect of polyphenols from green tea, oolong tea and black tea on human intestinal microbiota in vitro. *Journal of Food Science and Technology*, *55*, 399-407.
- Sun, M. F., Jiang, C. L., Kong, Y. S., Luo, J. L., Yin, P., & Guo, G. Y. (2022). Recent advances in analytical methods for determination of polyphenols in tea: A comprehensive review. *Foods*, *11*(10), 1425.
- Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, *71*(3), 209-249.
- Surh, Y. J. (2003). Cancer chemoprevention with dietary phytochemicals. *Nature Reviews Cancer*, *3*(10), 768-780.
- Suryadinata, R. V., & Wirjatmadi, B. (2020). Selenium linked to increased antioxidant levels and decreased free radicals in lung tissue of Wistar rats exposed to e-cigarette smoke. *Journal of Global Pharma Technology*, *12*(9), 32-39.
- Swetha, M., Keerthana, C. K., Rayginia, T. P., & Anto, R. J. (2022). Cancer chemoprevention: A strategic approach using phytochemicals. *Frontiers in Pharmacology*, *12*, 4044.
- Tadhani, M. B., Patel, V. H., & Subhash, R. (2007). *In vitro* antioxidant activities of *Stevia rebaudiana* leaves and callus. *Journal of food composition and Analysis*, *20*(3-4), 323-329.
- Takeo, T. (1992). Green and semi-fermented teas. *Tea: cultivation to consumption*, 413-457.

- Takino, Y., Imagawa, H., Horikawa, H., & Tanaka, A. (1964). Studies on the mechanism of the oxidation of tea leaf catechins: Part III. Formation of a reddish orange pigment and its spectral relationship to some benzotropolone derivatives. *Agricultural and Biological Chemistry*, *28*(1), 64-71.
- Talhout, R., Schulz, T., Florek, E., Van Benthem, J., Wester, P., & Opperhuizen, A. (2011). Hazardous compounds in tobacco smoke. *International journal of environmental research and public health*, *8*(2), 613-628.
- Tan, S., Gou, Q., Pu, W., Guo, C., Yang, Y., Wu, K., & Peng, Y. (2018). Circular RNA F-circEA produced from EML4-ALK fusion gene as a novel liquid biopsy biomarker for non-small cell lung cancer. *Cell research*, *28*(6), 693-695.
- Tanaka, T., & Matsuo, Y. (2020). Production mechanisms of black tea polyphenols. *Chemical and Pharmaceutical Bulletin*, *68*(12), 1131-1142.
- Tang, G. Y., Zhao, C. N., Xu, X. Y., Gan, R. Y., Cao, S. Y., Liu, Q., & Li, H. B. (2019). Phytochemical composition and antioxidant capacity of 30 Chinese teas. *Antioxidants*, *8*(6), 180.
- Tang, N., Wu, Y., Zhou, B., Wang, B., & Yu, R. (2009). Green tea, black tea consumption and risk of lung cancer: a meta-analysis. *Lung cancer*, *65*(3), 274-283.
- Tang, S., Qin, C., Hu, H., Liu, T., He, Y., Guo, H., & Zhou, H. (2022). Immune checkpoint inhibitors in non-small cell lung cancer: progress, challenges, and prospects. *Cells*, *11*(3), 320.
- Tas, F., Ciftci, R., Kilic, L., & Karabulut, S. (2013). Age is a prognostic factor affecting survival in lung cancer patients. *Oncology letters*, *6*(5), 1507-1513.
- Terwoord, J. D., Beyer, A. M., & Gutterman, D. D. (2022). Endothelial dysfunction as a complication of anti-cancer therapy. *Pharmacology & Therapeutics*, 108116.
- Teshome, K. (2019). Effect of tea processing methods on biochemical composition and sensory quality of black tea (*Camellia sinensis* (L.) O. Kuntze): A review. *Journal of Horticulture and Forestry*, *11*(6), 84-95.
- Tewari, D., Patni, P., Bishayee, A., Sah, A. N., & Bishayee, A. (2022, May). Natural products targeting the PI3K-Akt-mTOR signaling pathway in cancer: A novel therapeutic strategy. In *Seminars in cancer biology 80*, 1-17. Academic Press.
- Tewari, D., Sah, A. N., Pandey, H. K., Meena, H. S., Meena, R., Ramaswamy, R. S., & Murthy, P. H. (2012). A review on phytoconstituents of Ocimum (Tulsi). *International Journal of Ayurvedic Medicine*, *3*(1), 1-9.

- Thirunavukkarasu, P., Asha, S., Ramanathan, T., Manigandan, V., & Dinesh, P. (2013). Chemoprevention of Gastric Cancer by mangrove plant species *Bruguiera cylindrica* against Benzo (a)pyrene induced gastric cancer in albino mice.
- Thompson, H. J., & Lutsiv, T. (2023). Natural Products in Precision Oncology: Plant-Based Small Molecule Inhibitors of Protein Kinases for Cancer Chemoprevention. *Nutrients*, *15*(5), 1192.
- Tounekti, T., Joubert, E., Hernandez, I., & Munne-Bosch, S. (2013). Improving the polyphenol content of tea. *Critical reviews in plant sciences*, *32*(3), 192-215.
- Toyokuni, S., Okamoto, K., Yodoi, J., & Hiai, H. (1995). Persistent oxidative stress in cancer. *FEBS letters*, *358*(1), 1-3.
- Travis, W. D., Brambilla, E., Noguchi, M., Nicholson, A. G., Geisinger, K. R., Yatabe, Y., & Yankelewitz, D. (2011). International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. *Journal of thoracic oncology*, *6*(2), 244-285.
- Tungmunnithum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines*, *5*(3), 93.
- UlHaq, A., & Sehgal, A. (2020). Radical Scavenging Ability of Black Tea Infusions with or Without Milk in Combination with *Ocimum gratissimum*. *Plant Arch*, *20*, 2476-2480.
- Unsal, V., Cicek, M., & Sabancilar, İ. (2021). Toxicity of carbon tetrachloride, free radicals and role of antioxidants. *Reviews on environmental health*, *36*(2), 279-295.
- Vichai, V., & Kirtikara, K. (2006). Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nature protocols*, *1*(3), 1112-1116.
- Valavanidis, A., Vlachogianni, T., Fiotakis, K., & Loridas, S. (2013). Pulmonary oxidative stress, inflammation and cancer: respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. *International journal of environmental research and public health*, *10*(9), 3886-3907.
- Valko, M., Rhodes, C. J. B., Moncol, J., Izakovic, M. M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-biological interactions*, *160*(1), 1-40.

- Valsan, A., Athulya Bose, G. K., Amrutha, C. S., Anil Kumar, A. K., Jayamol, K. V., & Kumar, S. (2022). Preliminary Phytochemical Screening of Indigenous Medicinal Plants *Ocimum tenuiflorum*, *Ocimum basilicum*, and *Ocimum gratissimum*.
- Vargas, A. J., & Harris, C. C. (2016). Biomarker development in the precision medicine era: lung cancer as a case study. *Nature Reviews Cancer*, *16*(8), 525-537.
- Velli, S. K., Sundaram, J., Murugan, M., Balaraman, G., & Thiruvengadam, D. (2019). Protective effect of vanillic acid against benzo (a) pyrene induced lung cancer in Swiss albino mice. *Journal of biochemical and molecular toxicology*, *33*(10), e22382.
- Venugopal, R., Mahesh, V., Ekambaram, G., Aadithya, A., & Sakthisekaran, D. (2014). Protective role of *Solanum trilobatum* (Solanaeace) against benzo (a) pyrene-induced lung carcinogenesis in Swiss albino mice. *Biomedicine & Preventive Nutrition*, *4*(4), 535-541.
- Vermeire, E., Hearnshaw, H., Van Royen, P., & Denekens, J. (2001). Patient adherence to treatment: three decades of research. A comprehensive review. *Journal of clinical pharmacy and therapeutics*, *26*(5), 331-342.
- Vinci, G., D'Ascenzo, F., Maddaloni, L., Prencipe, S. A., & Tiradritti, M. (2022). The influence of green and black tea infusion parameters on total polyphenol content and antioxidant activity by ABTS and DPPH assays. *Beverages*, *8*(2), 18.
- Wachter, C., Hackner, K., Groissenberger, I., Jutz, F., Tschurlovich, L., Le, N. S., & Kreye, G. (2022). A retrospective, single-center analysis of specialized palliative care services for patients with advanced small-cell lung cancer. *Cancers*, *14*(20), 4988.
- Wakefield, M. A., Coomber, K., Durkin, S. J., Scollo, M., Bayly, M., Spittal, M. J., & Hill, D. (2014). Time series analysis of the impact of tobacco control policies on smoking prevalence among Australian adults, 2001? 2011. *Bulletin of the World Health Organization*, *92*, 413-422.
- Wallace, T. C., Bailey, R. L., Blumberg, J. B., Burton-Freeman, B., Chen, C. O., Crowe-White, K. M., & Wang, D. D. (2020). Fruits, vegetables, and health: A comprehensive narrative, umbrella review of the science and recommendations for enhanced public policy to improve intake. *Critical reviews in food science and nutrition*, *60*(13), 2174-2211.
- Wang, D., Chen, C., Wang, Y., Liu, J., & Lin, R. (2014). Effect of black tea consumption on blood cholesterol: a meta-analysis of 15 randomized controlled trials. *PLoS One*, *9*(9), e107711.

- Wang, G., Yang, F., Zhou, W., Xiao, N., Luo, M., & Tang, Z. (2023). The initiation of oxidative stress and therapeutic strategies in wound healing. *Biomedicine & Pharmacotherapy*, *157*, 114004.
- Wang, H., Shen, S., Wang, J., Jiang, Y., Li, J., Yang, Y., & Yuan, H. (2022). Novel insight into the effect of fermentation time on quality of Yunnan Congou black tea. *Lwt*, *155*, 112939.
- Wang, S., Meckling, K. A., Marcone, M. F., Kakuda, Y., & Tsao, R. (2011). Synergistic, additive, and antagonistic effects of food mixtures on total antioxidant capacities. *Journal of agricultural and food chemistry*, *59*(3), 960-968.
- Wang, Y., Zhang, S., Wang, Y., Wu, X., Zou, Y., Zhou, W., & Dong, S. (2022). Concentration and risk assessment of PCNs in green tea in different locations in China. *Science of The Total Environment*, *847*, 157587.
- Wang, Y., Zheng, P. C., Liu, P. P., Song, X. W., Guo, F., Li, Y. Y., & Jiang, C. J. (2019). Novel insight into the role of withering process in characteristic flavor formation of teas using transcriptome analysis and metabolite profiling. *Food Chemistry*, *272*, 313-322.
- Wang, Z. Y., Hong, J. Y., Huang, M. T., Reuhl, K. R., Conney, A. H., & Yang, C. S. (1992). Inhibition of N-nitrosodiethylamine-and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced tumorigenesis in A/J mice by green tea and black tea. *Cancer research*, *52*(7), 1943-1947.
- Wang, Z., Dabrosin, C., Yin, X., Fuster, M. M., Arreola, A., Rathmell, W. K., ... & Jensen, L. D. (2015, December). Broad targeting of angiogenesis for cancer prevention and therapy. In *Seminars in cancer biology* (Vol. 35, pp. S224-S243). Academic Press.
- Wattenberg, L. W., Wiedmann, T. S., Estensen, R. D., Zimmerman, C. L., Steele, V. E., & Kelloff, G. J. (1997). Chemoprevention of pulmonary carcinogenesis by aerosolized budesonide in female A/J mice. *Cancer Research*, *57*(24), 5489-5492.
- Wei, C., Yang, H., Wang, S., Zhao, J., Liu, C., Gao, L., & Wan, X. (2018). Draft genome sequence of Camellia sinensis var. sinensis provides insights into the evolution of the tea genome and tea quality. *Proceedings of the National Academy of Sciences*, *115*(18), E4151-E4158.
- Wei, Q. Y., Zhou, B., Cai, Y. J., Yang, L., & Liu, Z. L. (2006). Synergistic effect of green tea polyphenols with trolox on free radical-induced oxidative DNA damage. *Food Chemistry*, *96*(1), 90-95.

- Whitlock Jr, J. P. (1999). Induction of cytochrome P4501A1. *Annual review of pharmacology and toxicology*, *39*(1), 103-125.
- WHO methods and data sources for global burden of disease estimates 2000-2016. https://www.who.int/healthinfo/global_burden_disease/GlobalDALY_method_2000_2016.pdf. Accessed 21 July 2020
- Wills, R. B., Bone, K., & Morgan, M. (2000). Herbal products: active constituents, modes of action and quality control. *Nutrition research reviews*, *13*(1), 47-77.
- Wislocki, P. G., Kapitulnik, J., Levin, W., Lehr, R., Schaefer-Ridder, M., Karle, J. M., & Conney, A. H. (1978). Exceptional carcinogenic activity of benz [a] anthracene 3, 4-dihydrodiol in the newborn mouse and the bay region theory. *Cancer research*, *38*(3), 693-696.
- Wong, M., Sirisena, S., & Ng, K. (2022). Phytochemical profile of differently processed tea: A review. *Journal of Food Science*, *87*(5), 1925-1942.
- Wood, D. E., Kazerooni, E. A., Baum, S. L., Eapen, G. A., Ettinger, D. S., Hou, L., & Hughes, M. (2018). Lung cancer screening, version 3.2018, NCCN clinical practice guidelines in oncology. *Journal of the National Comprehensive Cancer Network*, *16*(4), 412-441.
- World Health Organization. (2018). WHO guidelines for the pharmacological and radiotherapeutic management of cancer pain in adults and adolescents.
- World Health Organization. (2019). International agency for research on cancer.
- World Health Organization. Global Adult Tobacco Survey (GATS) China 2010 Country Report [cited 14 January 2016]. Available online: http://www.who.int/tobacco/ surveillance/survey/gats/chn/en/
- World Health Organization. Global Adult Tobacco Survey (GATS) India 2009-2010 Country Report [cited 14 January 2016]. Available online: http://www.who.int/ tobacco/surveillance/survey/gats/ind/en/
- World Health Organization. WHO Mortality Database [cited 15 January 2016]. Available from: http://www.who. int/healthinfo/statistics/mortality_rawdata/en/
- Wu, Y., Zhang, D., & Kang, S. (2013). Black tea, green tea and risk of breast cancer: an update. *Springerplus*, *2*, 1-5.
- Wynder, E. L., & Graham, E. A. (1950). Tobacco smoking as a possible etiologic factor in bronchiogenic carcinoma: a study of six hundred and eighty-four proved cases. *Journal of the American medical association*, *143*(4), 329-336.
- Xu, N., & Chen, Z. M. (2002). Green tea, black tea and semi-fermented tea. *Tea: Bioactivity and therapeutic potential*, 35-57.

- Xu, Y., Ho, C. T., Amin, S. G., Han, C., & Chung, F. L. (1992). Inhibition of tobacco-specific nitrosamine-induced lung tumorigenesis in A/J mice by green tea and its major polyphenol as antioxidants. *Cancer Research*, *52*(14), 3875-3879.
- Xue, J., Yang, S., & Seng, S. (2014). Mechanisms of cancer induction by tobacco-specific NNK and NNN. *Cancers*, *6*(2), 1138-1156.
- Yadav, A. K., Singh, S., Dhyani, D., & Ahuja, P. S. (2011). A review on the improvement of stevia [*Stevia rebaudiana* (Bertoni)]. *Canadian journal of plant science*, *91*(1), 1-27.
- Yamagata, K., Tagami, M., & Yamori, Y. (2015). Dietary polyphenols regulate endothelial function and prevent cardiovascular disease. *Nutrition*, *31*(1), 28-37.
- Yan, X., Zhao, W., Wei, J., Yao, Y., Sun, G., Wang, L., & Li, Y. (2022). A serum lipidomics study for the identification of specific biomarkers for endometrial polyps to distinguish them from endometrial cancer or hyperplasia. *International Journal of Cancer*, *150*(9), 1549-1559.
- Yan, Y., Wang, Y., Tan, Q., Hara, Y., Yun, T. K., Lubet, R. A., & You, M. (2006). Efficacy of polyphenon E, red ginseng, and rapamycin on benzo (a) pyrene-induced lung tumorigenesis in A/J mice. *Neoplasia*, *8*(1), 52-58.
- Yan, Y., Wang, Y., Tan, Q., Lubet, R. A., & You, M. (2005). Efficacy of deguelin and silibinin on benzo (a) pyrene-induced lung tumorigenesis in A/J mice. *Neoplasia*, *7*(12), 1053-1057.
- Yang, C. S., Chung, J. Y., Yang, G. Y., Chhabra, S. K., & Lee, M. J. (2000). Tea and tea polyphenols in cancer prevention. *The Journal of nutrition*, *130*(2), 472S-478S.
- Yang, C. S., Kim, S., Yang, G. Y., Lee, M. J., Liao, J., Chung, J. Y., & Ho, C. T. (1999). Inhibition of carcinogenesis by tea: bioavailability of tea polyphenols and mechanisms of actions. *Proceedings of the Society for Experimental Biology and Medicine*, *220*(4), 213-217.
- Yang, C. S., Wang, X., Lu, G., & Picinich, S. C. (2009). Cancer prevention by tea: animal studies, molecular mechanisms and human relevance. *Nature Reviews Cancer*, *9*(6), 429-439.
- Yang, G. Y., Liao, J., Kim, K., Yurkow, E. J., & Yang, C. S. (1998). Inhibition of growth and induction of apoptosis in human cancer cell lines by tea polyphenols. *Carcinogenesis*, *19*(4), 611-616.
- Yang, G. Y., Liu, Z., Seril, D. N., Liao, J., Ding, W., Kim, S., & Yang, C. S. (1997). Black tea constituents, theaflavins, inhibit 4-(methylnitrosamino)-1-(3-pyridyl)-1-

butanone (NNK)-induced lung tumorigenesis in A/J mice. *Carcinogenesis*, *18*(12), 2361-2365.

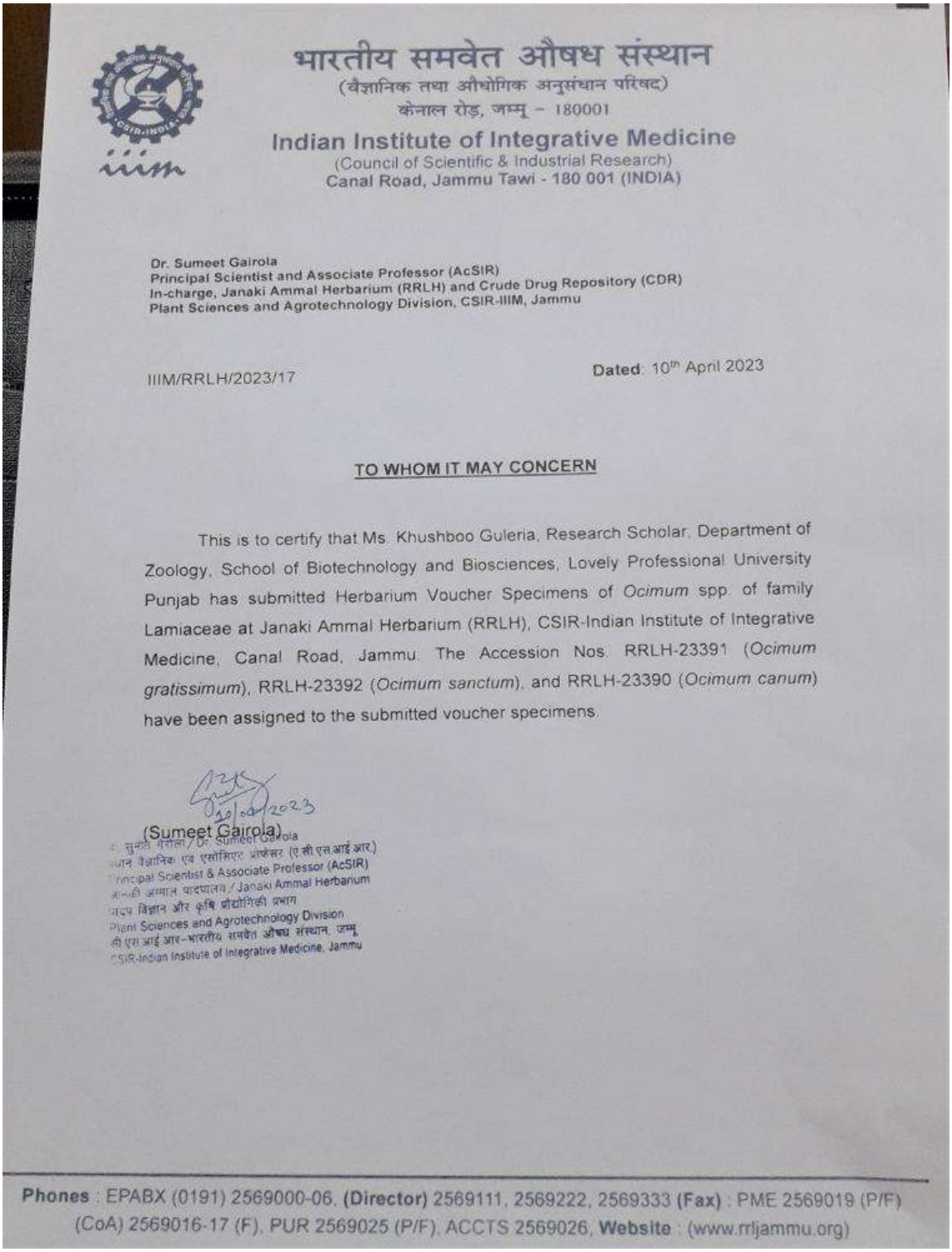
- Yeo, C. D., Kim, Y. A., Lee, H. Y., Kim, J. W., Kim, S. J., Lee, S. H., & Kim, Y. K. (2017). Roflumilast treatment inhibits lung carcinogenesis in benzo (a) pyrene-induced murine lung cancer model. *European journal of pharmacology*, *812*, 189-195.
- Yu, P., Huang, H., Zhao, X., Zhong, N., Zheng, H., & Gong, Y. (2020). Distinct variation in taste quality of Congou black tea during a single spring season. *Food Science & Nutrition*, *8*(4), 1848-1856.
- Yu, Y., Liu, H., Zheng, S., Ding, Z., Chen, Z., Jin, W., & Zhang, R. (2014). Gender susceptibility for cigarette smoking-attributable lung cancer: a systematic review and meta-analysis. *Lung cancer*, *85*(3), 351-360.
- Yun, J., Cui, C., Zhang, S., Zhu, J., Peng, C., Cai, H., & Hou, R. (2021). Use of headspace GC/MS combined with chemometric analysis to identify the geographic origins of black tea. *Food Chemistry*, *360*, 130033.
- Zahra, K. F., Lefter, R., Ali, A., Abdellah, E. C., Trus, C., Ciobica, A., & Timofte, D. (2021). The involvement of the oxidative stress status in cancer pathology: A double view on the role of the antioxidants. *Oxidative Medicine and Cellular Longevity*, *2021*.
- Zander, D. S., & Farver, C. F. (2018). Molecular basis of pulmonary disease. In *Molecular Pathology* (pp. 347-386). Academic Press.
- Zappa, C., & Mousa, S. A. (2016). Non-small cell lung cancer: current treatment and future advances. *Translational lung cancer research*, *5*(3), 288.
- Zhai, X., Zhang, L., Granvogl, M., Ho, C. T., & Wan, X. (2022). Flavor of tea (Camellia sinensis): A review on odorants and analytical techniques. *Comprehensive Reviews in Food Science and Food Safety*, *21*(5), 3867-3909.
- Zhan, F., Parajulee, A., Binnington, M. J., Gawor, A., & Wania, F. (2023). A multi-pathway exposure assessment for polycyclic aromatic hydrocarbons among residents in the Athabasca oil sands region, Canada. *Environmental Science: Processes & Impacts*, *25*(4), 755-766.
- Zhang, C., Qin, Y. Y., Wei, X., Yu, F. F., Zhou, Y. H., & He, J. (2015). Tea consumption and risk of cardiovascular outcomes and total mortality: a systematic review and meta-analysis of prospective observational studies. *European journal of epidemiology*, *30*, 103-113.
- Zhang, D., Chen, L., Cai, J., Dong, Q., Din, Z. U., Hu, Z. Z., & Cheng, S. Y. (2021). Starch/tea polyphenols nanofibrous films for food packaging application: From facile

construction to enhance mechanical, antioxidant and hydrophobic properties. *Food Chemistry*, *360*, 129922.

- Zhang, L., Ho, C. T., Zhou, J., Santos, J. S., Armstrong, L., & Granato, D. (2019). Chemistry and biological activities of processed Camellia sinensis teas: A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, *18*(5), 1474-1495.
- Zhang, X., Leng, S., Qiu, M., Ding, Y., Zhao, L., Ma, N., & Guo, X. (2023). Chemical fingerprints and implicated cancer risks of Polycyclic aromatic hydrocarbons (PAHs) from fine particulate matter deposited in human lungs. *Environment International*, *173*, 107845.
- Zhang, Y. F., Xu, Q., Lu, J., Wang, P., Zhang, H. W., Zhou, L., & Zhou, Y. H. (2015). Tea consumption and the incidence of cancer. *European Journal of Cancer Prevention*, *24*(4), 353-362.
- Zhao, J., He, B., Seshadri, V. D., & Xu, S. (2022). Anticancer Effect of Ruscogenin in B (a) P-Induced Lung Cancer in Mice via Modulation of Proinflammatory Cytokines and Mitochondrial Enzymes. *Applied Biochemistry and Biotechnology*, *194*(12), 5862-5877.
- Zhou, S., Huang, M., Xu, A., Yang, H., Duan, W., & Paxton, J. W. (2005). Prediction of herb–drug metabolic interactions: a simulation study. *Phytotherapy Research*, *19*(6), 464-471.
- Zhu, B., Guo, J., Dong, C., Li, F., Qiao, S., Lin, S., ... & Zhang, Z. (2021). CsAlaDC and CsTSI work coordinately to determine theanine biosynthesis in tea plants (*Camellia sinensis* L.) and confer high levels of theanine accumulation in a non-tea plant. *Plant biotechnology journal*, *19*(12), 2395.
- Zhu, Q. Q., Hou, J., Wang, G. Y., Xu, T., Yin, W., Cheng, J., & Yuan, J. (2022). Impaired lung function related to microenvironmental exposure to PAHs mixture in PM2. 5: A repeated measurement study. *Atmospheric Pollution Research*, *13*(1), 101273.
- Ziech, D., Franco, R., Pappa, A., Malamou-Mitsi, V., Georgakila, S., Georgakilas, A. G., & Panayiotidis, M. I. (2010). The role of epigenetics in environmental and occupational carcinogenesis. *Chemico-biological interactions*, *188*(2), 340-349.

APPENDIX II

PLANT IDENTIFICATION CERTIFICATE



भारतीय समवेत औषध संस्थान
(वैज्ञानिक तथा औद्योगिक अनुसंधान परिषद)
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Plant Sciences and Agrotechnology Division, CSIR-IIIM, Jammu

IIIM/RRLH/2023/17

Dated: 10th April 2023

TO WHOM IT MAY CONCERN

This is to certify that Ms. Khushboo Guleria, Research Scholar, Department of Zoology, School of Biotechnology and Biosciences, Lovely Professional University Punjab has submitted Herbarium Voucher Specimens of *Ocimum* spp. of family Lamiaceae at Janaki Ammal Herbarium (RRLH), CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu. The Accession Nos. RRLH-23391 (*Ocimum gratissimum*), RRLH-23392 (*Ocimum sanctum*), and RRLH-23390 (*Ocimum canum*) have been assigned to the submitted voucher specimens.

20/04/2023

(Sumeet Gairola)
डॉ. सुमीत गैरोला / Dr. Sumeet Gairola
प्रधान वैज्ञानिक एवं एसोसिएट प्रोफेसर (ए.सी.एस.आई.आर.)
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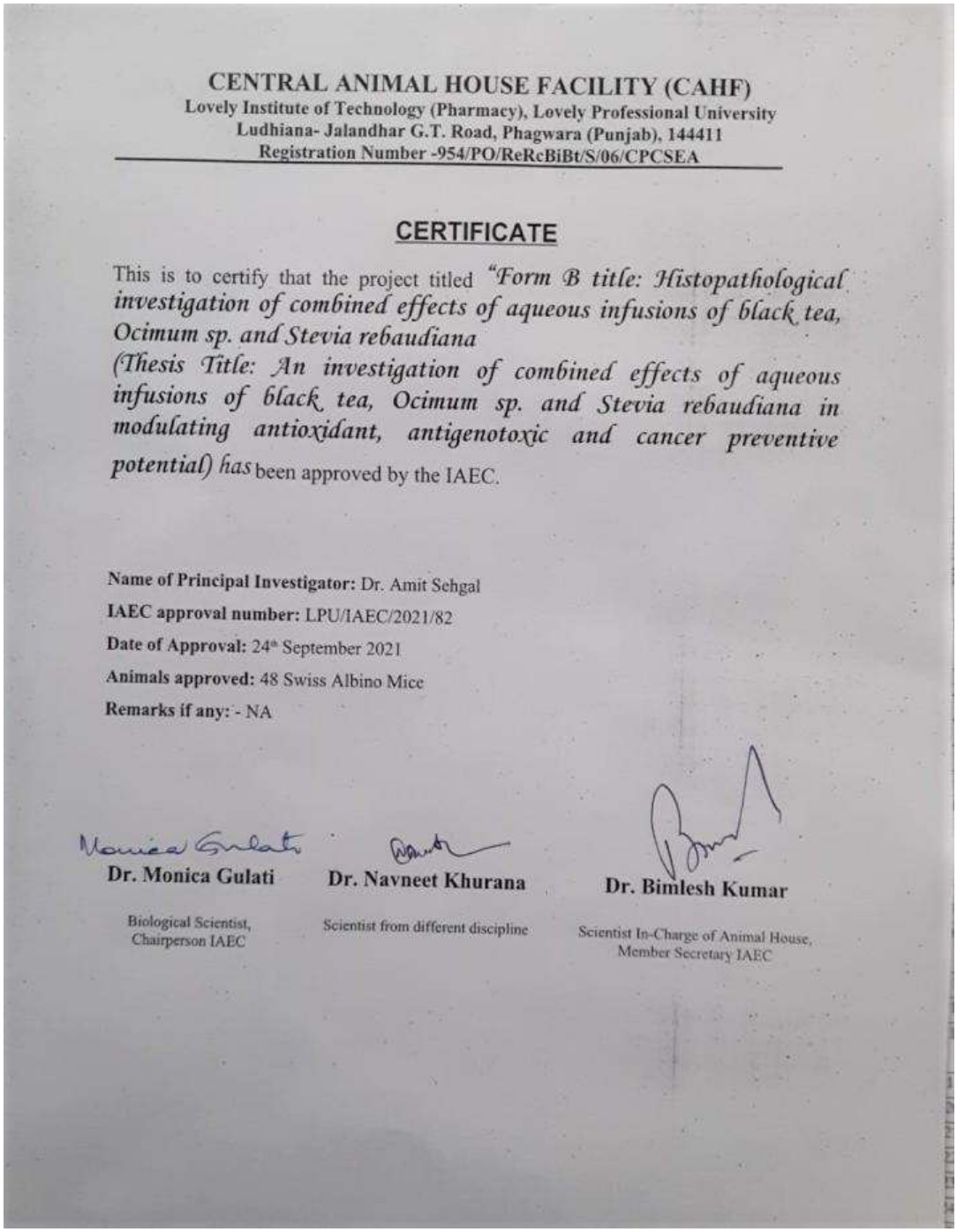
APPENDIX III

ANIMAL ETHICAL PERMISSION CERTIFICATE



CENTRAL ANIMAL HOUSE FACILITY (CAHF)
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Ludhiana- Jalandhar G.T. Road, Phagwara (Punjab), 144411
Registration Number -954/PO/ReRcBiBt/S/06/CPCSEA

CERTIFICATE

This is to certify that the project titled *"Form B title: Histopathological investigation of combined effects of aqueous infusions of black tea, Ocimum sp. and Stevia rebaudiana (Thesis Title: An investigation of combined effects of aqueous infusions of black tea, Ocimum sp. and Stevia rebaudiana in modulating antioxidant, antigenotoxic and cancer preventive potential)* has been approved by the IAEC.

Name of Principal Investigator: Dr. Amit Sehgal
IAEC approval number: LPU/IAEC/2021/82
Date of Approval: 24th September 2021
Animals approved: 48 Swiss Albino Mice
Remarks if any: - NA

Dr. Monica Gulati	Dr. Navneet Khurana	Dr. Bimlesh Kumar
Biological Scientist, Chairperson IAEC	Scientist from different discipline	Scientist In-Charge of Animal House, Member Secretary IAEC

LIST OF PUBLICATIONS

❖

Journal of Culinary Science & Technology

Taylor & Francis

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/wcsc20

Additive to Antagonistic Antioxidant Interaction of Black Tea with Three Different Species of *Ocimum*

Khushboo Guleria & Amit Sehgal

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Impact of Altering the Ratio of Black Tea Granules and *Ocimum gratissimum* Leaves in a Binary Infusion on Radical Scavenging Potential Employing Cell Free Models and Ex Vivo Assays

Khushboo Guleria [1], Amit Sehgal [1,*], Irshad Ahmad Bhat [2], Sandeep Kumar Singh [3], Emanuel Vamanu [4,*] and Mahendra P. Singh [1,*]

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Abstract: Black tea is one of the most popular beverages consumed in the world. It is stronger in taste as well as in flavour compared to other less oxidized teas. It is made from the leaves of the shrub *Camellia sinensis* var. *assamica*. Black tea can be supplemented with other plant parts to enhance its flavour and health-promoting properties. In India, *Ocimum* spp. leaves have been used for their medicinal properties since ancient times. These leaves can be added during black tea preparation to enhance their aroma and healing activities. *O. gratissimum*, known as "Scent Leaf", is traditionally used for the management of many diseases, such as the common cold and cough. This work was designed to evaluate the antioxidant interaction between black tea and *O. gratissimum* (leaves) at five different ratios (1:1, 1:2, 1:3, 2:1, and 3:1). To determine the antioxidant activity, chemical-based methods and ex vivo assays were conducted. The total phenolic and flavonoid contents were calculated by Folin's reagent and aluminium chloride colorimetric assays, respectively. The antioxidant interactions were determined by the combination index (CI), using CompuSyn software. The black tea exhibited higher radical quenching activity (DPPH, ABTS, and NO) and antihaemolytic and anti-lipid peroxidation potential compared to the *Ocimum gratissimum* infusion. Variation in the antioxidant capability was observed for various ratios of the black tea and *O. gratissimum* (BT+OG) combination.

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Citation: Guleria, K.; Sehgal, A.; Bhat, I.A.; Singh, S.K.; Vamanu, E.; Singh, M.P. Impact of Altering the Ratio of Black Tea Granules and *Ocimum gratissimum* Leaves in a Binary Infusion on Radical Scavenging Potential Employing Cell Free Models and Ex Vivo Assays. *Appl. Sci.* **2022**, *12*, 10632. https://

LIST OF CONFERENCES

- Participated in the International conference on **Innovative Strategies for Sustainable Water Management** held from 17-11-2017 to 18-11-2017 organized by School of Bioengineering and Biosciences in collaboration with Department of Bioresource Engineering, McGill University Canada at Lovely Professional University, Punjab.

- Poster presentation on my preliminary work titled "Appraisal of Antioxidant potential of binary combination of Black tea and different species of *Ocimum*" in **Integrated Conference on Ayurveda, Agriculture & Pharmaceutical Science** held on 13th and 14th October 2018 organised in Lovely Professional University, Punjab.

- Poster presentation on my research work titled "Effect of varying proportion in a binary mixture of black tea and *O. gratissimum* on possible antioxidant interactions" held from 20 – 12 – 2019 to 22 – 12 – 2019 organized by **14 JK Science Congress 2019 – A National Event on the Theme " Science and Technology for a Sustainable Future", University of Jammu, Jammu.**

- Oral presentation on my research work titled " The antioxidant activity of black tea with different species of *Ocimum* (*O. gratissimum*, *O. sanctum* & *O. canum* Sims)" in **2nd International Conference on Environmental, Agricultural, Chemical and Biological Sciences (ICEACBS2021)** held from 24 – 01 – 2021 to 26 – 01 – 2021 in support of United Nations SDGs organized by **V**oice **O**f **I**ndian **C**oncern for the **E**nvironment (VOICE) in association with CAFRE, University of Pisa, **Italy**. Murray State University, Murray, **Kentucky, USA**. Department of Biotechnology, GLA University, Mathura, **Uttar Pradesh, India**. Department of Zoology, Mercy College, Palakkad, **Kerala, India**. Department of Environmental Science, DM College of Science, Dhanamanjuri University, Imphal, **Manipur, India**. Department of Biochemistry, Vels University, Chennai, **Tamil Nadu, India**.

LIST OF WORKSHOPS

- Participation in **Workshop on Research Methodology using SPSS** organized at Lovely Professional University from February 24, 2017 to February 25, 2017.

- Participation in **Hands on Training to improve Quality of Research Conducted on Experimental Animals** organized by Human Resource Development Center, Lovely Professional University from March 23, 2018 to March 24, 2018 and obtained '**O**' Grade.

- Participation in Author workshop on "**How to Write and Publish Scientific Articles and Manuscripts"** organized by Lovely Professional University, Punjab and Springer Nature held on 15th April, 2018 at Lovely Professional University, Punjab.

- Participation in **Training on The Care and Use of Laboratory Animals in Accordance with Ethical Guidelines** organized by Lovely Professional University w.e.f. March 11, 2022 to March 12, 2022.