EVALUATION OF AMELIORATING POTENTIAL OF *RHEUM WEBBIANUM* RHIZOME EXTRACTS ON 1,2-DIMETHYLHYDRAZINE INDUCED COLORECTAL CARCINOGENESIS IN SWISS ALBINO RATS

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

In

ZOOLOGY

By

UMER MAJEED KHAJA

Registration Number: 11919685

Supervised By

Dr. Reena Singh

Department of Biotechnology
(Associate Professor)
School of Bioengineering and Biosciences
Lovely Professional University, Punjab
India

Co-Supervised by

Dr. Showkat Ahmad Ganie

Department of Clinical Biochemistry
(Assistant Professor)
University of Kashmir, J&K
India

LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

2025

DECLARATION

I hereby declare that the thesis titled **"Evaluation of ameliorating potential of *Rheum webbianum* rhizome extracts on 1,2-dimethylhydrazine induced colorectal carcinogenesis in Swiss albino rats"**, submitted in fulfillment of the requirements for the degree of Doctor of Philosophy (Ph.D.), is the result of my original research carried out under the supervision of Dr. Reena Singh, Associate Professor Department of Biotechnology, School of Bioengineering and Biosciences, Lovely Professional University, Punjab, India and Co-supervision of Dr. Showkat Ahmad Ganie, Assistant Professor, Department of Clinical Biochemistry, University of Kashmir, Srinagar, J&K, India. I have appropriately acknowledged the contributions and findings of other researchers wherever referenced in this thesis. I further certify that this work has not been submitted, either in part or in full, to any other university or institution for the award of any degree.

Name of scholar: Umer Majeed Khaja

Registration No: 11919685

Department: Zoology

School of Bioengineering and Biosciences

Lovely Professional University,

Punjab, India

CERTIFICATE

This is to certify that the Ph.D. thesis titled **"Evaluation of ameliorating potential of *Rheum webbianum* rhizome extracts on 1,2-dimethylhydrazine induced colorectal carcinogenesis in Swiss albino rats"**, submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Ph.D.) in the Department of Zoology of School of Bioengineering and Biosciences, represents the original research work conducted by **Umer Majeed Khaja**, 11919685 under our supervision. We confirm that this thesis is a genuine record of the candidate's independent research efforts and has not been submitted, either wholly or in part, for any other degree, diploma, or equivalent qualification.

Signature of Supervisor

Name of Supervisor

Dr. Reena Singh

Designation: Associate Professor

Dept. of Biotechnology,
School of Bioengineering and
Biosciences

Lovely Professional University

Signature of Co-Supervisor

Name of Co-supervisor

Dr. Showkat Ahmad Ganie

Designation: Assistant Professor

Dept. of Clinical Biochemistry

University of Kashmir

ABSTRACT

Cancer is one of the leading causes of death globally, with an alarming increase in mortality rates of about 40% over the past four decades. Current projections suggest that cancer deaths may reach up to 13 million annually by 2030, highlighting an urgent need for preventive and therapeutic measures. Among cancers, colorectal cancer (CRC) ranks as the third most prevalent form, with rapidly rising incidence and mortality rates, especially in developing countries. CRC currently accounts for around 10% of cancer-related deaths worldwide, and this increase is largely attributed to factors like aging, poor dietary habits, smoking, obesity, and sedentary lifestyles. The progression of colon cancer is complex and multi-staged, involving various pathological alterations, such as aberrant crypt foci (ACF), which are characteristic microscopic lesions that can develop into malignant tumors.

Interestingly, most cancer cases are not primarily driven by genetic factors but by environmental influences and lifestyle choices, opening significant avenues for preventive strategies. Chemoprevention-using agents to inhibit, slow down, or reverse cancer progression at early stages has emerged as a promising field. Many plants rich in bioactive compounds have demonstrated potential as chemopreventive agents. *Rheum webbianum* Royle (RW), in particular, has garnered attention for its extensive ethnopharmacological background. Cultivated for over 5000 years, RW utilized in traditional medicine systems like Ayurveda and Unani. Known for its culinary and medicinal applications, RW exhibits numerous therapeutic properties, including anticancer, gastrointestinal, anti-inflammatory, and anti-fibrotic activities. Despite these known bioactivities, RW's potential in combating CRC has remained largely unexplored until now.

This present study evaluates the chemopreventive potential of RW rhizome extracts against CRC for the first time. The research involved preparing four RW extracts using series of solvents like ethylacetate, ethanol, hexane and methanol. These extracts were then tested for their cytotoxic effects on HCT-116 human colorectal cancer cell lines, identifying methanolic and ethanolic extracts as particularly promising due to their pronounced cytotoxic effects. To further investigate these findings, an in-vivo study was conducted using a rat model of CRC induced by 1,2-dimethylhydrazine (DMH). This model helped assess RW extracts' anticancer and antioxidant

properties, as well as their impact on liver function and blood parameters, which often deteriorate in cancer patients.

In vitro tests on HCT-116 cells showed that methanolic and ethanolic extracts of RW significantly reduced cell viability in a dose- and time-dependent manner. At a concentration of 200 µg/mL, these extracts achieved reductions in cell viability of up to 85% and 90%, respectively. Moving to in-vivo experiments, histopathological analysis of colon tissue from RW-treated groups revealed substantial improvements compared to rats treated only with DMH. Notably, RW-treated animals displayed fewer structural irregularities, reduced congestion, and less inflammation, suggesting that the RW extracts were effective in mitigating cancer-induced damage to colon tissue. On the contrary, DMH-only treated animals exhibited severe structural abnormalities, mucosal destruction, and other cancer-related tissue alterations. The histological findings thus underscore the potential role of RW extracts in reducing cancer-related changes in colon tissue structure.

The DMH-induced CRC model also led to significant changes in hematological and hepatic markers. There was a marked decline in erythrocytes (49.13%), hemoglobin (32.18%), and hematocrit (26.79%), while white blood cell counts and platelets surged by 79.62% and 68.96%, respectively. The administration of RW extracts stabilized the hematological parameters closer to normal levels, underscoring their protective effects against CRC-induced blood abnormalities. The study also observed improvements in liver health, with a reduction in elevated ALT and AST levels by 36.78% and 33.12%, respectively. This reduction reflects RW's hepatoprotective properties, mitigating the liver toxicity associated with DMH-induced CRC. Additionally, RW treatment was found to significantly lower serum cholesterol and triglyceride levels, contrasting with the elevated lipid profiles observed in the DMH-only group, suggesting that RW may also reduce the risk of atherosclerosis associated with cancer progression.

An important aspect of this research was assessing the antioxidant potential of RW extracts. Cancer progression, including CRC, is associated with high oxidative stress levels due to excessive free radical production. DMH treatment in the CRC model showed a 195.59% increase in MDA levels, indicating lipid peroxidation, while antioxidant defenses, such as SOD, CAT, GSH, and GR activities, were significantly reduced. RW extract treatment, however, resulted in notable decreases in MDA levels and enhancements in antioxidant enzyme activities, suggesting a strong antioxidative capacity. This antioxidative effect may contribute to RW's chemopreventive

properties, as reducing oxidative stress can prevent the cellular damage that drives cancer progression.

To explore the molecular mechanisms underlying RW's anticancer effects, this study investigated two critical signaling pathways responsible for CRC: the Wnt/β-catenin and TGF-β pathways. Both pathways are known for their roles in promoting tumor growth and metastasis. Western blot analyses revealed that RW extracts effectively inhibited β-catenin expression, especially at higher doses, implying suppression of Wnt signaling. This suppression is significant, as hyperactivation of the Wnt pathway is a hallmark of CRC progression. Additionally, RW extracts reduced TGF-β1 expression, a signaling molecule involved in cancer progression and metastasis, thereby indicating a downregulation of this pro-tumorigenic pathway. By targeting these key pathways, RW extracts may interfere with critical molecular events that drive CRC development.

The implications of this study are substantial. The findings underscore the potential of RW rhizome extracts as natural agents in CRC prevention, capable of not only reducing cancer cell viability but also alleviating cancer-related tissue damage, oxidative stress, and hematological imbalances. These results lay the groundwork for further investigations to identify the active compounds within RW which are responsible for anticancer effects. Such research could lead to the development of novel, plant-based chemopreventive agents that offer safer, more accessible alternatives to conventional therapies.

This study's hypothesis centers on the potential of *Rheum webbianum* (RW) rhizome extracts to exert cytotoxic effects on CRC cells and to inhibit CRC progression through antioxidant and molecular pathway modulation. Specifically, RW extracts are anticipated to reduce oncogenic effects induced by DMH in a CRC model. This hypothesis was supported by the study's findings, which demonstrated that RW extracts significantly improved histopathological, hematological, hepatic, and oxidative stress markers in a CRC context. The research objectives aimed to establish RW's cytotoxic effect on HCT-116 cells, to evaluate its impact on DMH-induced colon alterations, and to assess RW's influence on antioxidant levels, signaling pathway expressions, and blood and liver health. By addressing these objectives, the study provides compelling evidence for RW's chemopreventive properties against CRC, with the potential to contribute valuable insights to both cancer biology and ethnopharmacology.

Overall, this study contributes to the understanding of plant-based interventions in cancer prevention, especially for CRC. It provides a foundation for the potential therapeutic use of *Rheum webbianum*, not only in preventive strategies but also in integrative cancer treatment approaches that emphasize the use of natural products. The findings could inspire further exploration of RW and similar plants in the context of cancer and beyond, driving future studies that may eventually lead to the development of affordable, accessible cancer prevention options with minimal side effects.

Acknowledgements

All praise to Almighty Allah, the Most Gracious and the Most Merciful, whose countless blessings and guidance throughout the journey of this research endeavor have enabled me to complete this thesis.

In the journey of my academic pursuits, there are no words sufficient to express my deepest gratitude to my beloved parents, Father **Abdul Majeed Khaja** and Mother **Khursheeda Begum**. Your unwavering support, encouragement, and sacrifices have been the cornerstone of my achievements. Your love and prayers have been my greatest source of strength throughout this journey.

I extend my heartfelt gratitude to my esteemed supervisor, **Dr. Reena Singh**, and co-supervisor, **Dr. Showkat Ahmad Ganie**. Their expertise, guidance, and encouragement have been instrumental in shaping this research, and their unwavering support throughout my PhD tenure has been invaluable. Further, I would also like to express my sincere gratitude to the **Department of Clinical Biochemistry, University of Kashmir especially Dr. Showkat Ahmad Ganie**, for providing all the necessary facilities, resources, and support essential for the successful completion of my research work.

I am also grateful to **Prof. Joydeep Dutta**, Head of the Department of Zoology, LPU, and **Department of Zoology, University of Kashmir** especially **Prof. Fayaz Ahmad Bhat** for their moral support, which provided me with the motivation and confidence to persevere through the challenges of my research journey.

I extend my heartfelt gratitude to all distinguished members of my research committee, for their instrumental role in shaping my research and for their invaluable suggestions. I wish to extend my heartfelt gratitude to all the esteemed faculty members for their invaluable guidance throughout the course of my research endeavors. Their profound expertise, unwavering commitment, and insightful suggestions have been pivotal in shaping the quality and direction of my work.

Finally, I extend my heartfelt thanks to my family, relatives, friends and colleagues for their continuous support, encouragement, and companionship. Their kindness and camaraderie have enriched this journey, and I am truly blessed to have them by my side. Thank you all for your contributions and unwavering support.

Umer Majeed Khaja

Table of Contents

List of abbrevations

IAEC (Institutional Animal Ethics Committee)

CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals)

DMH (1,2-Dimethylhydrazine)

RW (*Rheum webbianum*)

HCT-116 (Human Colorectal Cancer Cell Line)

MDA (Malondialdehyde)

SOD (Superoxide Dismutase)

CAT (Catalase)

GSH (Reduced Glutathione)

GR (Glutathione Reductase)

ALT (Alanine Transaminase)

AST (Aspartate Transaminase)

PBS (Phosphate-Buffered Saline)

PFA (Paraformaldehyde)

RBC (Red Blood Cell)

Hb (Hemoglobin)

HCT (Hematocrit)

MCV (Mean Corpuscular Volume)

MCH (Mean Corpuscular Hemoglobin)

MCHC (Mean Corpuscular Hemoglobin Concentration)

Plt (Platelet Count)

WBC (White Blood Cell)

SGPT (Serum Glutamic Pyruvic Transaminase)

SGOT (Serum Glutamic Oxaloacetic Transaminase)

LPO (Lipid Peroxide)

PVDF (Polyvinylidene Fluoride)

Wnt (Wingless/Integrated)

TGF-β (Transforming Growth Factor Beta)

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)

List of Tables

List of Figures

CHAPTER 1

INTRODUCTION

Introduction

Cancer is one of the leading cause of deaths worldwide and mortality related to cancer has increased by almost 40% over the past 40 years. In coming 10 years, 60% increase is expected with the estimated death of 13 million people due to cancer in 2030 (Kuipers et al. 2013). Further, Colorectal cancer (CRC) is the 3rd most common cancer worldwide. A rapid rise in the incidence and mortality due to CRC is observed in various developing countries (Benarba et al. 2014). CRC is 2nd in terms deaths and now approximately accounting for 10% of mortality related to cancer and reasons for this increased incidence include poor dietary habits, smoking, obesity, less physical activity and ageing (Li et al. 2021).

The progression of colorectal cancer is characterized by the gradual accumulation of genetic and epigenetic alterations, leading to tumor enabling characteristics like genomic instability as well as activation of oncogenic pathways, notably the RNA-activated signaling system (RAS). Mutations in critical genes such as P53, PI3K, APC, and BRAF are associated with the deactivation of tumor-suppressor mechanisms (Markowitz and Bertagnolli 2009).The process of CRC induced by DMH involves multiple steps and exhibits morphological and histological characteristics akin to those observed in human sporadic colon carcinogenesis (Tanaka 2009). Colon cancer development involves a complex sequence of pathological changes, ranging from early microscopic mucosal abnormalities to the formation of malignant tumors (Rodrigues et al. 2002).Today, it is widely acknowledged that the sequence of adenoma to carcinoma is typified by identifiable histological alterations, commencing with intraepithelial neoplasia or dysplastic aberrant crypts (Mori et al. 2005). Subsequently, these lesions possess the potential to advance into advanced adenomas, which carry a notable propensity for transformation into adenocarcinomas (Tanaka 2009).

Colorectal cancer (CRC) encompasses a range of genetic classifications, divided into three primary groups. "Sporadic" CRC occurs in individuals without a family history of the disease. "Familial" CRC involves individuals with at least one blood relative diagnosed with CRC. "Hereditary" CRC arises from germline mutations (Kheirelseid et al. 2013). Empirical research underscores that a significant portion of cancer cases can be linked to environmental and lifestyle factors, with genetic abnormalities contributing minimally. Consequently, these findings offer promising avenues for cancer prevention. Chemoprevention, therefore, has garnered substantial attention from

researchers and the general public alike for its potential to forestall cancer initiation, slow down its progression, or even halt carcinogenesis during its incipient stages (Sporn and Suh 2002; Anand et al. 2008). Numerous studies attest to the remarkable chemopreventive properties found in a diverse array of plants rich in phytochemicals (Figure 1).

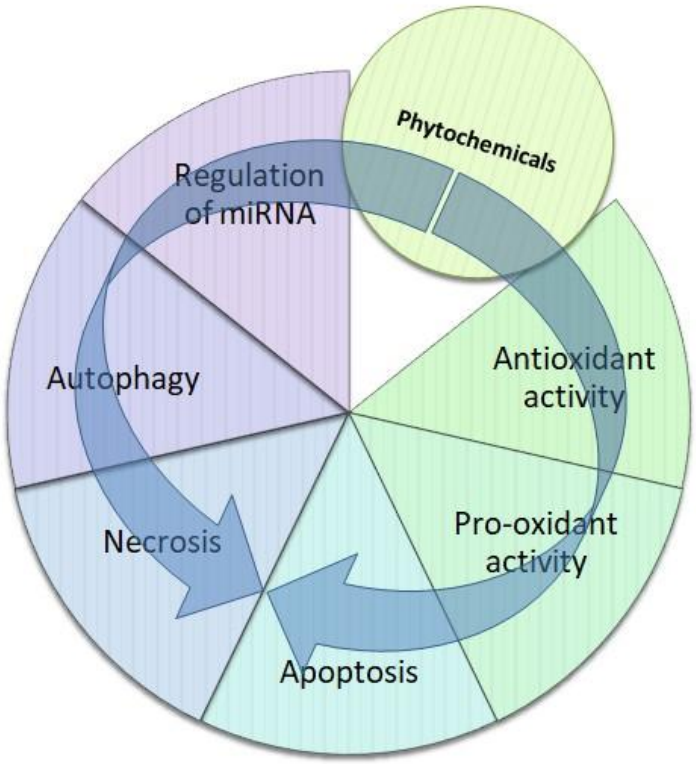


Figure 1: Role of Phytochemicals in Cancer Prevention and Treatment.

Analysis of phytochemicals proves the presence of alkaloids, terpenoids, flavonoids, and polyphenolic substances within these plants. The identified bioactive compounds demonstrate specificity toward various cellular processes, including but not limited to the cell cycle, apoptosis, cell proliferation, DNA repair, as well as TS gene modulation and oncogene-activation (Pandey and Rizvi 2009; Prakash et al. 2013). One of the primary mechanistic effects of anticancer phytochemicals is exhibited through the amelioration of carcinogen-mediated oxidative stress. Many studies reveal that free radicals are vital in the formation of cancer, invasion and metastatic spread (Kumari et al. 2018). The reactive free radicals bind cellular biochemical components as

well as alter the homeostasis of the cell. In addition, the nitric oxide synthase expression is also increased by the reactive oxygen species which elevates the metabolic rates of the cell and further activate the signaling pathways that support the cancer cell proliferation (Sever and Brugge 2015). Therefore, vital role can be played in the chemoprevention of CRC by the suppression of the oxidative stress.

Rheum webbianum Royle. (RW) (source: http://www.worldfloraonline.org/taxon/wfo-0001101732, Accessed on: 24 May 2024) is a perennial leafy herb in the family of Polygonaceae, thrives in the temperate and subtropical regions of the Himalayas, specifically at altitudes between 2800 to 3800 meters. Commonly known as Himalayan rhubarb and "Pambhak" (leaves) or "Pambchalan" (rhizome), this plant is indigenous to the Himalayan belt, spanning China (particularly southern Tibet), Bhutan, Myanmar, India (notably Kashmir and Sikkim), Nepal, and Pakistan (Tayade et al. 2012). Apart from its traditional use as a food source, RW has a rich history of medicinal applications. Revered in the Unani and Ayurvedic medical systems, this plant boasts significant therapeutic potential. Various studies have extensively documented the multifaceted pharmacological properties of *Rehum* species, showcasing their antifungal, antibacterial, anti-inflammatory, antioxidant, antiulcer, hepatoprotective, immunoenhancing, and anticancer effects (Cao et al. 2017). This plant holds significant medicinal value and is widely utilized in the pharmaceutics for the development of drugs aimed at combating cancer and reducing body cholesterol (Basant Ballabh and Chaurasia 2009).

Traditional medicinal practices also highlight its effectiveness in treating abdominal disorders, boils, wounds, indigestion and gastritis. These traditional uses are supported by scientific evidence, as documented in multiple studies. For instance, Chaurasia et al detailed the traditional applications of *Rheum webbianum* in treating digestive and abdominal issues, while Tabin et al and Wani et al corroborated its use in managing boils, wounds, and gastritis through ethnobotanical research that aligns with internationally accepted scientific standards (Basant Ballabh and Chaurasia 2009; Tabin et al. 2016). A recent study highlights the rich medicinal knowledge of the Ladakh people, documenting 176 medicinal plants used in primary health care, including the traditional use of *Rheum webbianum*. The study underscores the significance of local traditional knowledge in treating various ailments, noting that *Rheum webbianum* is one of the commonly used species across all eight regions studied in Ladakh (Wani et al. 2022; Batool et al. 2023). Despite its

widespread traditional usage, there has been no previous scientific validation of its traditional applications. This calls for further phytochemical and pharmacological investigations to provide scientific evidence supporting its traditional uses and potentially incorporating it into clinical trials. Despite this extensive literature, there remains a paucity of research investigating the chemopreventive capabilities of *Rheum webbianum* (RW) extracts concerning colorectal cancer.

Thus, the presented study investigates the potential preventive effects of *Rheum webbianum* rhizome extracts on 1,2-dimethylhydrazine (DMH)-induced colorectal carcinogenesis in Swiss albino rats. This research endeavors to elucidate the potential role of RW extracts in inhibiting the progression of colorectal cancer, thereby advancing our understanding of its chemopreventive properties.

CHAPTER 2

REVIEW OF LITERATURE

Review of Literature

Cancer is one of the major causes of death worldwide. Globally it is among the primary diseases that cause mortality and morbidity in millions of people. Further, colorectal carcinoma (CRC) is listed as the third most fatal type of cancer causing mortality in both males and females worldwide (Siegel et al. 2011). CRC is fundamentally caused by gene mutations, environmental and physiological factors like radiations, smoking, alcohol consumption, excess red meat intake and microbial infection (Siegel et al. 2011; Redondo-Blanco et al. 2017). Increasing ratios of incidence and deaths related to CRC has been observed in many developing countries and CRC is now accounting for approximately 10% of mortality related to cancer. CRC occurs due to the genomic instability caused by the factors of genetic and epigenetic alterations and their accumulation which further leads to the activation of oncogenes like P53, PI3K and APC, BRAF due to mutation(Benarba et al. 2014; Akimoto et al. 2021).

2.1 Incidence and Mortality Rates

CRC incidences are strongly linked to a higher prevalence of adenomatous polyps and advanced carcinoma stages (both are pathological features) (Markowitz and Bertagnolli 2009; Akimoto et al. 2021). In 2020, about 1.9 million fresh cases of CRC and 930,000 related deaths were reported globally, and the incidence and mortality rates vary significantly across different regions worldwide. The lowest incidence rates were observed in Southern Asia and African countries, while the highest were in Europe, New Zealand and Australia (Morgan et al. 2023). Mortality rates followed similar patterns, with Southern Asia showing the lowest and Eastern Europe the highest rates. Notably, CRC incidence and mortality rates were higher in males compared to females across all regions (Morgan et al. 2023). It is also projected that by the year 2040, there will be 3.2 million new incidences and 1.6 million deaths due to CRC, predominantly in countries with high Human Development Index (Figure 2.1) (Xi and Xu 2021).

Figure 2.1 (A): Estimation of new cases from 2022 to 2045, Both sexes, age [0-85+] Colorectum.

(A)

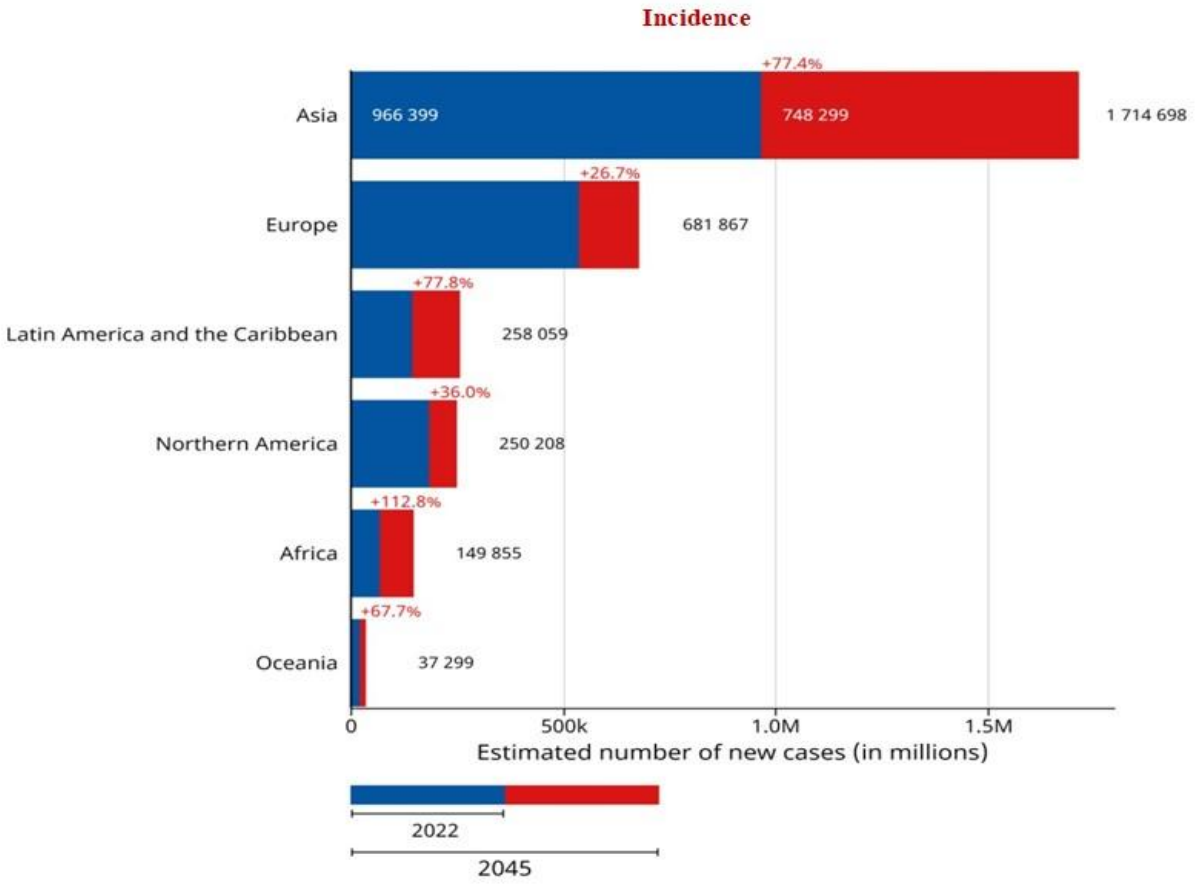


Figure 2.1 (B): Estimation of deaths from 2022 to 2045, Both sexes, age [0-85+] Colorectum.

(B)

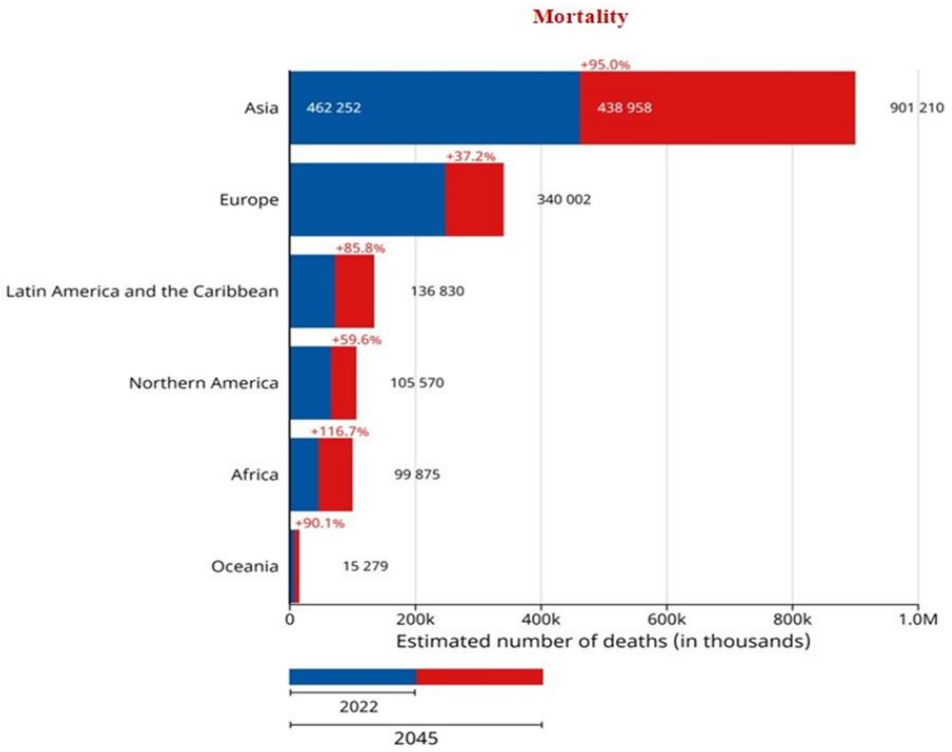


Figure 2.1 (A, B): Predictions of the future worldwide cancer incidence and mortality burden up until 2050. Data extracted from GLOBOCAN: Cancer Surveillance Branch of the International Agency for Research on Cancer. Cancer Tomorrow | IARC - https://gco.iarc.who.int

2.2 Risk Factors

A variety of risk factors can contribute to the CRC development, which is a diverse disease. The risk of colorectal cancer is considerably elevated in those with a history of inflammatory bowel disease (Hnatyszyn et al. 2019). Additional risk factors encompass tobacco use, alcohol intake, excess red and processed meat intake, prolonged exposure to antibiotics since a young age, diabetes mellitus, sedentary lifestyle, and elevated BMI (body mass index) (Johnson et al. 2013). In a study reported by Murphy et al., a study on the cohort of 3,50,000 subjects revealed that the people maintaining a healthy lifestyle, having a normal BMI, performing recommended physical activity, non-smokers and alcohol non or low consumers and maintaining a nutritious diet had a lower hazard ratio of merely 0.63 (95% CI, 0.54–0.74) as compared to those who followed only one factor or no factors (Murphy et al. 2013). The Global Burden of Disease Study 2010 identified a diet which lacks milk and whole grains as significant risk factors for early-onset CRC (EOCRC) worldwide (Pan et al. 2022).

2.3 Molecular Network of CRC

Colorectal cancer is a complex disease at the molecular level, driven by three main pathways (Figure 2.3). The common most pathway in CRC is the CIN pathway which is an acronym for chromosomal instability pathway. This pathway is seen in 85% of the sporadic CRC cases. CIN involves abnormalities in chromosome structure and number, leading to gene mutations in key oncogenes and tumor suppressor genes such as APC, PIK3CA, KRAS, BRAF, SMAD4, and p53, which are crucial for cell proliferation and CRC progression (Fearon 2011; Nguyen and Duong 2018 May 9). The 2nd pathway in CRC is the microsatellite instability (MSI), resulting from the dysfunctional DNA mismatch repair genes, leading to genetic hypermutability (Schmitt and Greten 2021). The 3rd pathway is related to the CIMP phenotype, which is short for CpG island methylator phenotype. This pathway can further be categorized into high CIMP and low CIMP tumors, each associated with specific gene mutations. CRC pathogenesis often involves multiple pathways, with about a third of the cases developing via a pathway linked to KRAS or BRAF mutations and CIMP alterations.

Figure 2.3

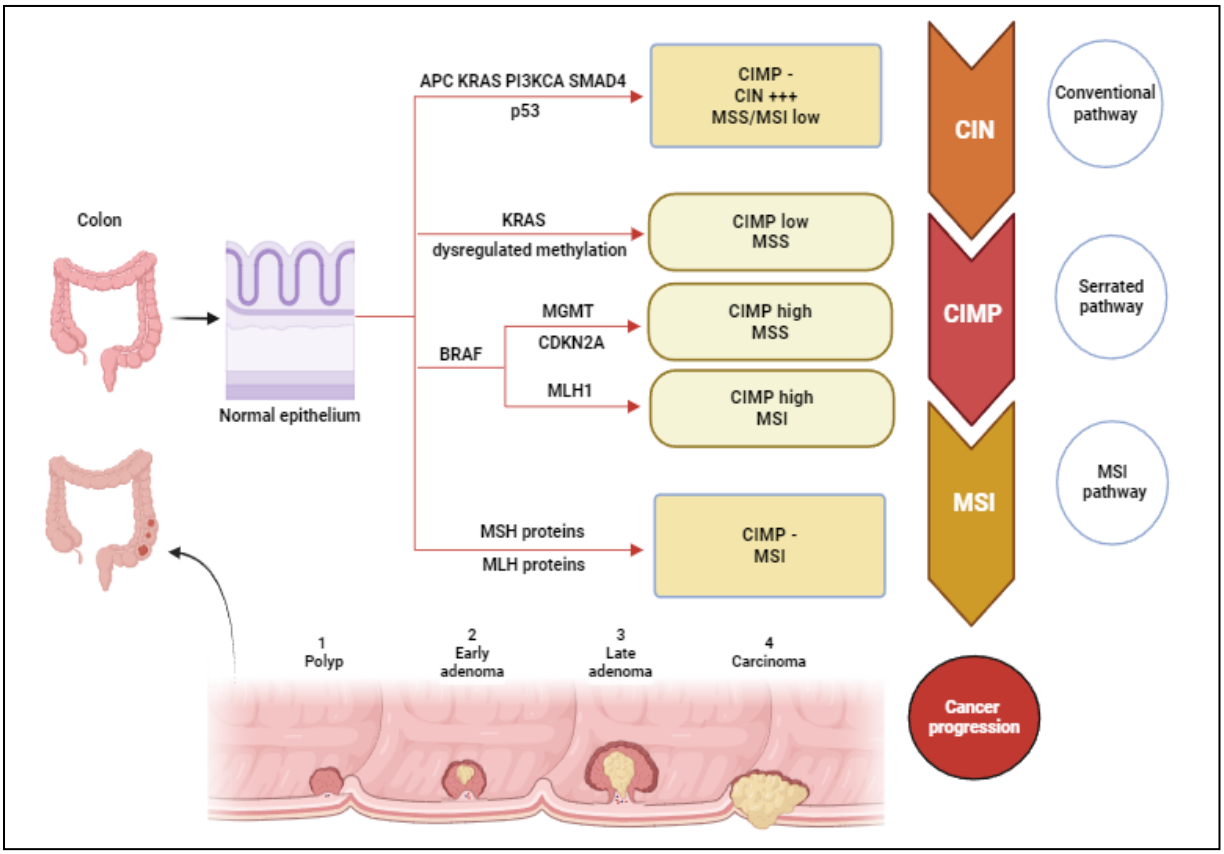


Figure 2.3: Depiction of the three main pathways involved in CRC. The Chromosomal Instability pathway (CIN), common in CRC, is initiated by gene mutations of APC, followed sequentially by changes in PIK3CA, KRAS, and SMAD4, with frequent loss of p53 function. This pathway typically shows minimal or absent CpG island methylation ($CIMP^{-}$), microsatellite stability-MSS, and pronounced chromosomal instability (CIW^{3+}). Around one-third of the CRC cases follow the serrated pathway, further divided into KRAS-mutated, low-CIMP MSS tumors; BRAF-mutated, high-CIMP MSS tumors; and BRAF-mutated, high-CIMP tumors with microsatellite instability (MSI). This serrated pathway often involves silencing of genes such as MGMT, CDKN2A, or MLH1. The third pathway, known as the MSI pathway, arises from defects in the mismatch repair (MMR) genes, affecting proteins like MutL homolog (MLH) and MutS homolog (MSH).

CRC typically develops from normal colorectal epithelial cells that undergo transformation due to spontaneous mutations, exposure to environmental mutagens, or genetic and epigenetic changes. These altered cells can expand quickly, forming aberrant crypt foci and early adenomas. This growth is driven by mutations, such as those in the APC gene, and influenced by pathways like WNT-β-catenin, as well as factors from the tumor microenvironment (TME), including chemokines, cytokines and growth factors (Papanikolaou et al. 2000). Mutations in genes like SMAD4, CDC4, and TGFBR2, along with chromosomal changes such as loss of heterozygosity (LOH) on chromosome 18q, contribute to the progression from early lesions to late adenomas and malignant tumors (through the process of tumor promotion). Further mutations, including those in p53 and BAX, along with pro-angiogenic factors and extracellular matrix-degrading enzymes, enhance invasive potential, leading to the spread of CRC cells to other tissues and organs, a phase referred to as tumor progression.

2.4 Cellular Signaling Pathways

Colorectal cancer (CRC) develops through a complex, multistage process driven by sequential genetic mutations. CRC progression involves activation of key signaling pathways that regulate cell proliferation, differentiation, survival and apoptosis. These pathways include the prominent epidermal growth factor EGFR-Ras-MAP Kinase pathway, Wnt/β-catenin pathway, PI3K/Akt pathway, and the TGF-β pathway, Notch signaling, and the nuclear-factor kappa light chain enhancer of activated B cells -NF-κB pathway (Koveitypour et al. 2019).

2.4.1 EGFR-Ras-MAPK Pathway

The epidermal growth factor receptor is a transmembrane (TM) protein that works as a receptor tyrosine kinase (RTK) with an extracellular domain that binds specific ligands. Upon ligand attachment, EGFR undergoes activation and dimerization, leading to autophosphorylation at multiple tyrosine residues within its intracellular region. This phosphorylation event recruits adaptor proteins, such as Grb2 and SOS, which activate the rat sarcoma virus (RAS) protein by facilitating the exchange of GDP for GTP. Activated RAS initiates a phosphorylation cascade through a series of kinases, beginning with Raf (MAPKKK), followed by MEK (MAPKK), and then MAPK/ERK (Ahmad et al. 2021). This ERK signaling pathway plays a vital role in managing cell proliferation, differentiation, and survival. Abnormal EGFR/MAPK pathway activation is

commonly observed in several cancers, including colorectal cancer (CRC), where it contributes to cancerous transformation and subsequent progression of tumor by incrementing cell proliferation, angiogenesis, cellular survival, resistance to programmed cell death, invasion, and metastasis (Barbosa et al. 2021). Research has highlighted the EGFR/MAPK signaling pathway as a significant contributor to CRC tumor development and progression, positioning it as a critical target for therapeutic interventions aimed at controlling CRC growth and spread (Frattini et al. 2015 Jan; Jeong et al. 2018).

2.4.2 Wnt/β-catenin Pathway

The Wnt family comprises 19 glycoproteins that play pivotal roles in regulating developmental processes and cancer-related activities like cell proliferation, migration, and division. The signaling pathway of Wnt/β-catenin is crucial for tissue maintenance and the regeneration of structures like the intestinal lining, skin, and hair. Dysregulation of this pathway, often due to mutations, is commonly associated with sporadic colorectal cancer (sCRC). Upon accumulation, Wnt ligands bind to Frizzled (Fz) receptors, triggering a cascade that inactivates glycogen synthase kinase-3β (GSK-3β). This inactivation stabilizes β-catenin, which functions both as a cell adhesion molecule through its interaction with E-cadherin and as a transcriptional regulator. Stabilized β-catenin translocates towards the nucleus, where it partners with T-cell factor-TCF or lymphoid enhancer factor-LEF to activate genes required for cell signaling and proliferation. Without Wnt in the extracellular environment, β-catenin undergoes phosphorylation by casein kinase 1-CK1 and the APC-Axin-GSK-3β complex, leading to its ubiquitination and subsequent degradation by the proteasome. Overactivation of Wnt signaling contributes significantly to cancer cell proliferation, supporting tumor progression in advanced colorectal cancer (Koveitypour et al. 2019; Świerczyński et al. 2021).

2.4.3 PI3K-Akt Signaling Pathway

Phosphoinositide 3-kinase (PI3K) is an essential intracellular lipid kinase which regulates growth, differentiation, proliferation, migration, and survival (Narayanankutty 2019). Structurally, PI3K functions as a heterodimer consisting of a regulatory sub-unit (p85; a protein of molecular weight 85 kilodaltons) and a catalytic unit (p110). Protein kinase B (AKT/PKB), a downstream serine/threonine kinase, is a key mediator of PI3K's role in tumor growth and progression (Temiz

et al. 2014). In CRC, AKT phosphorylation is linked to enhanced cell proliferation and the apoptosis inhibition. Therefore, the PI3K/AKT pathway has emerged as a significant therapeutic target in cancer treatment (Castel et al. 2021). The activation of PI3K occurs when ligands bind to receptor tyrosine kinases-RTKs on the cell surface. This activation leads PI3K to phosphorylate the membrane phospholipid phosphatidylinositol-4,5-bisphosphate (a.k.a. PIP2), converting it into a secondary messenger phosphatidylinositol-3,4,5-trisphosphate (a.k.a. PIP3). PIP3 subsequently binds and activates AKT by phosphorylating its threonine and serine residues, facilitating processes that support the proliferation and survival of cells. Activated AKT regulates various downstream targets, including the mammalian target of rapamycin-mTOR, a key regulator of the cell cycle that drives cell growth, angiogenesis, protein synthesis, and cell survival. The tensin and phosphatase homolog (PTEN) which is a negative regulator and tumor suppressor of the PI3K pathway, dephosphorylates PIP3, thereby inhibiting PI3K signaling. In colorectal cancer (CRC), dysregulation of components within this pathway often leads to uncontrolled cell proliferation and cancer progression. Thus, the PI3K pathway plays a critical part in the onset and the further development of CRC.

2.4.4 Transforming Growth Factor Beta (TGF-β) signaling Pathway

The TGF-β signaling pathway plays a central role in controlling cell processes like cell division, proliferation, growth, migration, and adhesion. This pathway is activated when a TGF-β ligand binds to its receptors, leading to receptor dimerization and the formation of a receptor complex. Upon activation through phosphorylation, these receptors stimulate downstream transcription factors, particularly the SMAD proteins. Specifically, SMAD2 and SMAD3 undergo phosphorylation and form heterodimers, which then associate with SMAD4 to create a heterotrimer. This complex upon entering the nucleus binds to target genes regulated by TGF-β, thereby controlling their transcription. Recent research highlights TGF-β's tumor-suppressive functions, particularly in managing division of cells, cellular differentiation and apoptosis in epithelial cells of colon (Jung et al. 2017). In the early CRC stages, TGF-β signaling is often lost, resulting in resistance to growth inhibition. Conversely, in late stages of CRC, TGF-β expression is increased, leading to epithelial-to-mesenchymal transition (EMT). This increased TGF-β expression reduces the normal cellular immune response and enhances cell migration and invasion. TGF-β can also trigger epithelial-mesenchymal transition (EMT) through an alternative pathway

that bypasses SMAD4, utilizing the signaling pathway of Ras homolog family member A (Levy and Hill 2005).

2.4.5 Notch Signaling Pathway

The signaling pathway notch is an evolutionarily conserved mechanism which regulates key cellular processes like development, differentiation, proliferation, growth, and apoptosis (Previs et al. 2015). It comprises four receptors (Notch1–4) and two ligand families: the Jagged family (JAG1-JAG2) and Delta-like family (DLL1, DLL3, and DLL4). The notch receptors are the TM proteins composed of intracellular and extracellular domains, whereas the ligands are TM proteins characterized by putative epidermal growth factor-like repeat motifs (Previs et al. 2015).

The notch signaling pathway begins after the activation of notch ligands via ubiquitination mediated by the MIB protein (mind bomb protein). Upon activation, these ligands bind to the ectodomain of the notch receptor causing cleavage of the receptor's extracellular domain by a metalloproteinase-ADAM protease and disintegrin. This is followed by the cleavage of the notch intracellular domain (a.k.a. NICD) by γ-secretase, releasing it from receptor's transmembrane-domain. The released protein domain is translocated into the nucleus, where it binds the transcription factor CSL (CBF-1/suppressor of hairless/LAG1). This interaction forms a transcriptional activation complex with transcriptional co-activator proteins such as mastermind like proteins-MAML and the histone-acetylase p300. Histone acetyltransferase-HAT and p300 further activate transcription factors, facilitating the expression of Notch target genes (Carulli et al. 2015; Borggrefe and Oswald 2016). Research has shown that several proteins of the Notch signaling pathway such as the receptor, ligand and transduction proteins are frequently overexpressed in CRC cases (Tiwari et al. 2018). Recent findings suggest that Notch signaling contributes to the progression of CRC by modulating the cell cycle and apoptosis, particularly through the regulation of genes like p21 and p53 upregulated modulator of apoptosis (PUMA) (Liao et al. 2018). As a result, targeting and inhibiting Notch signaling may offer a promising therapeutic approach for CRC patients.

2.4.6 NF-kB Signaling Pathway

The NF-κB protein complex consists of two key subunits, p65 and p50, which play a critical role in its activation and nuclear translocation. The NF-κB family includes five major transcription

factors like RelA/p65, RelB, c-Rel, NF-κB1 (p50/p105), and NF-κB2 (p52/p100). These factors are regulate various cellular processes, which includes development of cell and its differentiation, cell cycle regulation, and migration, functioning as heterodimers within the NF-κB signaling cascade (Wong et al. 2011) .

Activation of the NF-κB pathway is initiated by extracellular signals like cytokines, growth factors, viral components, lipopolysaccharides-LPS, and Toll like receptor-TLR ligands. These stimuli phosphorylate and activate the IκB thorugh the IκK complex. This phosphorylated kappa B is further lysed through the ubiquitination-dependent proteasomal degradation, releasing the NF-κB into the nucleus. Once activated, NF-κB induces the transcription of target genes linked to the initiation and progression of CRC (Figure 2.4) (Soleimani et al. 2020).

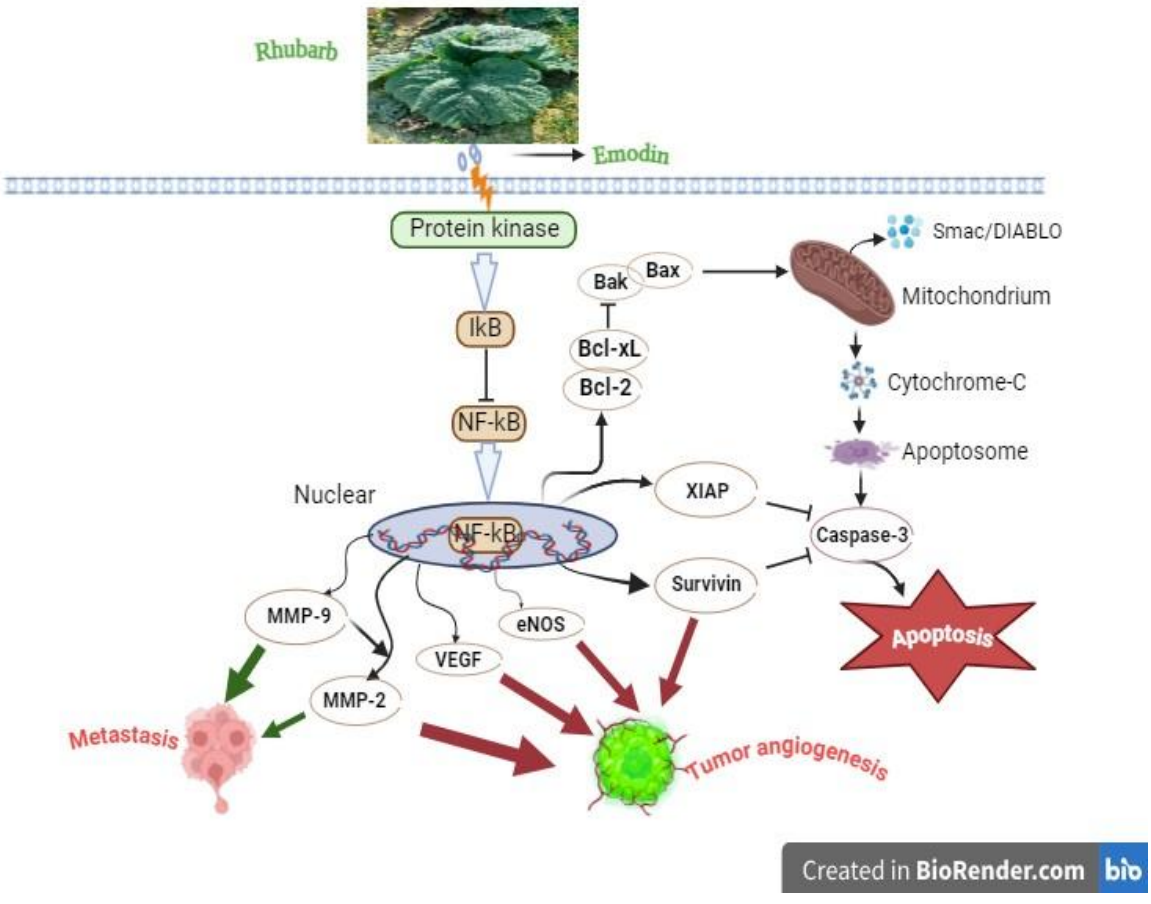


Figure 2.4: Schematic illustrations of possible antitumor mechanisms of *Rheum*.

This pathway is activated by ligands such as CD40L, BAFF, RANKL, and the RelB (p100; protein with molecular weight 100 kDa) subunits (Martin et al. 2021). Upon activation, NIK phosphorylates IKKα, which subsequently phosphorylates p100, leading to its processing into p52. The resulting RelB/p52 complex translocates to the nucleus, where it regulates the transcription of specific target genes. Studies indicate that NF-κB signaling plays a pivotal role in the progression of colorectal cancer (CRC), influencing malignancy across different stages (Soleimani et al. 2020). This pathway contributes towards the CRC cell proliferation, supports anchorage independence, and facilitates cellular migration, and possibly invasion (Hartley et al. 2020). Consequently, targeting the NF-κB pathway has emerged as a potential therapeutic approach for CRC management.

2.5 Current therapeutic approaches for the CRC treatment

CRC is conventionally treated by surgery, chemotherapy and radiotherapy, while all the methods have not shown any complete successful outcome (Higashi et al. 2011). CRC patients are usually treated conventionally using cytotoxic chemotherapy, irrespective of their ailment onset duration and is a way of an overtreatment in the young patients (Baskar et al. 2012). One of the latest tools for detecting colorectal neoplasm is CT (Computed tomographic) colonography, which has a good acceptance among people but may have adverse effects on the health of the patients. The adverse effects reported in the patients who underwent the colorectal tomographic colonography were vasovagal reactions and had an occasional diagnosis of colorectal perforations. For surgical treatment of colorectal cancer this is one of the drawbacks reported, also with the limitation of the possibility of recurrence of the disease.

The most common treatment known for CRC is chemotherapy, which is done upon the progression of cancer to the level when it could not be treated by radiotherapy or surgery (Alexandrescu et al. 2017). The chemicals used in the treatment of CRC include the antitubulin agents such as taxanes, doxorubicin and cisplatin- the DNA interactive chemicals, antimetabolites like methotrexate, and molecular targeting agents and hormones (Fan et al. 2017). Upon the use of these drugs in the clinical treatment of CRC, many adverse effects like neurological dysfunction, bone marrow suppression, gastrointestinal lesion, cardiac toxicity and drug resistance arise constantly (Nussbaumer et al. 2011). Furthermore, resistance to drugs used in chemotherapy is one of the

major challenges in CRC metastases management, which contributes to the higher death rates (Hammond et al. 2016).

In spite of the advancement in the therapeutic interventions, approximately about 40% of the colorectal cancer patients are expected to die mainly due to metastasis (Siegel et al. 2011). Although CRC at the early stage can be resected surgically, but at the advanced stage, CRC recurs frequently and becomes deadly even in the chemotherapy receiving patients (Chung and Saltz 2007). Development of CRC is due to the result of cascade of genetic events (ER 1990) . Some forms of this disease are genetically heritable and mostly sporadic cases of CRC are linked to the type of diet and lifestyle (Burn et al. 2011; Louis et al. 2014). In this study, we utilized the established colon carcinogen, 1,2-dimethylhydrazine-DMH to induce carcinogenesis and the development of preneoplastic lesions in the colon. The administration of DMH leads to the generation of ROS (reactive oxygen species), which ultimately result in oxidative stress (Martin et al. 1973). Administration of DMH cause significant increase in the level of oxidative stress marker like MDA (Malondialdehyde) (Gaweł et al. 2004) and reduction in the cellular antioxidants like GSH (reduced glutathione), SOD (superoxide dismutase), CAT (catalase) levels (Pence 1991). DMH is metabolized into azoxymethane, which induces DNA base methylation in the colon epithelial cells. This methylation leads to the formation of dysplastic aberrant crypts, followed by the development of adenomas and adenocarcinomas, which are characteristic features of colon cancer (Wolter and Frank 1982; Tanaka 2009).

To overcome the problems of these inefficient methods of treatment and to minimize their adverse reactions, the plant origin compounds and antioxidant compounds need to be investigated and explored for the treatment of CRC.

2.6 Plant derived compounds in CRC treatment

Recent focus has been on the discovery of therapeutic agents against the colorectal cancer cells by making use of plant products and phytochemicals as active compounds. Ample of studies and evidences indicate clearly that dietary consumption of vegetables and fruits has link with the low risk of CRC (Liu et al. 2017).

There are evidences also suggesting that the activity of antioxidants in plants play a significant role in anti-proliferative effects, and hence justifying the potential of antioxidants against the

growth of cancer cells (Patta and Fakih 2011). These bioactive compounds besides playing role in prevention, can also modulate the signaling pathways possibly to trigger apoptosis in CRC cells (Fulda 2010). Studies of chemoprevention which use plant-derived vitamins, compounds and supplements has been explored extensively in recent years after considering the role played by the inflammation in the formation of tumors. CRC takes about 20 years before the occurrence of initial symptoms, to inhibit the CRC metastasis in such a long time period chemoprevention is vital in function (Connell et al. 2017; Redondo-Blanco et al. 2017). The metastasis and inflammatory reaction of CRC is attributed to the cell signaling pathways which are regulated by the reaction oxygen species (ROS) or free radicals (Arulselvan et al. 2012; Arulselvan et al. 2016). The etiological factor of CRC is oxidative stress, which causes mutation of oncogenes and DNA damage, which leads to the activation of pathways responsible for the proliferation of cancer cells (Khlebnikov et al. 2007; Redondo-Blanco et al. 2017).

Recent studies suggest the inclusion of exogenous natural antioxidants in supplementation for chemoprevention of CRC, which has the potential for cell signaling prevention and anti-inflammatory effects (Figure 2.6) (Connell et al. 2017; Redondo-Blanco et al. 2017; Świerczyński et al. 2021).

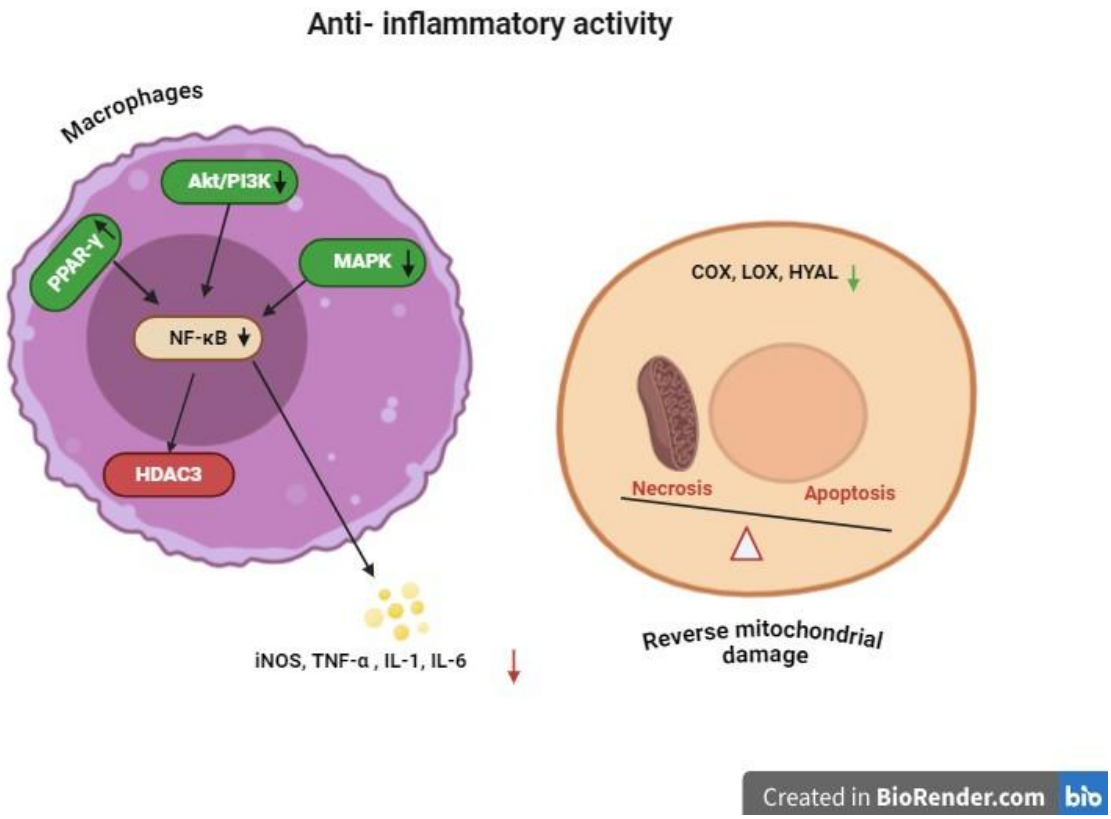


Figure 2.6: Overview of the anti-inflammatory effects of *Rheum*.

Exogenous antioxidants consist of large group of molecules which are diverse in their biochemical properties (Arulselvan et al. 2016). They are broadly grouped into Polyphenols, vitamins and derivatives, and minerals. Polyphenols being the largest antioxidant group, is further divided into phenolic acids and flavonoids. Flavonoids are majorly comprised of polyphenols in them and are found commonly in all plants (Li et al. 2010) . It has been well observed that antioxidant compounds of plants derivatives are significantly researched for treatment of various cancers, that also include CRC (Connell et al. 2017; Redondo-Blanco et al. 2017; Świerczyński et al. 2021) . Flavonoid rich diet consumption helps to reduce the severity of cancer and also lowers the risk of colorectal cancer (van der Stok et al. 2017). Phytochemicals- non-nutritive component of the plants, recently have been garnering significant scientific attention worldwide for their amazing chemopreventive potential (Abuajah et al. 2015; Benarba et al. 2015). Hence chemoprevention via phytochemicals can be a suitable alternative approach for preventing and treating colorectal cancer.

As per the studies, *Rheum* contains various types of compounds like flavonoids, tannins, anthraquinone glycosides, volatile oils and saponins (Lu and Chen 1989; Aslam et al. 2012). These herbs possesses antifungal, antimicrobial, wound healing, immune enhancing, antioxidant and anticancer activities and is often called as "the wondrous drug" because of its common worldwide and extensive medicinal use (Bilal et al. 2014) (Table 2.6). Additionally, these herbs have been reported to exhibit a range of biological activities, including laxative, diuretic, antioxidant, and anti-cancer effects, as well as in vivo inhibitory activity against P388 leukemia in mice (Venkatadri Rajkumar et al. 2011). Upon testing the methanol fraction and aqueous *Rheum emodi* extracts in human liver (Hep3B) and breast carcinoma (MDA-MB 435S) cell lines, it was found that the extracts of this herb showed extensive cytotoxicity in the tested cells (Venkatadri Rajkumar et al. 2011; V. Rajkumar et al. 2011). The rhizomes of *Rheum* are officially recognized in the Indian Pharmacopoeia and hold considerable importance due to their traditional use in Ayurvedic, Unani, and folk medicine. They are also a key ingredient in various polyherbal formulations, which are utilized for managing blood fat levels, treating hepatitis, and addressing cancer (Singh et al. 2005; Singh et al. 2010). Scientific studies following the traditional use of *R. webbianum* have reported that crude polar extracts of this plant exhibit promising anti-inflammatory, antioxidant, and cytotoxic properties (Basant Ballabh and Chaurasia 2009; Venkatadri Rajkumar et al. 2011). A new research report showcases the extensive medicinal expertise of Ladakhi communities, detailing 176 medicinal plants employed in basic healthcare, including the traditional application of *Rheum webbianum*. The study emphasizes the importance of indigenous knowledge in treating diverse health conditions, highlighting *Rheum webbianum* as prominently utilized across all eight regions surveyed in Ladakh (Wani et al., 2022; Batool et al., 2023).

Table 2.6: Anti-Cancer effects of *Rheum* derivatives against various cancer types

Type of Cancer	*Rheum* Species/ Compounds	Type of Extract used	Outcomes
Breast Cancer	*R. Palmatum* *R. undulatum* *R. emodi* *R. tanguticum* *R. officinale*	Ethanol, aqueous and ethyl acetate extracts.	Inhibited migration, motility, and invasion in highly aggressive MDA-MB-231 human breast cancer cells. Also caused cancer-specific cytotoxicity, inhibited the proliferation, cell growth and induced apoptosis in MCF-7 cancer cells (Li et al. 2009; Nho et al. 2015).
Colorectal cancer	*Rheum ribes*, *Rheum palmatum*, Emodin, Aloe-emodin	Crude extracts.	Inhibits HT-29 colorectal cancer cell growth in a dose-dependent manner. Inhibited cell migration and invasion in LS1034 cell (Ma et al. 2015; Erdoğan et al. 2020).
Lung cancer	*Rheum officinale*, *Rheum palmatum*	Water extract	Significant decrease in the cell number. Suppress cell proliferation and reduce clone formation rate by induction of apoptosis in A549 cancer cells (Zhang et al. 2020).
Hepatic cancer	*Rheum emodi* *Rheum palmatum.*	Methanolic and aqueous extracts of rhizomes	Revealed considerable cytotoxicity on the Hep3B cell line. Inhibited SMMC-7721 and HepG2 cell survival, migration, and invasion (Venkatadri Rajkumar et al. 2011; Ma et al. 2015; Tan et al. 2019).

Blood cancer	*Rheum turkestanicum*	Hexane, ethyl acetate and H2O extracts	Reduced cell viability in HeLa and MCF-7 cancer cell lines (Shiezadeh et al. 2013).
Pancreatic cancer	*Rheum officinale* Emodin	Gemcitabine coupled with emodin	Effectively inhibited tumor growth in mice model (Wei et al. 2011).
Oral cancer	*Rheum undulatum* *Rheum palmatum* Emodin, Aloe-emodin Rhein	Hexane extract	Induced apoptosis in HN22 and SCC15 cancer cell lines, Inhibited invasion and migration of human tongue cancer SCC-4 cells, Inhibited SCC-9 and SAS cell motility, migration and invasiveness by lowering the enzyme activities of MMP-2 (matrix metalloproteinase) (Choi et al. 2011; Chen et al. 2017).
Stomach cancer	Rhapontin	-	Rhapontin suppresses KATO III cell growth by inducing apoptosis (Hibasami et al. 2007).

Screening of medicinal plants under in vitro situations is a very quick task to determine their medicinal importance but however, results obtained from in vitro experiments may not fully or accurately predict. *In-vivo* animal studies have been fundamental to biological and biomedical research, especially in clinical medicine and pharmaceutical development. As per the best of our knowledge, no *in-vivo* studies have been conducted in relation to the chemopreventive potential of *R. webbianum* extracts in colorectal cancer formation.

Therefore, the potential of *Rheum webbianum* as a prevention agent in CRC requires thorough investigation. Hence this study is aimed to investigate the chemopreventive potential of *R. webbianum* rhizome extracts, on 1,2 dimethylhydrazine mediated colorectal carcinogenesis in Swiss albino rats by analyzing the oxidative stress inhibition and the level of expression of various proteins.

Hypothesis

Colorectal cancer (CRC) is one of the leading causes of morbidity and mortality related to cancer across the globe. The exploration of natural compounds with chemopreventive properties offers a promising approach to addressing this significant health burden.

Rheum webbianum (RW) apart from its traditional use as a food source, have demonstrated potential anticancer activities, making them a subject of interest in the treatment of various cancers, including CRC. Revered in the Unani and Ayurvedic medical systems, this plant boasts significant therapeutic potential. Despite this extensive literature, there remains a paucity of research investigating the chemopreventive capabilities of *Rheum webbianum* (RW) extracts concerning colorectal cancer.

This study hypothesizes that *Rheum webbianum* rhizome extracts may exert a cytotoxic effect on HCT-116 human colon cancer cell lines, demonstrating their broad-spectrum anti-cancer potential. Furthermore, it is anticipated that RW extracts may possess significant chemopreventive properties capable of inhibiting the initiation and progression of colorectal cancer. Specifically, it is proposed that RW extracts, through their modulation of oxidative stress and key signaling pathways like WNT/β-catenin and TGF-β, can reduce the oncogenic effects induced by the carcinogen 1,2-dimethylhydrazine (DMH) in Swiss albino rats.

Additionally, it is expected that RW extracts will lead to significant improvement in histopathological alterations in the colon and positively affect hematological and hepatic markers, further supporting their chemopreventive potential.

This study aims to comprehensively assess the role of RW extracts in CRC prevention by integrating cell line-based (in-vitro) and murine (in-vivo) models, elucidating their mechanisms of action, and providing a foundation for their potential use in future therapeutic strategies.

CHAPTER 3

OBJECTIVES

Research Objectives

1. **To determine the cytotoxic effect of *R. webbianum* rhizome extracts on HCT116 human colon cancer cell line.**

2. **To evaluate the in vivo effect of *R. webbianum* extract showing highest cytotoxic activity on dimethylhydrazine (DMH) induced histopathological alterations in colon.**

3. **To determine enzymatic and non-enzymatic endogenous antioxidants modulation in colon.**

4. **To analyse the expression of key cell signalling proteins such as wnt & TGF-β in colon cancer employing western blotting technique.**

5. **To assess the hematological and hepatic markers in different groups of animals.**

CHAPTER 4

METHODS AND MATERIALS

4. Methods and Materials

4.1 Chemicals

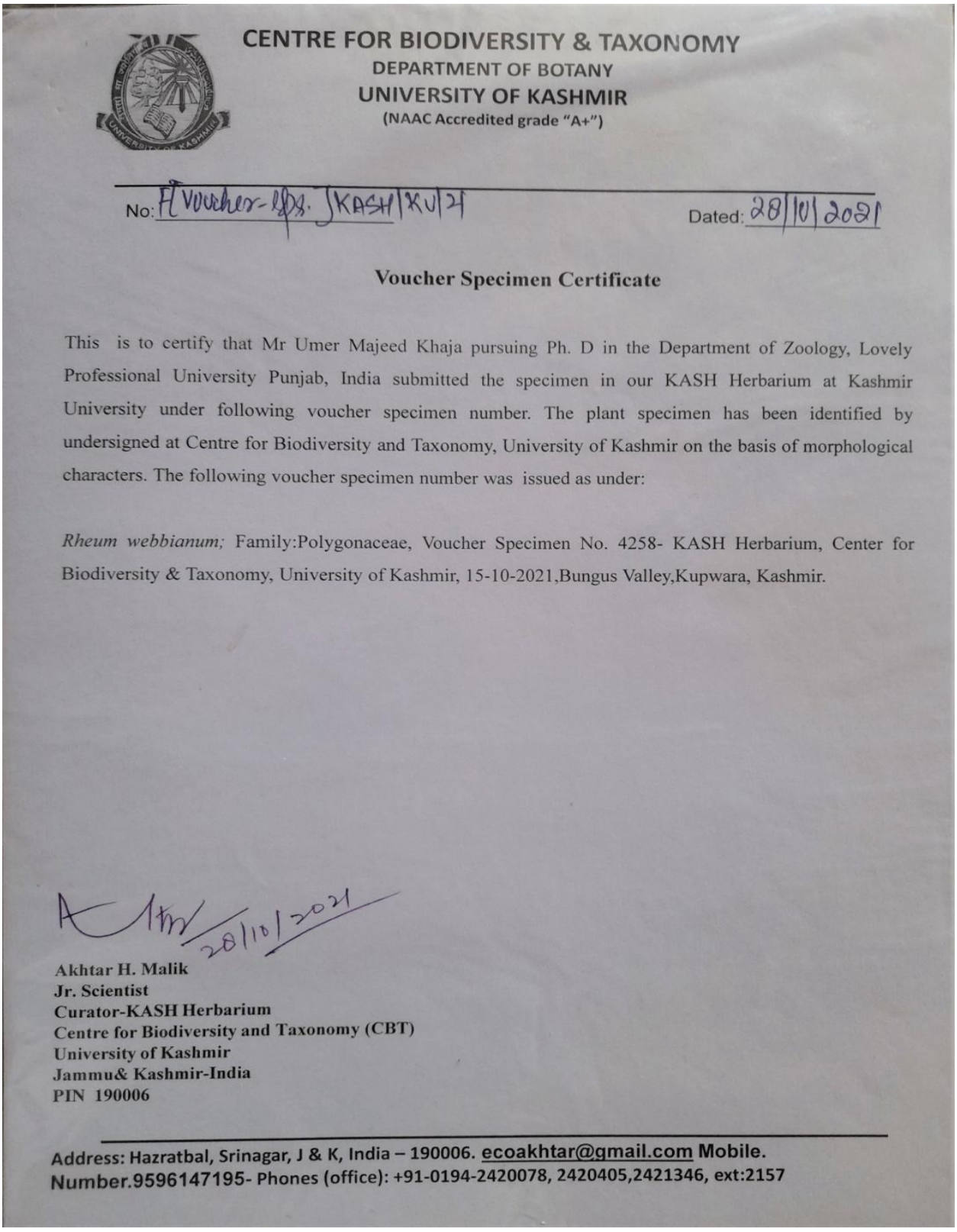
DMH: 1,2-Dimethylhydrazine and other chemicals needed were purchased from the registered pharmaceutical company.

4.2 Plant material

Specimens of *Rheum webbianum* were procured from their natural habitat in Bungus Valley, situated in the Jammu and Kashmir region of India. These specimens were then transported to the KASH-Herbarium, housed within the Centre for Biodiversity and Taxonomy at the University of Kashmir, for accurate identification. Following meticulous examination, the plant was identified as *Rheum webbianum* Royle, a member of the Polygonaceae family, and assigned the Voucher Specimen No. 4258.

Picture of *Rheum webbianum* in natural habitat

Plant Identification Certificate



CENTRE FOR BIODIVERSITY & TAXONOMY
DEPARTMENT OF BOTANY
UNIVERSITY OF KASHMIR
(NAAC Accredited grade "A+")

No: FI Voucher-Rpts. JKASH/KU/21 Dated: 28/10/2021

Voucher Specimen Certificate

This is to certify that Mr Umer Majeed Khaja pursuing Ph. D in the Department of Zoology, Lovely Professional University Punjab, India submitted the specimen in our KASH Herbarium at Kashmir University under following voucher specimen number. The plant specimen has been identified by undersigned at Centre for Biodiversity and Taxonomy, University of Kashmir on the basis of morphological characters. The following voucher specimen number was issued as under:

Rheum webbianum; Family:Polygonaceae, Voucher Specimen No. 4258- KASH Herbarium, Center for Biodiversity & Taxonomy, University of Kashmir, 15-10-2021,Bungus Valley,Kupwara, Kashmir.

28/10/2021

Akhtar H. Malik
Jr. Scientist
Curator-KASH Herbarium
Centre for Biodiversity and Taxonomy (CBT)
University of Kashmir
Jammu& Kashmir-India
PIN 190006

Address: Hazratbal, Srinagar, J & K, India – 190006. ecoakhtar@gmail.com Mobile. Number.9596147195- Phones (office): +91-0194-2420078, 2420405,2421346, ext:2157

4.3 Extraction of *Rheum webbianum* rhizomes

The rhizomes of the plant were carefully dried in shaded conditions and subsequently ground into a fine powder. This powder underwent extraction using a Soxhlet apparatus with a series of solvents including Hexane, Ethyl acetate, Ethanol, and Methanol. The resulting extracts were concentrated in a hot air incubator at a controlled temperature of 45°C. Ultimately, the concentrated extracts were stored in a refrigerator set at 4°C to preserve their integrity and were freshly prepared before experiments.

4.4 Cell lines and culture

For our study, we utilized the HCT-116 human cell line, which is derived from colorectal cancer and was received from the "National Centre for Cell Science" in Pune. The cells were grown in DMEM medium (Sigma Life Science, D0819) with the addition of 10% fetal bovine serum (Sigma Life Science, F9665), 1% penicillin-streptomycin combination (Himedia laboratories, India), and 1% (w/v)of L-Glutamine. The cells were grown in a controlled environment with high (95%) humidity and a 5% concentration of carbon dioxide at a temperature of 37°C.

4.5 Cell Viability Assay (*in-vitro*)

The cells were seeded into 96-well plates at a density of 10,000 cells per well. On next day, the cells were treated with increasing concentrations of RW extracts or dimethylsulfoxide (DMSO) for the control wells for one day. Following incubation, the medium was removed, and the cells were treated with 100 µL MTT for 3 hours (working concentration 0.5 mg/mL) per well (procured from Sigma Aldrich). The MTT tetrazolium salt was metabolized by viable cells, resulting in the formation of the purple formazan product. Following incubation, the purple-formazan crystals were dissolved in 100 µL of solution comprising 11% SDS, isopropyl alcohol, and N/100 HCl. The absorbance of the resulting formazan product was measured at 570 nm with a reference wavelength of 690 nm using an ELISA reader (Biotek Synergy 2, 266,278). The percentage of cell viability was calculated using the formula: [(Mean OD of treated cells − Mean OD of blank) / (Mean OD of control − Mean OD of blank)] × 100, where the blank refers to the solubilization solution (Venkatadri Rajkumar et al. 2011).

4.6 Experimental design (*in-vivo*)

Forty-two rats procured from IIIM, Jammu, were assigned randomly to six groups, and each group consisted of seven rats. The sample sizes were determined based on preliminary data, ensuring that the study has sufficient power to detect significant differences between experimental conditions, thereby enhancing the reliability and reproducibility of the findings. The rats were kept in a controlled laboratory environment with a temperature of 22 ± 2°C, relative humidity of 50% ± 10%, and a 12-hour cycle of light and darkness. Animals had unrestricted access to water and food. Prior to commencing the experiments, the animals underwent a one-week acclimatization period to the laboratory environment. The experimental phase lasted for 10 weeks, and the treatment protocols are outlined in (Table 4.1). Upon completion of the period of treatment, animals were anesthetized with ether and euthanized via cervical decapitation. All procedures involving animal subjects adhered to the guidelines established by the Institutional Animal Ethics Committee of Kashmir University, with Approval No: F(IAEC-Approval) KU/2021/09 (Mishra et al. 2014; Narota et al. 2020).

➢ Experimental design (*In-vivo*)

The animals were equally divided into six groups of seven animals each.

- **Group 1 (Control)**
- **Group 2 (Only DMH treated)**
- **Group 3 (DMH + *R. webbianum* ethanolic extract 1)**
- **Group 4 (DMH + *R. webbianum* ethanolic extract 2)**
- **Group 5 (DMH + *R. webbianum* methanolic extract 1)**
- **Group 6 (DMH + *R. webbianum* mthanolic extract 2)**

Ethical Certificate

INSTITUTIONAL ANIMAL ETHICS COMMITTEE
DEPARTMENT OF PHARMACEUTICAL SCIENCES
UNIVERSITY OF KASHMIR
Registration No. 801/GO/Re/S/03/CPCSEA

Chairperson
Prof.Nahida Tabassum
Deptt.of Pharm Sciences
University of Kashmir

Member Secretary/ Scientist Incharge
Dr.M. Iqbal Zargar

Members
Dr.Asgar Samoon (Main nominee)
Dr.Mukund Lal Sharma
Prof.Khalid Fazili
Dr.S.M.M. Qadri
Dr.Manzoor Ur Rehman Mir
Dr.Govind Yadev
Prof.Afzal Zargar

F(IAEC-Approval)KU/2021/09
Dated: 16-11-2021

CERTIFICATE

Name of the Investigator: Umer Majeed Khaja
Designation: Research Scholar
Department: Clinical Biochemistry, University of Kashmir.

This is to certify that the project title: **"Evaluation of ameliorating potential of *Rheum webbianum* rizome extracts on 1,2-dimethylhydrazine induced colorectal carcinogenesis in Swiss albino rats."** has been approved by IAEC of the department on 16-11-2021.

No. of animals approved: 60
Species: Swiss albino rats/Wistar rats
Period of the Project: 3 years

(Dr. M.Iqbal Zargar)
Member Secretary

(Prof. Nahida Tabassum)
Chairperson

- ✓ Project leader and the Investigator are impressed upon to observe the prescribed ethical guidelines while using the laboratory animals for research work in letter and spirit.
- ✓ Investigator and HOD are requested to preserve a copy of the approved letter for reference and record.
- ✓ Preserve a copy of approval letters with investigator and Department master file and keep one copy at the site of experimentation.

Table 4.1: Animal groups and treatment procedure

Group Description		Approach
1	Control	Injected weekly with 1 mM EDTA, pH 6.5 (DMH vehicle) via intraperitoneal route for seven weeks.
2	DMH only	Received weekly DMH injection in 1mM EDTA (40 mg/kg of body weight) via intraperitoneal route consecutively for 7 weeks.
3	DMH + RWEE-1	Daily oral dose of RWEE-1 for 14 days before the first DMH injection and administered till the final dose of DMH.
4	DMH + RWEE-2	Received a daily oral dose of RWEE-2 for 14 days before the first DMH injection and administered till the final dose of DMH.
5	DMH + RWME-1	Received a daily oral dose of RWME-1 for 14 days before the first DMH injection and administered till the final dose of DMH.
6	DMH + RWME-2	Received a daily oral dose of RWME-2 for 114 days before the first DMH injection and administered till the final dose of DMH.

Table 4.1: EDTA, Ethylene diamine tetraacetic acid, DMH, 1,2-Dimethylhydrazine, RWEE-1, *Rheum webbianum* ethanol extract-1 (50mg/kg body weight), RWEE-2, *Rheum webbianum* ethanol extract-2 (100mg/kg body weight), RWME-1, *Rheum webbianum* methanol extract-1 (50mg/kg body weight), RWME-2, *Rheum webbianum* methanol extract-2 (100mg/kg of average body mass).

All the groups of animals were kept for the 10-week time period and later sacrificed via cervical decapitation. Blood was collected in non-EDTA and EDTA coated tubes. Colon was removed and washed with saline solution and further processed for histopathology (Mishra et al. 2014; Narota et al. 2020).

4.7 Body Mass Gain in Rats

Weekly body weight measurements were recorded, and the average change in body mass was calculated for each group of animals by subtracting the initial weight from the end weight during the whole treatment duration. Dietary intake was also monitored and observed on a daily basis.

4.8 Blood samples

Blood samples were taken using the retro-orbital method and collected into tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant for hematological analysis, while samples without anticoagulant were collected for blood chemistry assessment.

4.9 Haematological parameters

An automated haematology analyzer (Beckman Coulter, USA) was used to determine various haematological parameters. These conventional parameters included (as quoted) "red blood cell count (RBC, 10^12/L), haemoglobin concentration (Hb, g/dL), hematocrit (Ht, %), mean corpuscular volume (MCV, fL), mean corpuscular haemoglobin (MCH, pg), mean corpuscular haemoglobin concentration (MCHC, g/dL), platelet count (plt, 10^9/L), and white blood cell count (WBC, 10^9/L)".

4.10 Biochemical parameters

For the purpose of performing biochemical analysis, blood samples were placed in empty tubes and kept undisturbed for a duration of 3 hours to facilitate the formation of clots. Afterwards, the tubes underwent centrifugation at a rate of 5000 revolutions per minute for a period of 15 minutes using a tabletop centrifuge type (HUMAX-K) produced by HUMAN-GmbH in Germany. The resulting plasma was transferred to sterile vials and stored at a temperature of -20°C until further analysis. The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and blood lipid profiles were measured automatically using Cobas Integra-400 plus analyzer.

4.11 Histopathological Examination

The colonic tissues were obtained and preserved by immersing them in a 4% solution of paraformaldehyde (PFA) for an entire night. Following dehydration using ethanol and xylene, the

tissues underwent staining with a 5% hematoxylin-eosin solution for a duration of 10 minutes. After the staining process, the samples were washed with distilled water for 5 minutes and then soaked in a solution of 0.1% HCl-ethanol for 30 seconds. Following additional washing and dehydration procedures, the materials were analysed using a microscope (Make-Nikon) (Narota et al. 2020).

4.12 Assessment of oxidative stress in colon homogenate

Following the process of homogenizing the colon tissue in ice-cold PBS, the resulting sample was subjected to centrifugation for a duration of 15 minutes at a temperature of 4°C and a speed of 4500 revolutions per minute (rpm). The concentrations of oxidative stress indicators, such as reduced glutathione (GSH), glutathione reductase (GR), malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT), were quantified in the supernatant (Clairbone, 1985; Flohe´ & Gu¨ nzler, 1984 & Beauchamp & Fridovich, 1971).

4.13 Evaluation of the expression level of proteins by western blotting analysis

Proteins were separated from fresh colon tissue homogenate prepared by SDS-PAGE and subsequently transferred to a PVDF membrane. The membrane was blocked with 5% non-fat dry milk in Tris-buffered saline with 0.1% Tween-20 (TBST). Following the blocking step, the membrane was washed and incubated with primary antibodies like anti-Wnt3a and anti-TGF beta specific to Wnt/β-catenin and TGF-β respectively. After primary antibody incubation, the membrane was washed and then incubated with an appropriate HRP-conjugated secondary antibody. The membrane was developed using a chemiluminescent substrate until intense bands appeared. The levels of Wnt/β-catenin and TGF-β were quantified using the Gel Doc XR+ system with Image Lab Software version 2.0.1 (Narota et al. 2020).

4.14 Statistical Analysis

The data underwent analysis using Microsoft Excel. Every experiment was reproduced independently, with a minimum of two replications. Statistical significance was established using a criterion of $p < 0.05$.

CHAPTER 5

RESULTS AND DISCUSSION

Results

5.1 Antiproliferative Effects of various *Rheum webbianum* rhizome Extracts

Four types of extracts were prepared from the rhizome of *Rheum webbianum* using four different solvents: 1. Hexane; 2. Ethyl-acetate; 3. Ethanol; 4. Methanol. Colorectal cancer cells HCT-116 were subjected to treatment with an increasing concentration ranging from 10 to 200 µg/ml of different fractions. The cellular viability was measured using the standard MTT assay after 24 hours of treatment. The ethanolic and methanolic extracts exhibited significant cytotoxic effect on HCT-116 colorectal cancer cells (Figure 5.1A and B). Based on these noteworthy findings with the ethanolic and methanolic extracts, we proceeded with an *in-vivo* study focusing on these two extracts to evaluate their effect on DMH-induced colorectal carcinogenesis in Swiss albino rats (Figure 5.1 A&B).

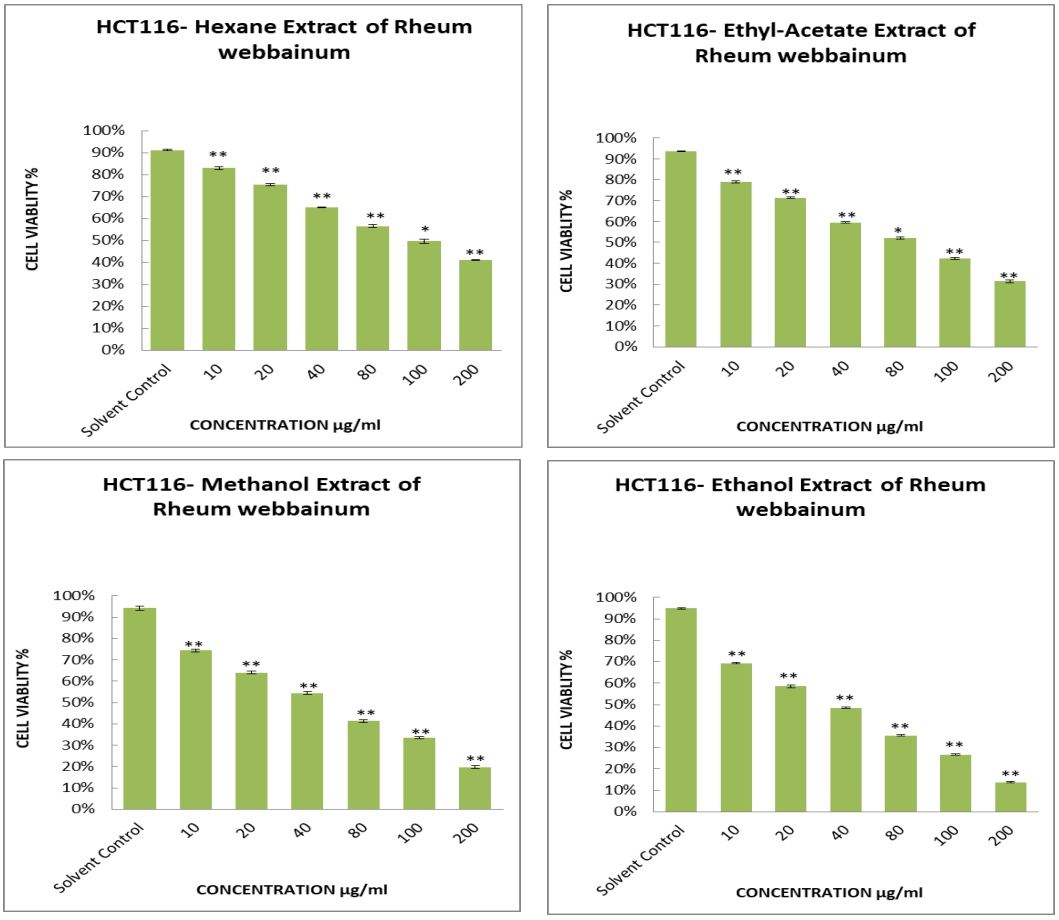


Figure 5.1 (A): Column graphs of the cytotoxicity of RW extracts on HCT-116 cells

Figure 5.1 (B)

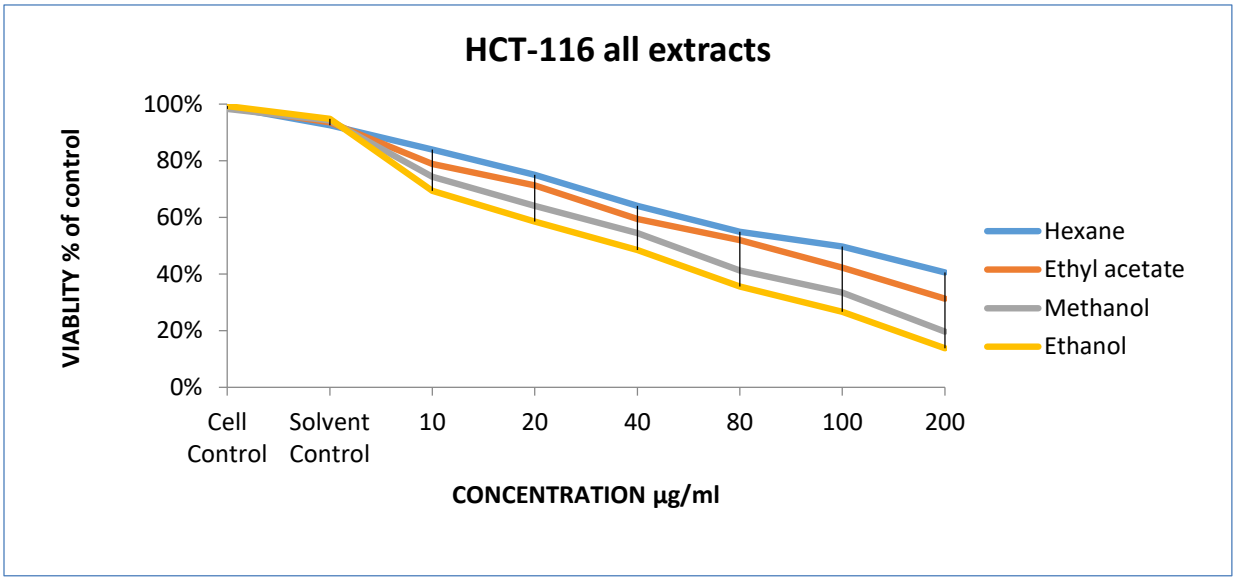


Figure 5.1 (B): Scatter plot of cell viability of HCT-116 in response to treatment with different RW extracts

5.2 **Effect of RW on body weight gain**

The weekly average increase in body weight was measured and the findings are presented in Fig 3. The animals in the control group experienced a considerable rise in average body mass over the DMH treatment period and did not show any significant differences among themselves. However, rats treated with DMH saw a reduction in the average body mass gain. Animals that received DMH + RW treatment exhibited a notable increase in average body weight compared to group treated just with DMH (Figure 5.2)

Figure 5.2

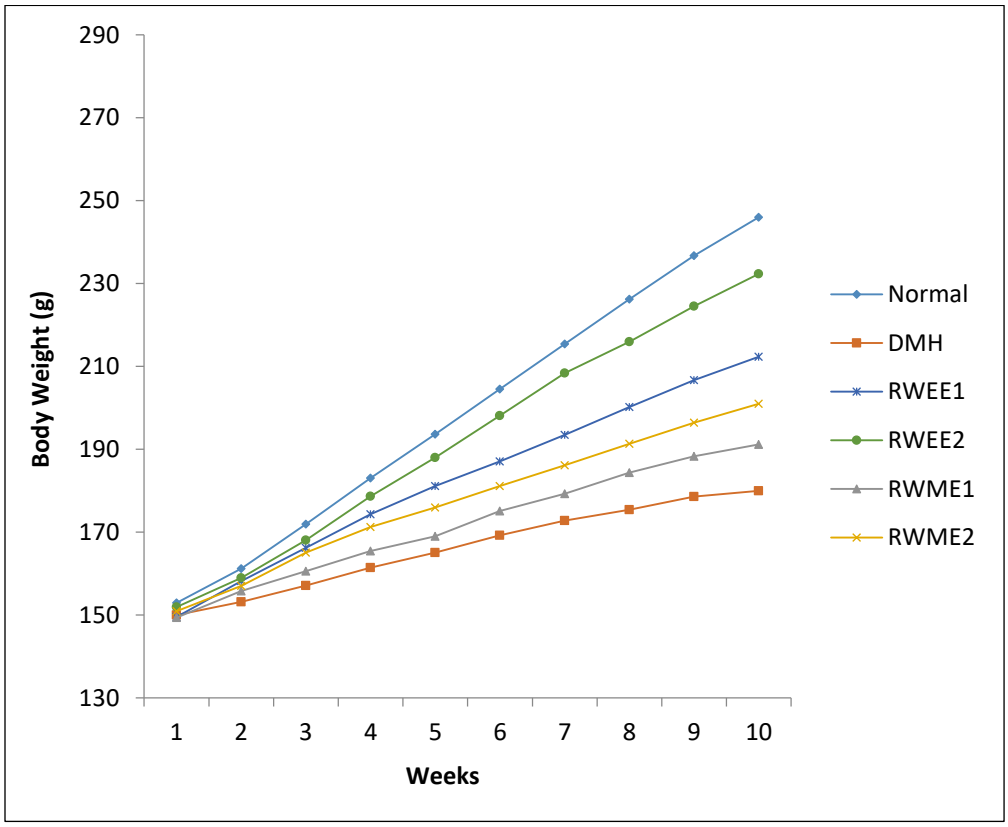


Figure 5.2: Average change in body weight for each group of animals

5.3 Haematological parameters

Treatment with DMH resulted in a decrease in red blood cell count-RBC, hemoglobin-Hb, and hematocrit-Hct by 20% each, while white blood cell count-WBC increased by 60% and platelet count (Plt) increased by 180% compared to the control group. However, pre-treatment with RW restored the entire hematological test profile of the animals at par with control group (Table 5.3 & Figure 5.3).

Table 5.3: Effect of RW extracts on haematological parameters

Haematological Parameters						
Parameter	**Control**	**DMH Only**	**Methanolic Extract 1**	**Methanolic Extract 2**	**Ethanolic Extract 1**	**Ethanolic Extract 2**
Hb (g/dL)	12.96	9.2	11.12	11.59	12.25	12.38
	13.24	8.56	10.88	11.12	11.96	12.89
Average	13.1	8.88	11	11.355	12.105	12.635
STDEV	0.1979899	0.45254834	0.16970563	0.33234019	0.20506097	0.36062446
SE	0.14	0.32	0.12	0.235	0.145	0.255
p-value		NS	NS	NS	NS	NS
RBC $10^{\wedge 6}$/Ml	9.73	5.35	7.25	7.95	8.01	8.84
	9.87	4.62	6.86	7.21	8.25	9.05
Average	9.8	4.985	7.055	7.58	8.13	8.945
STDEV	0.09899495	0.51618795	0.27577164	0.52325902	0.16970563	0.14849242
SE	0.07	0.365	0.195	0.37	0.12	0.105
p-value		NS	NS	NS	0.01905478	0.02604592
HCT (%)	38.03	28.02	32.21	34.58	33.25	37.3
	37.44	27.22	32.41	34.84	33.12	36.63
Average	37.735	27.62	32.31	34.71	33.185	36.965
STDEV	0.417193	0.56568542	0.14142136	0.18384776	0.09192388	0.47376154
SE	0.295	0.4	0.1	0.13	0.065	0.335
p-value		0.00660827	0.04627131	NS	0.03215341	0.03304146
WBC ($10^{\wedge 3}$/μL)	9.48	17.28	14.05	13.07	12.51	10.68
	9.12	16.11	14.21	13.32	11.85	10.06

Average	9.3	16.695	14.13	13.195	12.18	10.37
STDEV	0.25455844	0.82731493	0.11313708	0.1767767	0.46669048	0.4384062
SE	0.18	0.585	0.08	0.125	0.33	0.31
p-value		0.03483079	0.03423634	0.04974932	0.03312735	NS
Plt ($10^{\wedge}5/\mu L$)	5.67	9.01	7.87	6.4	7.2	6.15
	5.51	9.88	7.55	6.44	7.13	6.22
Average	5.59	9.445	7.71	6.42	7.165	6.185
STDEV	0.11313708	0.6151829	0.22627417	0.02828427	0.04949747	0.04949747
SE	0.08	0.435	0.16	0.02	0.035	0.035
p-value		NS	0.02401199	NS	0.01818419	NS
MCV (fL)	55	48	49.05	52.12	52.18	54.36
	57	46	50.1	53.11	53.33	54.66
Average	56	47	49.575	52.615	52.755	54.51
STDEV	1.41421356	1.41421356	0.74246212	0.70003571	0.8131728	0.21213203
SE	1	1	0.525	0.495	0.575	0.15
p-value		NS	0.04697981	NS	NS	NS
MCH(pg)	17.07	13	15.17	15.89	16.24	16.96
	17	11.96	14.32	16.01	15.25	17
Average	17.035	12.48	14.745	15.95	15.745	16.98
STDEV	0.04949747	0.73539105	0.60104076	0.08485281	0.70003571	0.02828427
SE	0.035	0.52	0.425	0.06	0.495	0.02
p-value		NS	NS	NS	NS	NS

MCHC	30.85	35.04	32.12	31.74	32.1	31.21
	29.45	37	33.65	29.66	31.32	30.02
Average	30.15	36.02	32.885	30.7	31.71	30.615
STDEV	0.98994949	1.38592929	1.08187338	1.4707821	0.55154329	0.84145707
SE	0.7	0.98	0.765	1.04	0.39	0.595
p-value		NS	NS	NS	NS	NS

Figure 5.3

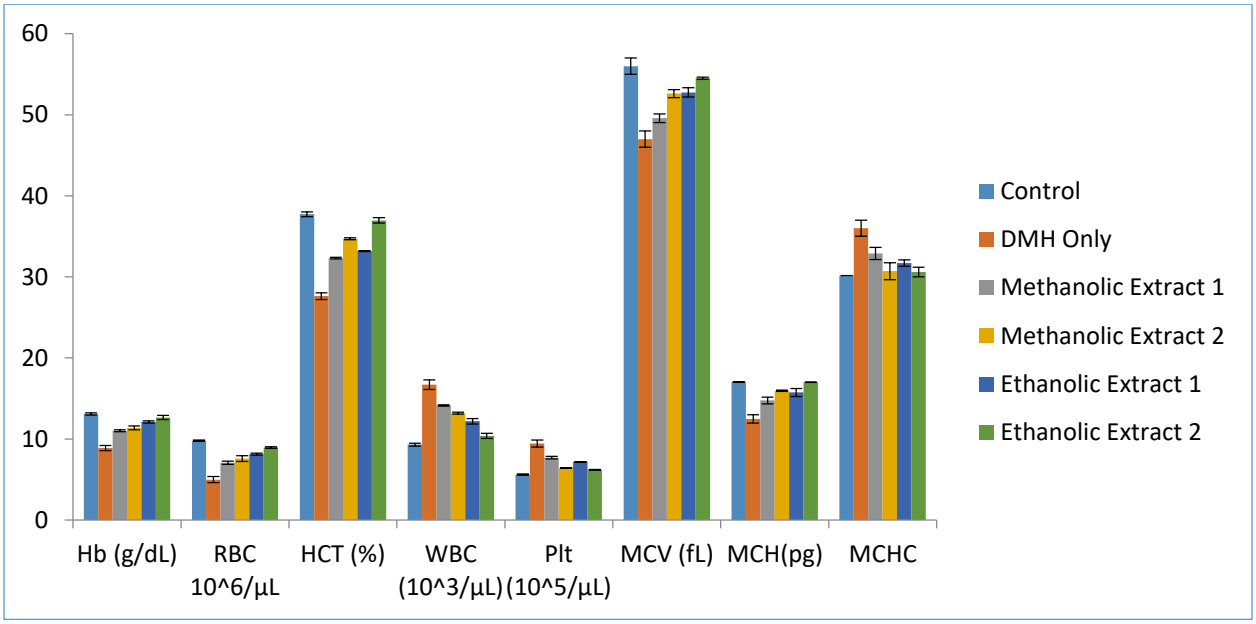


Figure 5.3: Column graph showing data represented as average values of hematological parameters. Control; DMH, 1,2-dimethyl-hydrazine; Methanolic Extract 1 (50mg/kg BW); Methanolic Extract 2 (100mg/kg BW); Ethanolic Extract 1 (50mg/kg BW); Ethanolic Extract 2 (100mg/kg BW); RBC, red blood cell; Hb, haemoglobin; HCT, haematocrit.

Oxidative stress induced by DMH in rats led to changes in their hematological profile. We observed a reduction in RBC count, Hb levels, and HCT, which is indicative of anemia. Additionally, there was a considerable rise in WBC and PLT counts, suggesting activation of the immune response. The rise in WBCs (leukocytosis) and platelets (thrombocytosis) is typically seen during the tumor promotion phase. WBCs, which are phagocytic, move to sites of inflammation to target altered or infected cells, including tumor cells. Their migration to these sites is often enhanced by platelets.

5.4 Biochemical parameters

In DMH-treated rats, there was a notable increase in the activity of serum hepatic aminotransferases (ALT and AST) compared to the control group of normal rats. However, administration of RW to the DMH-treated rat groups significantly alleviated these hepatic impairments, as indicated in Table 3. Furthermore, the blood lipid profiles of rats affected by DMH exhibited early signs of atherosclerosis, characterized by a substantial rise in serum total cholesterol and triglyceride levels. In contrast, the DMH groups treated with RW demonstrated effective recovery of lipid profile indices compared to the group solely treated with DMH, as depicted in (Table 5.4 & Figure 5.4).

Table 5.4: Effect of RW extracts on Serum biochemical parameters. Control; DMH, 1,2-dimethyl-hydrazine; Methanolic Extract 1 (50mg/kg BW); Methanolic Extract 2 (100mg/kg BW); Ethanolic Extract 1 (50mg/kg BW); Ethanolic Extract 2 (100mg/kg BW); aminotransferases (ALT and AST)

Serum Biochemical Parameters						
Parameter	**Control**	**DMH Only**	**Methanolic Extract 1**	**Methanolic Extract 2**	**Ethanolic Extract 1**	**Ethanolic Extract 2**
SGPT(ALT) (IU/L)	62.2	86	79.2	67.4	75.3	64.9
	63	85.25	77.32	66.56	74.23	65
Average	62.6	85.625	78.26	66.98	74.765	64.95
STDEV	0.56568542	0.53033009	1.32936075	0.5939697	0.75660426	0.07071068
SE	0.4	0.375	0.94	0.42	0.535	0.05
p-value		0.00452056	0.01055074	0.00661645	0.00696832	0.00110503
SGOT(AST) (IU/L)	103	138	115.3	109.4	112	106.3
	102.33	140	116.12	108.02	111.5	105.2
Average	102.665	139	115.71	108.71	111.75	105.75
STDEV	0.47376154	1.41421356	0.57982756	0.97580736	0.35355339	0.77781746
SE	0.335	1	0.41	0.69	0.25	0.55
p-value		0.0045824	0.00204372	0.00489153	0.00199821	0.00411359

Total protein (g/L)	64.5	70.9	68.6	82.9	76.2	80.8
	63.3	75.5	65	70	73.2	85
Average	63.9	73.2	66.8	76.45	74.7	82.9
STDEV	0.84852814	3.25269119	2.54558441	9.12167748	2.12132034	2.96984848
SE	0.6	2.3	1.8	6.45	1.5	2.1
p-value		0.01098774	0.00235746	0.00740468	0.00305003	0.01091735
Total cholesterol (mg/dL)	94	180.2	123.1	100.5	114.7	108
	93	176.12	120.05	99.5	110	98
Average	93.5	178.16	121.575	100	112.35	103
STDEV	0.70710678	2.88499567	2.15667568	0.70710678	3.32340187	7.07106781
SE	0.5	2.04	1.525	0.5	2.35	5
p-value		0.00701211	0.00762603	0.00224606	0.01340379	0.03271937
Triglycerides(mg/dL)	93.8	165.1	135.6	116.8	124.3	112.5
	91	162.55	136	118	120	110

Average	92.4	163.825	135.8	117.4	122.15	111.25
STDEV	1.97989899	1.80312229	0.28284271	0.84852814	3.04055916	1.76776695
SE	1.4	1.275	0.2	0.6	2.15	1.25
p-value		0.01083281	0.01233372	0.01239637	0.00852467	0.00959656

Figure 5.4

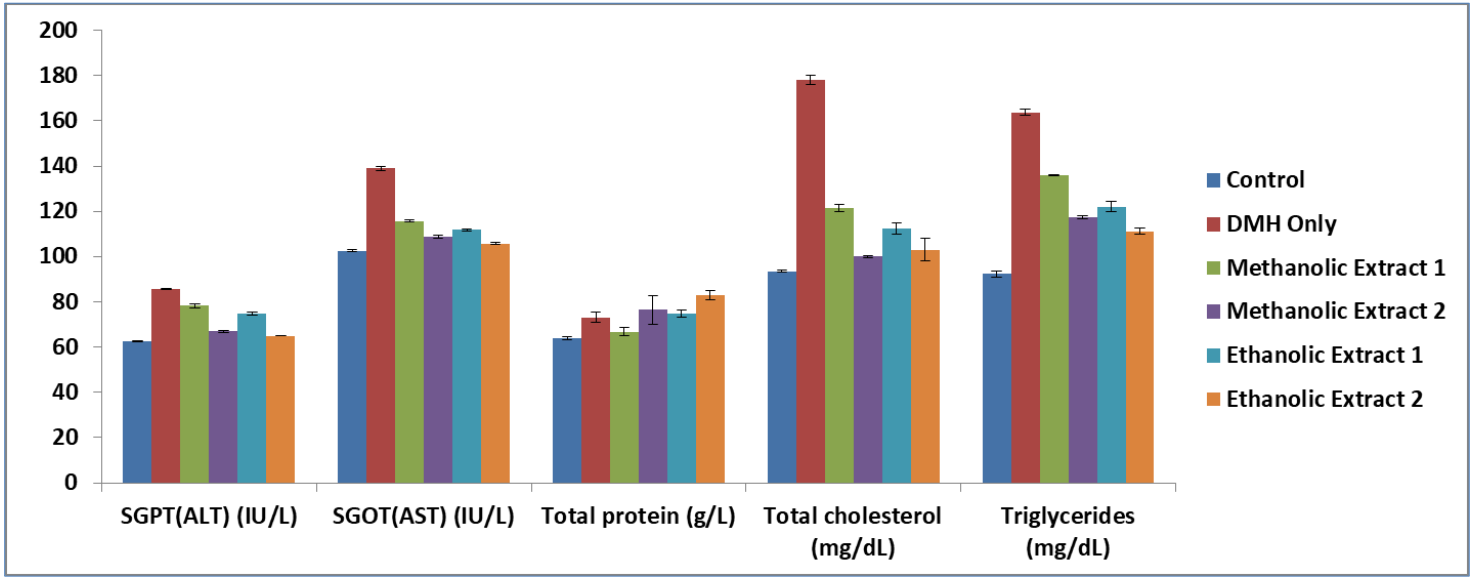
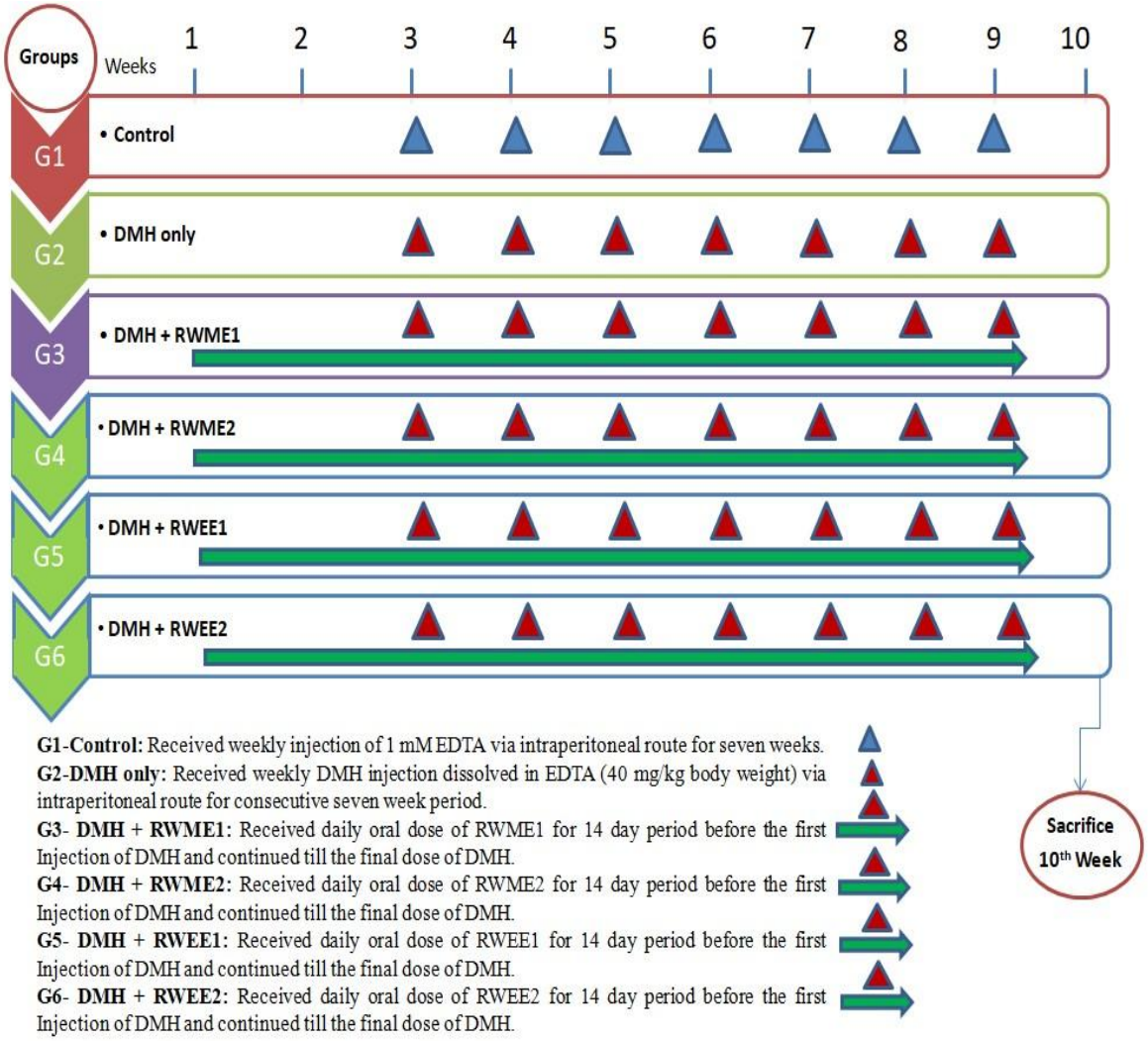


Figure 5.4: Column graph of the serum biochemical parameters in response to treatment with different extracts of R. webbianum.

5.5 *Rheum webbianum* rhizome extracts alleviate DMH-Induced Colorectal Cancer in Rats

In the control group, histological sections displayed the typical architecture of the colon. In contrast, the DMH-treated group exhibited irregular glandular structure, regional mucosal destruction, and substantial infiltration of inflammatory cells in both mucosal and submucosal layers, along with crypt abscess formation and intestinal gland cells showing dysplasia and hyperchromasia. However, in groups treated with methanolic and ethanolic extracts, histological sections revealed that RW rhizome extracts conferred protection against mucosal damage, accompanied by a notable decrease in inflammatory cell infiltration and minimal crypt abscess formation (Figure 5.6B)

(A)



(B)

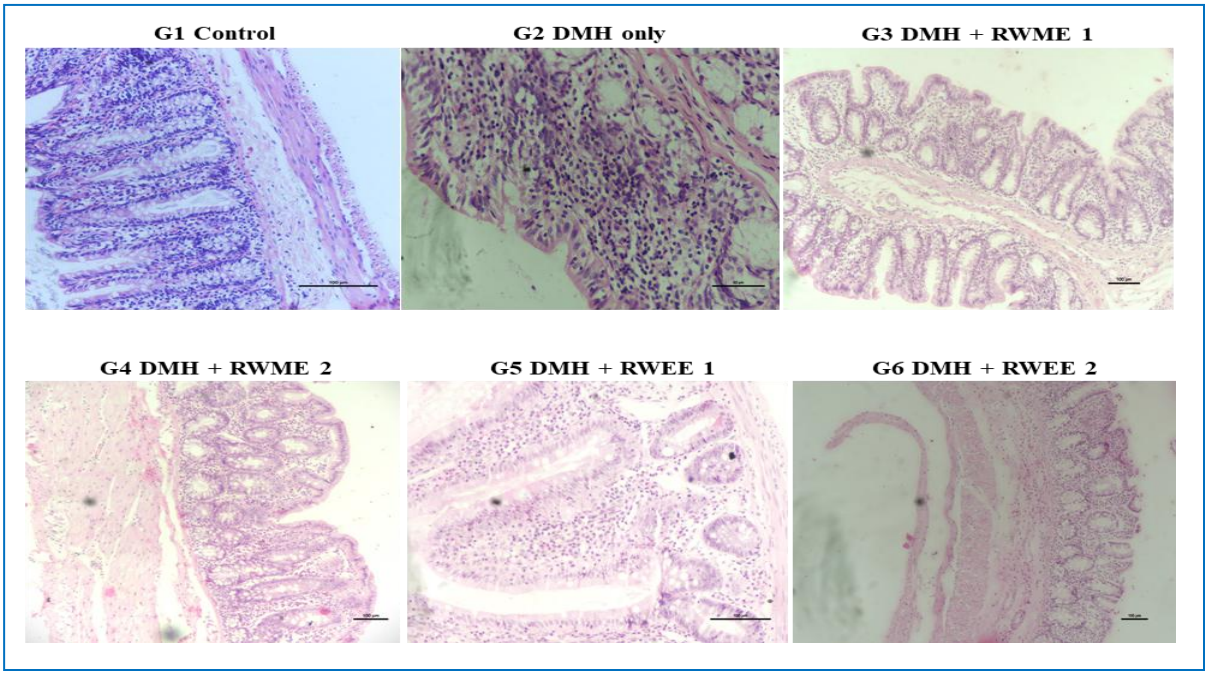


Figure 5.6: (A) – Experimental Protocol: EDTA- Ethylene diamine tetraacetic acid, DMH-1,2-Dimethylhydrazine, RWME1- *Rheum webbianum* methanolic extract 1 (50mg/kg body weight), RWME2- *Rheum webbianum* methanolic extract 2 (100mg/kg body weight), RWEE1- *Rheum webbianum* ethanolic extract 1 (50mg/kg body weight), RWEE2- *Rheum webbianum* ethanolic extract 2 (100mg/kg body weight).

5.6 (B): Histopathological examination of Colon. G1: The colon exhibited normal histoarchitecture. G2: In the DMH-treated group, there was evidence of irregular glandular structures, localized mucosal damage, severe infiltration of inflammatory cells in both the mucosal and submucosal layers, and the formation of crypt abscesses and dysplasia. G3-G6: In the groups treated with methanolic and ethanolic extracts, the RW rhizome extracts provided protection against mucosal damage, significantly reducing the inflammatory cell infiltration and resulting in minimal crypt abscess formation and dysplasia.

5.6 Effect of RW rhizome extracts on oxidative stress

MDA is a key aldehyde produced during lipid peroxidation and reacts rapidly with various biomolecules, including proteins, nucleic acids, lipids and carbohydrates, disrupting cellular balance. In the DMH-treated group, MDA levels were significantly higher compared to the control animal group, indicating the free radical generation. Additionally, there was a marked decrease in the activity of CAT and SOD, lower GSH and GR levels in the DMH group, all of which contributed to oxidative stress in these animals (Table 5.7). In contrast, treatment with RW extracts in DMH-treated animals led to a notable reduction in MDA levels. Moreover, there was a significant improvement in SOD and CAT activity, increased GSH and GR levels in the DMH + RW extract groups compared to the DMH only group, suggesting the antioxidant effects of RW extracts (Figure 5.7). The antioxidant assay results corroborated with the results obtained through the rat model.

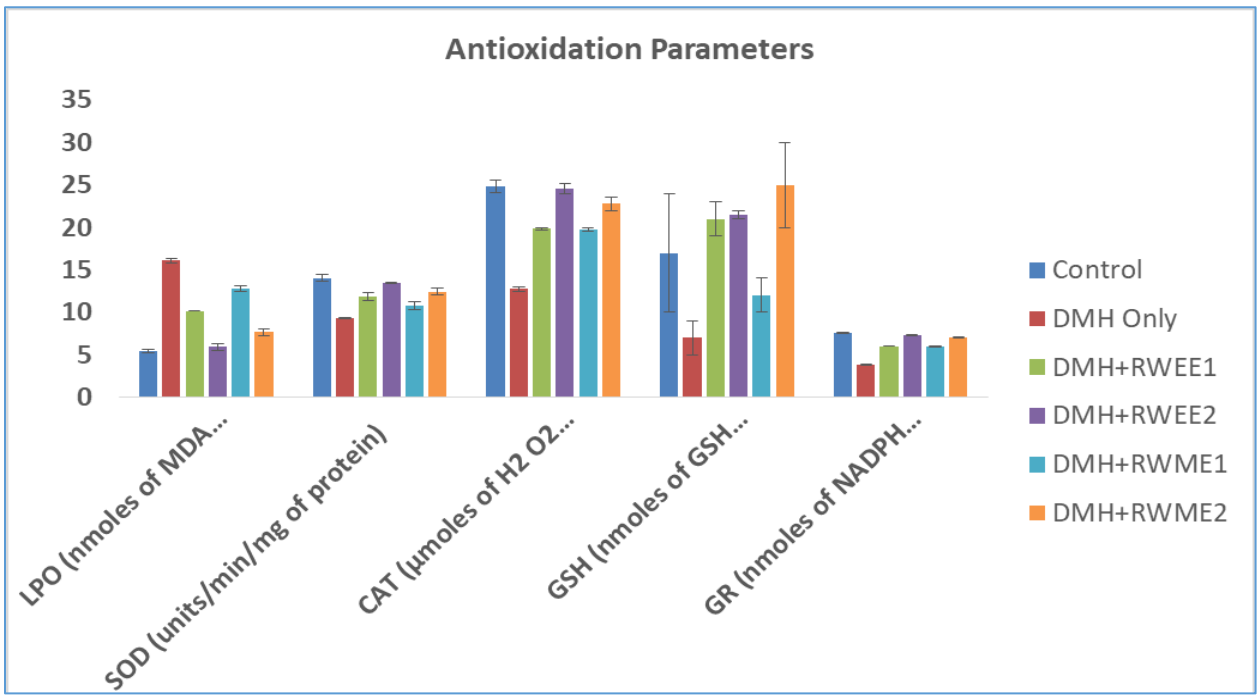


Figure 5.7: Column graph of the Antioxidatino parameters in response to treatment with different extracts. Values are Average±SD of two observations in each animal group.

Table 5.7: Oxidative stress evaluation in animal groups

ANTIOXIDANT PARAMETERS						
Parameters	**Control**	**DMH Only**	**DMH+RWEE1**	**DMH+RWEE2**	**DMH+RWME1**	**DMH+RWME2**
LPO (nmoles of MDA formed/min/mg of protein)	5.65	16.4	10.2	6.34	12.4	7.23
	5.21	15.76	10.14	5.56	13.12	8.1
Average	5.43	16.08	10.17	5.95	12.76	7.665
STDEV	0.31112698	0.45254834	0.04243	0.55154	0.50912	0.61518
SE	0.22	0.32	0.03	0.39	0.36	0.435
p-value		0.00597747	0.0255	NS	0.05027	NS
SOD (units/min/mg of protein)	14.43	9.35	11.4	13.5	10.36	12
	13.63	9.21	12.32	13.42	11.2	12.86
Average	14.03	9.28	11.86	13.46	10.78	12.43
STDEV	0.56568542	0.09899495	0.65054	0.05657	0.59397	0.60811
SE	0.4	0.07	0.46	0.04	0.42	0.43
p-value		0.04415737	NS	NS	NS	NS
	24.06	12.5	19.65	25.23	20.01	22

Average	24.835	12.75	19.825	24.615	19.755	22.765
STDEV	1.09601551	0.35355339	0.24749	0.86974	0.36062	1.08187
SE	0.775	0.25	0.175	0.615	0.255	0.765
p-value		0.02763884	NS	NS	NS	0.00308
GSH (nmoles of GSH formed/min/mg of protein)	24	9	19	21	14	20
	10	5	23	22	10	30
Average	17	7	21	21.5	12	25
STDEV	9.89949494	2.82842712	2.82843	0.70711	2.82843	7.07107
SE	7	2	2	0.5	2	5
p-value		NS	NS	NS	NS	NS
GR (nmoles of NADPH oxidized/sec/mg of protein)	7.52	3.80	6.07	7.35	5.93	7.02
	7.67	3.83	6.00	7.25	6.00	7.08
Average	7.59	3.82	6.03	7.30	5.97	7.05
STDEV	0.10606602	0.02357023	0.04714	0.07071	0.04714	0.04714
SE	0.075	0.01666667	0.03333	0.05	0.03333	0.03333
p-value		0.00983661	0.04419	NS	0.01632	0.04887

5.7 Expression of Wnt/β-catenin and TGF-β in Colon Tissue Using Western Blotting Technique.

5.7.1 Western Blot Analysis of Wnt/β-Catenin Signaling Pathway

Expression levels of Wnt/β-catenin pathway proteins in colon tissues from four experimental groups were analyzed via Western blotting (Figure 5.8). The normal group (G1) displayed baseline levels of β-catenin expression, reflecting normal tissue homeostasis. In the DMH-induced CRC group (G2), β-catenin levels were significantly upregulated, indicating the activation of the Wnt signaling pathway, which is a hallmark of colorectal cancer progression. The densitometric analysis of protein bands revealed that this group had the highest expression of β-catenin among all the groups, confirming enhanced Wnt/β-catenin signaling due to carcinogenic induction.

Treatment with ethanolic extracts of *Rheum webbianum* rhizomes in DMH-induced rats (G3 and G4) showed a dose-dependent decrease in β-catenin expression. The third group (G3), that was treated with 50 mg/kg of body mass of the extract, a moderate reduction in β-catenin levels was observed compared to G2. This suggests partial inhibition of the Wnt signaling pathway by the lower dose of the extract. The group treated with the higher dose of the extract (G4; 100 mg/kg of the body mass) exhibited the most pronounced suppression of β-catenin expression. This group had the lowest levels of β-catenin, approaching near-normal levels seen in the control group (G1). The significant reduction in β-catenin levels in G4 indicates a potent inhibitory effect of the higher concentration of *Rheum webbianum* extract on the Wnt/β-catenin pathway, suggesting its potential role in attenuating tumor progression in CRC. This dose-dependent effect of the extract implies its ability to modulate the aberrant Wnt/β-catenin signaling pathway, which is crucial in colorectal carcinogenesis.

5.7.2 Western Blot Analysis of TGF-β1 Expression

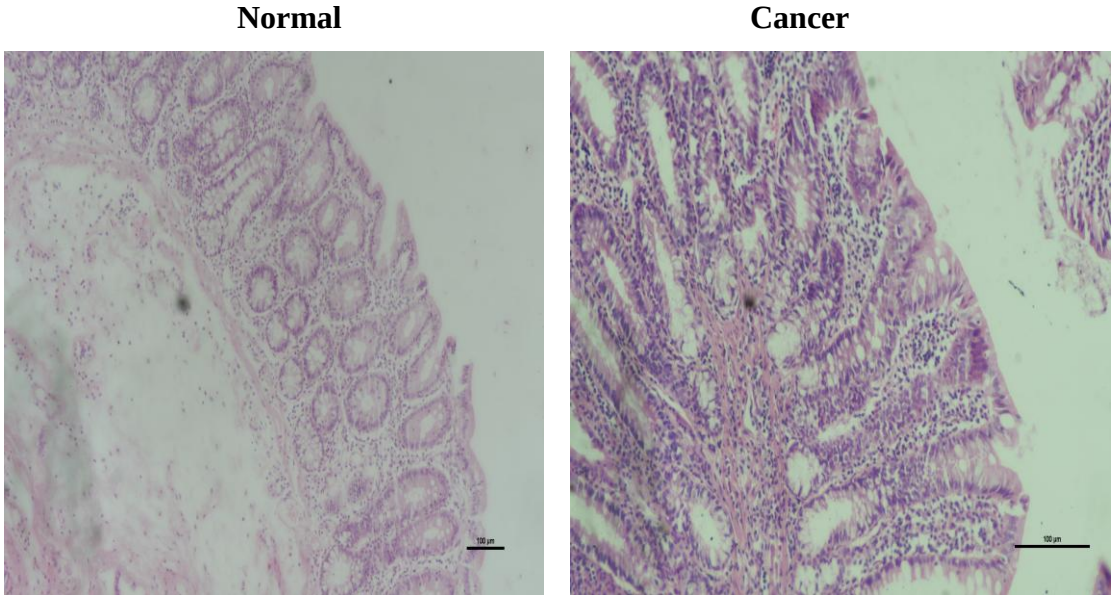
To determine whether TGF-β signaling was activated in the colon tissues of the experimental groups, we examined the expression levels of TGF-β1 through Western blotting (Figure 5.8). The control group (G1) exhibited minimal expression of TGF-β1, consistent with the normal, non-pathological state of the tissue.

In contrast, the DMH-induced CRC group (G2) showed a significant upregulation of TGF-β1 expression, reflecting the activation of TGF-β signaling pathway, which is commonly associated

with tumor progression and fibrosis in colorectal cancer. The heightened levels of TGF-β1 in this group suggest its involvement in promoting the malignant transformation and progression of CRC in the DMH model. Treatment with ethanolic extracts of *Rheum webbianum* (RW) rhizomes significantly suppressed the elevated TGF-β1 levels in a dose-dependent manner. In the group treated with 50 mg/kg of RW extract (G3), TGF-β1 expression was moderately reduced relative to the treatment group, indicating the extract's potential to inhibit the pro-tumorigenic effects of TGF-β1. The group treated with 100 mg/kg of RW extract (G4) demonstrated the most marked reduction in TGF-β1 expression. This significant inhibition suggests that higher doses of RW extract exert a stronger suppressive effect on TGF-β1 signaling, which may contribute to mitigating the tumor-promoting activities of TGF-β1 in CRC.

These findings indicate that *Rheum webbianum* ethanolic extracts are effective in downregulating TGF-β1 expression, potentially offering therapeutic benefits by inhibiting the TGF-β signaling pathway involved in colorectal cancer progression (Figure 5.8).

Figure 5.8 A



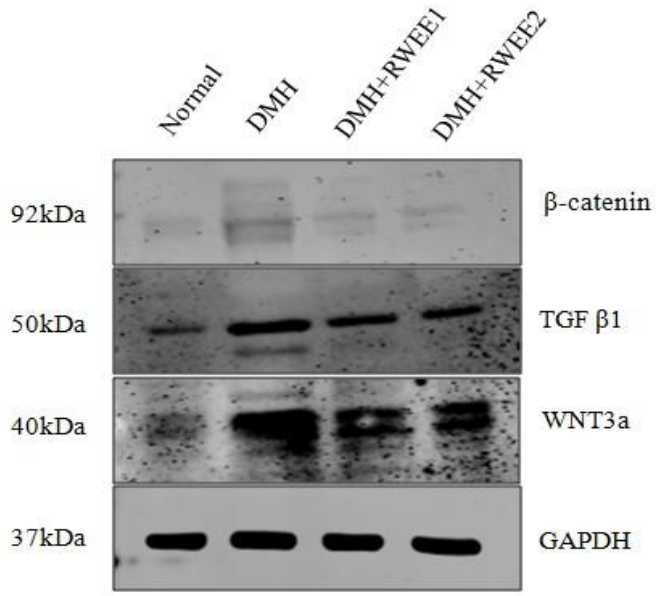


Figure 5.8: A: Histopathology showing cancer. B: Western blot analysis of Wnt/β-Catenin and TGF-β1 Signaling Pathways in Colon Tissues: **The normal group (G1) showed baseline levels of both Wnt/β-Catenin β-catenin and TGF-β1.The DMH-induced CRC group (G2) showed significantly elevated levels of Wnt/β-Catenin and TGF-β1, indicating activation of both pathways. Treatment with RW extracts resulted in a dose-dependent decrease in both Wnt/β-Catenin and TGF-β1 expression. G3 (50 mg/kg B.wt) exhibited moderate reduction, while G4 (100 mg/kg B.wt) showed the most pronounced inhibition,, suggesting a potential therapeutic effect of RW in modulating these signaling pathways in CRC.**

6. Discussion

Colorectal cancer (CRC) remains a substantial health concern across the globe, with less options for effective and adverse effect-free treatment (Li et al. 2021; Yan et al. 2023). Medicinal plants have yielded natural chemicals that show great potential as options for anticancer treatment because of their diverse chemical compositions and biological interactions (Liu et al. 2022) . Research has made investigating the key mechanisms by which these chemicals act against colorectal cancer a central focus.

Rheum species, belonging to the Polygonaceae family, have demonstrated significant inhibitory effects against various cancers, including human lung adenocarcinoma, nasopharyngeal carcinoma (Shi et al. 2001), pancreatic carcinoma (Pan et al. 2016), stomach cancer (Hibasami et al. 2007), and ovarian carcinoma (Zhou et al. 2017). Several anticancer compounds derived from *Rheum* species, such as emodin, rhein, and aloe emodin, are believed to be primarily responsible for eliciting various anticancer responses (Xiang et al. 2020). These plant species from the genus *Rheum* have been shown to reduce tumor invasion and migration, inhibit tumor neovascularization, and impede tumor cell proliferation (Chen et al. 2010). To date, no study has investigated the chemopreventive or chemotherapeutic potential of *Rheum webbianum* (RW) in an animal model of colorectal cancer (CRC), particularly in the dimethylhydrazine (DMH) model. Therefore, this study was conducted to assess the potential chemopreventive activity of RW extracts in DMH-induced colorectal cancer in Swiss albino rats, which closely mirrors the adenoma-carcinoma sequence observed in human CRC.

6.1 In-vitro Study

Considering that the solvents employed in the process of extraction may selectively dissolve specific active phytochemicals, thereby potentially influencing the range of activities exhibited by the extract (Tiwari et al. 2011; Altemimi et al. 2017), our study commenced by preparing four distinct types of RW extracts from rhizomes using solvents with varying polarities like hexane, ethyl acetate, ethanol, and methanol. An initial screening was carried out to evaluate the potential anti-proliferative effects of these four extracts on the colorectal cancer cell line HCT-119. Among them, only the ethanol and methanol extracts demonstrated anti-proliferative effects, exhibiting a dose-dependent reduction in the viability of HCT-116 cells. Our findings can be elucidated by the

bioactive compounds present in the ethanolic extract, which exert cytotoxic activity. It is a well-established fact that fatty acids and terpenoids are predominantly extracted in relatively non-polar solvents, whereas polyphenolic compounds, anthocyanins, flavonoid compounds, and various other compounds are extracted in alcohol-based solvents preferentially (Altemimi et al. 2017). The observed activity of these extract appears to be attributed to their polyphenolic compounds.

For example, the methanolic extract from the rhizomes of *Rheum emodi* have the double amount of total phenolic content- TPC as comparison to its aqueous extract. The study (V. Rajkumar et al. 2011) found that this was linked to higher levels of antioxidants and had two to four times stronger ability to inhibit the growth of breast and liver cancer cells. In a separate investigation (Cinar Ayan et al. 2021) discovered that the ethanolic extract of *Rheum ribes* L. roots had higher level of total phenolic content (TPC) in comparison to the nonpolar petroleum ether extract. The rise in TPC was linked with enhanced antioxidant effects of the ethanolic extract. Cinar Ayan et al found similar results, showing that methanolic extract of *Rheum ribes* L. roots included a greater amount of phenolic chemicals compared to the hexane and ethyl acetate extracts. Nevertheless, the antioxidant capacity of the methanolic and ethyl acetate extracts exhibited a high degree of similarity (Cinar Ayan et al. 2021).

Building upon the notable *in-vitro* findings of the ethanolic and methanolic extracts, it became imperative to conduct further investigations using *in-vivo* cancer models to ascertain whether the observed effects were not specific to the cell line. Consequently, we opted to proceed with an *in-vivo* study utilizing these two RW rhizome extracts to assess their impact on DMH induced CRC in Swiss albino rats.

6.2 In-Vivo Study

In cancer patients, one of the initial observable changes is the loss of appetite and weight as the disease progresses, a condition known as cancer cachexia. In our study, DMH treatment led to the significant decrease in body weight, consistent with previous research findings (Thangaraj et al. 2018). The process of carcinogenesis in the colon is often linked with reduced appetite and diminished food intake (Vinothkumar et al. 2014; Sivaranjani et al. 2016). However, RW supplementation appeared to mitigate weight loss, possibly owing to its chemo preventive potential, which is evidenced by the reduced inflammatory cell infiltration, crypt abscess formation, mucosal and glandular structure destruction. DMH acts as a procarcinogen, generating

carcinogenic metabolites primarily in the liver. Among these metabolites, azoxymethane-AOM, methyl diazonium ions, and carbonium ions are particularly significant. The other two are electrophiles which are excreted via bile and reach to the intestine.

Red blood cells (RBCs) in the body are typically the first cells to encounter stressful stimuli. Oxidative stress of erythrocytes is known to contribute to hematological abnormalities and the progression of various diseases, including carcinogenesis (Shiono et al. 2003; Childress 2012). In this study, our focus was on assessing the impact of RW on hematological disruptions during the promotion of CRC induced by DMH (Gali-Muhtasib et al. 2008; Ghadi et al. 2009). DMH-induced oxidative stress in rats was associated with alterations in hematological parameters. We observed reduced levels of blood pathology markers especially "erythrocyte count, hemoglobin level and hematocrit value", suggesting a clinical anemia. Additionally, there was a significant increase in WBC and platelet count, signifying the activation of defense system. Increases in WBCs (leukocytosis) and platelets (thrombocytosis) are expected during the tumor development process. The white blood cells have phagocytosis functions and can migrate to inflammation areas to target infected/altered cells as well as cancerous cells, with platelets facilitating their attraction to these sites (Laoui et al. 2011; Vieira-de-Abreu et al. 2012). The objective of this study was to assess the effect of RW on chemical-induced hematological changes (by DMH) during the carcinogenesis process of rat colorectal tissue. We evaluated the efficacy of RW as a pre-treatment, which successfully mitigated DMH-induced hematological abnormalities (anemia, leukocytosis, and thrombocytosis).

DMH administration also resulted in hepatotoxicity, as evidenced by elevation in the activity of serum ALT and AST. In the activation of DMH, the liver plays a crucial role, leading to the formation of reactive electrophiles. These electrophiles can leak and cause damage to hepatocytes, leading to disintegration of the membrane and leakage of hepatic enzymes into the plasma (Abd-Elmoneim et al. 2013). However, treatment of RW to DMH treated rats reduced serum ALT and AST activity, indicating significant hepatoprotective and/or stabilizing effects of RW. Similar results have been reported before also in the same animal model (Rani et al. 2014).

The histopathological analysis of colon tissues from the control group of rats did not indicate any abnormalities in the morphology and structure of the crypts. However, the rats treated with DMH exhibited the presence of several malignant lesions, such as hyperplasia, which confirmed the

development of colon cancer in these rats. Prior studies have documented similar findings (Tanaka 2009) Remarkably, the histological examination demonstrated a decrease in cancerous areas, such as abnormal cell growth, in the colon sections after receiving RW treatment. In summary, our data suggest that RW has the ability to prevent the formation of tumors in the colon that are produced by DMH, hence showing its anti-tumor properties and potential.

The administration of DMH resulted in a substantial elevation in MDA levels. MDA serves as a marker for the process of lipid peroxidation, which causes damage to certain components of the mitochondrial membrane, including polyunsaturated fatty acids and membrane-specific proteins. This damage leads to a loss of integrity in the mitochondria (Gaweł et al. 2004). MDA ultimately disrupts the structure of the colon and the balance of redox reactions, leading to harmful effects (Marnett 1999). The current investigation demonstrated that the extract derived from RW rhizome effectively mitigated OS (oxidant stress), as evidenced by a reduced MDA levels.

Reduced glutathione is a significant intracellular antioxidant that eliminates free radicals by converting them into disulfide form, thereby helping to maintain cellular homeostasis (Lv et al. 2019) . The administration of DMH has been found to disrupt the glutathione reservoir and cause a decrease in the amounts of reduced glutathione (Pence 1991). Studies have shown that bioactive chemicals derived from plants can counteract the reduction of cellular antioxidants, such as reduced glutathione, CAT and SOD activities, caused by DMH (Hamiza et al. 2014). The current study found that treatment with RW rhizome extract restored GSH levels compared to the DMH group.

We evaluated antioxidant enzyme activities, in addition to glutathione. We noticed a decrease in the SOD and CAT activity in the colon homogenate of animals treated with DMH. This decrease would worsen the imbalance in redox reactions. The administration of RW rhizome extract resulted in an elevation in SOD and CAT activity, leading to a decrease in oxidative damage caused by free radicals to cellular structures such as plasma membrane, mitochondria, nucleic acids and other organelles. Moreover, the chemopreventive impact of RW rhizome extract was demonstrated by its ability to restore SOD and CAT activity, as well as its significant free radical scavenging activity.

The findings of this study provide novel insights into the potential mechanisms by which *Rheum webbianum* (RW) extracts prevent the development of CRC, particularly via modulation of the

key Wnt/β-catenin signaling pathway. This pathway is essential for maintaining normal intestinal homeostasis, as it regulates the renewal of the intestinal epithelium, which is crucial for tissue development and repair (Shtutman et al. 1999). However, aberrant activation of Wnt signaling, marked by the excessive accumulation of nuclear β-catenin, has been well-documented as a key driver of colorectal carcinogenesis (de Lau et al. 2007).

In this study, the expression level of Wnt/β-catenin signaling varied significantly across the four experimental groups, reflecting both normal intestinal homeostasis and the pathological changes associated with colorectal cancer (CRC). The control group (G1) exhibited baseline levels of β-catenin, indicative of normal Wnt signaling required for the regulation of intestinal epithelium renewal and homeostasis. These findings underscore the importance of balanced Wnt signaling in maintaining healthy colon tissue without any carcinogenic alterations. In stark contrast, the DMH-induced CRC group (G2) showed a marked increase in β-catenin expression, reflecting hyperactivation of the Wnt pathway, which is well-documented to drive colorectal carcinogenesis. This elevated β-catenin accumulation in G2 confirms that DMH-induced carcinogenesis leads to uncontrolled cell proliferation, a key factor in tumor progression. Treatment with *Rheum webbianum* (RW) ethanolic extracts significantly mitigated this aberrant Wnt signaling in a dose-dependent manner. In the group treated with 50 mg/kg of RW extract (G3), β-catenin levels were noticeably lower than those in G2, indicating that even at a lower dose, RW extracts can suppress the overactivation of this oncogenic pathway. However, the most substantial inhibition of β-catenin was observed in the group treated with 100 mg/kg RW extract (G4), where the expression was nearly normalized, approaching the levels seen in the control group (G1).

These results suggest that *Rheum webbianum* ethanolic extracts effectively downregulate the Wnt/β-catenin pathway, thus preventing the accumulation of β-catenin that typically leads to CRC. The dose-dependent reduction in β-catenin across the treated groups indicates that RW extracts may play a crucial role in halting or delaying colorectal carcinogenesis by restoring normal Wnt signaling activity. This comparison highlights the therapeutic potential of RW extracts in preventing tumor progression by targeting key molecular pathways associated with CRC.

TGF-β plays a double role in tumorigenesis, by acting as both tumor promoter and suppressor, depending on the stage of cancer development. In the early stages, TGF-β inhibits cell proliferation

and triggers apoptosis, helping to prevent tumor formation. However, as the tumor progresses, TGF-β switches roles and begins to promote cancer growth by enhancing invasion and metastasis. In colorectal cancer (CRC), elevated levels of TGF-β1 are frequently observed and are closely linked to aggressive tumor behavior, increased risk of recurrence, and poorer patient survival outcomes. This shift underscores the complex, context-dependent role of TGF-β in cancer biology (Picon et al. 1998).

In this study, we systematically examined TGF-β1 protein levels across the four animal groups, focusing on its role in DMH-induced colorectal cancer (CRC). Our findings revealed a significant increase in TGF-β1 expression in the DMH-induced CRC group (G2) compared to the normal control group (G1), where baseline levels of TGF-β1 were observed, reflecting normal cellular regulation. This elevated expression in G2 aligns with previous studies highlighting the role of TGF-β1 in promoting tumor progression.

Notably, treatment with *Rheum webbianum* (RW) ethanolic extracts in groups G3 (50 mg/kg) and G4 (100 mg/kg) led to a substantial reduction in TGF-β1 levels, demonstrating the extract's ability to inhibit this signaling pathway. The higher dose of RW extract in G4 was particularly effective, almost normalizing TGF-β1 expression. These results indicate that RW extracts possess strong antitumor properties, likely by targeting and downregulating the TGF-β1 signaling pathway. Overall, the modulation of TGF-β1 and its receptors by RW extracts highlights its potential therapeutic role in preventing CRC progression.

7. Summary

Colorectal cancer remains a significant global health challenge, with limited effective options of treatment. The exploration of medicinal plants, particularly the *Rheum* species, has opened avenues for potential anticancer treatments due to their diverse bioactive compounds. Previous studies have demonstrated the anticancer effects of compounds like emodin, rhein, and aloe emodin from *Rheum* species against various cancers. However, the chemopreventive potential of *Rheum webbianum* (RW) against CRC, particularly in the dimethylhydrazine (DMH)-induced animal model, remained unexplored. This research work investigated the chemopreventive properties of RW extracts on DMH-induced CRC in Swiss albino rats, a model closely resembling human CRC.

The research was conducted in two stages: cell line-based (*in-vitro)* and murine model (*in-vivo)*. In the *in-vitro* stage, ethanolic and methanolic extracts of RW demonstrated dose-dependent anti-proliferative effects on the HCT-116 colorectal cancer cell line, attributed to the polyphenolic compounds present in the extracts. Building on these promising *in-vitro* results, an *in-vivo* study was conducted to assess the effects of these extracts on DMH-induced CRC in rats.

In the *in-vivo* study, DMH administration caused significant reductions in body weight, anemia, leukocytosis, and hepatotoxicity, typical signs of CRC progression. RW supplementation mitigated these harmful effects, suggesting its chemopreventive potential. The treatment reduced DMH-induced oxidative stress, as evidenced by the decrease in MDA levels and restoration of antioxidant enzymes (SOD, CAT, and GSH), indicating an improved redox balance in the rats. Moreover, histopathological analysis revealed fewer malignant lesions in RW-treated rats compared to the DMH group, highlighting RW's ability to prevent tumor formation.

At the molecular level, RW extracts were found to modulate key signaling pathways involved in colorectal carcinogenesis, including the Wnt/β-catenin and TGF-β pathways. Aberrant activation of the Wnt/β-catenin pathway in DMH-treated rats was significantly reduced by RW extracts, particularly at higher doses. Additionally, RW treatment downregulated TGF-β1 expression, which is known to promote tumor growth in advanced CRC stages. These findings suggest that RW's chemopreventive effects may be mediated through the modulation of these critical oncogenic pathways.

8. Conclusion and Future Scope

This research provides compelling evidence for the chemopreventive potential of *Rheum webbianum* (RW) extracts against colorectal cancer (CRC), particularly in the 1,2-dimethylhydrazine (DMH)-induced CRC model in Swiss albino rats. The study systematically evaluated RW extracts through both *in vitro* and *in vivo* models, demonstrating their ability to inhibit cancer cell proliferation, restore antioxidant balance, and mitigate the adverse hematological and hepatic effects induced by carcinogen exposure. The findings reveal that RW extracts, particularly in ethanolic and methanolic forms, exert anticancer effects by modulating key molecular pathways such as Wnt/β-catenin and TGF-β1, which are critically involved in CRC progression. Furthermore, histopathological assessments confirmed a significant reduction in tumor burden and structural abnormalities in the colon tissue of RW-treated groups, underscoring its potential as a natural chemopreventive agent.

Despite these promising findings, several aspects warrant further exploration. Future research should focus on isolating and characterizing the specific bioactive compounds responsible for the anticancer effects of RW extracts. Advanced pharmacokinetic and toxicological studies are essential to establish the safety and efficacy of RW-based formulations before progressing to clinical trials. Additionally, mechanistic studies at the genetic and proteomic levels could provide deeper insights into how RW interacts with cellular pathways involved in carcinogenesis. Expanding research into other cancer models and evaluating synergistic effects with conventional chemotherapeutics could further enhance the clinical applicability of RW extracts.

In conclusion, this study contributes to the growing body of evidence supporting the role of plant-derived compounds in cancer prevention and therapy. With further validation, *Rheum webbianum* holds significant promise as a natural, cost-effective, and accessible alternative in the fight against colorectal cancer.

9. Bibliography

Abd-Elmoneim MA, Bakar AA, Awad IM, Moharib SA, Mohamed EM. 2013. Anticarcinogenic effect of *Raphanus sativus* on 1, 2 dimethylhydrazine (DMH) induced colon cancer in rats. Egypt J Hosp Med. 51(1):473–486.

Abuajah CI, Ogbonna AC, Osuji CM. 2015. Functional components and medicinal properties of food: a review. J Food Sci Technol. 52(5):2522–2529. doi:10.1007/s13197-014-1396-5.

Ahmad R, Singh J, Wunnava A, Al-Obeed O, Abdulla M, Srivastava S. 2021. Emerging trends in colorectal cancer: Dysregulated signaling pathways (Review). Int J Mol Med. 47(3):14. doi:10.3892/ijmm.2021.4847.

Akimoto N, Ugai T, Zhong R, Hamada T, Fujiyoshi K, Giannakis M, Wu K, Cao Y, Ng K, Ogino S. 2021. Rising incidence of early-onset colorectal cancer—a call to action. Nat Rev Clin Oncol. 18(4):230–243.

Alexandrescu S, Diaconescu A, Ionel Z, Zlate C, Grigorie R, Hrehoret D, Brasoveanu V, Dima S, Botea F, Ionescu M. 2017. Comparative analysis between simultaneous resection and staged resection for synchronous colorectal liver metastases-a single center experience on 300 consecutive patients. Chirurgia (Bucur). 112(3):278–288.

Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. 2017. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants. 6(4):42.

Anand P, Kunnumakara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB. 2008. Cancer is a Preventable Disease that Requires Major Lifestyle Changes. Pharm Res. 25(9):2097–2116. doi:10.1007/s11095-008-9661-9.

Arulselvan P, Fard MT, Tan WS, Gothai S, Fakurazi S, Norhaizan ME, Kumar SS. 2016. Role of Antioxidants and Natural Products in Inflammation. Ojha S, editor. Oxid Med Cell Longev. 2016(1):5276130. doi:10.1155/2016/5276130.

Arulselvan P, Wen C-C, Lan C-W, Chen Y-H, Wei W-C, Yang N-S. 2012. Dietary administration of *scallion* extract effectively inhibits colorectal tumor growth: cellular and molecular mechanisms in mice. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0044658.

Aslam M, Dayal R, Javed K, Fahamiya N, Mujeeb M, Husain A. 2012. Pharmacognostical and phytochemical evaluation of *Rheum emodi* Wall. J Curr Pharma Res. 2(2):471.

Barbosa R, Acevedo LA, Marmorstein R. 2021. The MEK/ERK network as a therapeutic target in human cancer. Mol Cancer Res. 19(3):361–374.

Basant Ballabh BB, Chaurasia OP. 2009. Medicinal plants of cold desert Ladakh used in the treatment of stomach disorders.https://www.cabidigitallibrary.org/doi/full/10.5555/20093162470.

Baskar R, Lee KA, Yeo R, Yeoh K-W. 2012. Cancer and radiation therapy: current advances and future directions. Int J Med Sci. 9(3):193.

Batool Z, Singh K, Gairola S. 2023. Medicinal plants traditionally used in the health care practices by the indigenous communities of the Trans-Himalayan region of Ladakh, India. J Ethnopharmacol. 317:116837.

Beauchamp, C., & Fridovich, I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry, 44, 276–287.

Benarba B, Belabid L, Righi K, amine Bekkar A, Elouissi M, Khaldi A, Hamimed A. 2015. Ethnobotanical study of medicinal plants used by traditional healers in Mascara (North West of Algeria). J Ethnopharmacol. 175:626–637.

Benarba B, Meddah B, Hamdani H. 2014. Cancer incidence in North West Algeria (Mascara) 2000-2010: results from a population-based cancer registry. EXCLI J. 13:709.

Bilal S, Bhat SA, Ahanger AA, Hussain I, Ahmad SP, Mir MR. 2014. Healing potential of *rheum emodi* (Rhubarb) root powder on excision wounds in rabbit. World J Pharm Pharm Sci. 3:1317–1323.

Borggrefe T, Oswald F. 2016. Setting the stage for Notch: the Drosophila Su (H)-hairless repressor complex. PLoS Biol. 14(7):e1002524.

Burn J, Gerdes A-M, Macrae F, Mecklin J-P, Moeslein G, Olschwang S, Eccles D, Evans DG, Maher ER, Bertario L. 2011. Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. The Lancet. 378(9809):2081–2087.

Cao Y-J, Pu Z-J, Tang Y-P, Shen J, Chen Y-Y, Kang A, Zhou G-S, Duan J-A. 2017. Advances in bio-active constituents, pharmacology and clinical applications of rhubarb. Chin Med. 12(1):36. doi:10.1186/s13020-017-0158-5.

Carulli AJ, Keeley TM, Demitrack ES, Chung J, Maillard I, Samuelson LC. 2015. Notch receptor regulation of intestinal stem cell homeostasis and crypt regeneration. Dev Biol. 402(1):98–108.

Castel P, Toska E, Engelman JA, Scaltriti M. 2021. The present and future of PI3K inhibitors for cancer therapy. Nat Cancer. 2(6):587–597.

Clairbone, A. 1985. Catalase activity. In R. A. Greenwald (Ed.), Handbook of methods for oxygen radical research (pp. 283–284). Boca Raton, FL: CRC Press.

Chen Y-Y, Chiang S-Y, Lin J-G, Ma Y-S, Liao C-L, Weng S-W, Lai T-Y, Chung J-G. 2010. Emodin, aloe-emodin and rhein inhibit migration and invasion in human tongue cancer SCC-4 cells through the inhibition of gene expression of matrix metalloproteinase-9. Int J Oncol. 36(5):1113–1120.

Chen Y-Y, Hsieh M-J, Hsieh Y-S, Chang Y-C, Chen P-N, Yang S-F, Ho H-Y, Chou Y-E, Lin C-W. 2017. Antimetastatic effects of *Rheum palmatum* L. extract on oral cancer cells: CHEN et al. Environ Toxicol. 32(10):2287–2294. doi:10.1002/tox.22444.

Childress MO. 2012. Hematologic abnormalities in the small animal cancer patient. Vet Clin Small Anim Pract. 42(1):123–155.

Choi E-S, Cho S-D, Jeon J-G, Cho N-P. 2011. The apoptotic effect of the hexane extract of *Rheum undulatum* L. in oral cancer cells through the down-regulation of specificity protein 1 and survivin. Lab Anim Res. 27(1):19–24.

Chung KY, Saltz LB. 2007. Adjuvant therapy of colon cancer: current status and future directions. Cancer J. 13(3):192–197.

Çınar Ayan İ, Çetinkaya S, Dursun HG, Süntar İ. 2021. Bioactive Compounds of *Rheum ribes* L. and its Anticancerogenic Effect via Induction of Apoptosis and miR-200 Family Expression in Human Colorectal Cancer Cells. Nutr Cancer. 73(7):1228–1243. doi:10.1080/01635581.2020.1792947.

Connell LC, Mota JM, Braghiroli MI, Hoff PM. 2017. The Rising Incidence of Younger Patients With Colorectal Cancer: Questions About Screening, Biology, and Treatment. Curr Treat Options Oncol. 18(4):23. doi:10.1007/s11864-017-0463-3.

ER F. 1990. A genetic model for colorectal tumorigenesis. Cell. 61. [accessed 2025 Feb 24]. https://cir.nii.ac.jp/crid/1573387449726570112.

Erdoğan MK, Agca CA, Geçibesler İH. 2020. The antiproliferative potential of isolated emodin and aloe-emodin from *Rheum ribes* on different cancer cell lines. Biol Divers Conserv. 13(2):160–168.

Fan YX, Abulimiti P, Zhang HL, Zhou YK, Zhu L. 2017. Mechanism of reversal of multidrug resistance by curcumin in human colorectal cancer cell line HCT-8/5-FU. Genet Mol Res. 16(2):16029414.

Fearon ER. 2011. Molecular Genetics of Colorectal Cancer. Annu Rev Pathol Mech Dis. 6(1):479–507. doi:10.1146/annurev-pathol-011110-130235.

Flohe´ , L., & Gu¨ nzler, W. A. I. 1984. Assays of glutathione peroxidise. Methods in Enzymology, 105, 114–121.

Frattini M, Saletti P, Molinari F, De Dosso S. 2015 Jan. EGFR signaling in colorectal cancer: a clinical perspective. Gastrointest Cancer Targets Ther.:21. doi:10.2147/GICTT.S49002.

Fulda S. 2010. Modulation of Apoptosis by Natural Products for Cancer Therapy. Planta Med. 76(11):1075–1079. doi:10.1055/s-0030-1249961.

Gali-Muhtasib H, Ocker M, Kuester D, Krueger S, El-Hajj Z, Diestel A, Evert M, El-Najjar N, Peters B, Jurjus A, et al. 2008. Thymoquinone reduces mouse colon tumor cell invasion and inhibits tumor growth in murine colon cancer models. J Cell Mol Med. 12(1):330–342. doi:10.1111/j.1582-4934.2007.00095.x.

Gaweł S, Wardas M, Niedworok E, Wardas P. 2004. Malondialdehyde (MDA) as a lipid peroxidation marker. Wiadomosci Lek Wars Pol 1960. 57(9–10):453–455.

Ghadi FE, Ghara AR, Bhattacharyya S, Dhawan DK. 2009. Selenium as a chemopreventive agent in experimentally induced colon carcinogenesis. World J Gastrointest Oncol. 1(1):74.

Hamiza OO, Rehman MU, Tahir M, Khan R, Lateef A, Khan AQ, Sultana S. 2014. Methanolic extract of *Terminalia chebula* protects against DMH-induced colon damage in Wistar rats by restoring antioxidant enzyme activities and suppressing inflammation. Int J Drug Dev Res. 6:54–69.

Hammond WA, Swaika A, Mody K. 2016. Pharmacologic resistance in colorectal cancer: a review. Ther Adv Med Oncol. 8(1):57–84. doi:10.1177/1758834015614530.

Hartley A-V, Wang B, Jiang G, Wei H, Sun M, Prabhu L, Martin M, Safa A, Sun S, Liu Y. 2020. Regulation of a PRMT5/NF-κB Axis by Phosphorylation of PRMT5 at Serine 15 in Colorectal Cancer. Int J Mol Sci. 21(10):3684.

Hibasami H, Takagi K, Ishii T, Tsujikawa M, Imai N, Honda I. 2007. Induction of apoptosis by rhapontin having stilbene moiety, a component of rhubarb (*Rheum officinale* Baillon) in human stomach cancer KATO III cells. Oncol Rep. 18(2):347–351.

Higashi D, Futami K, Ishibashi Y, Egawa Y, Maekawa T, Matsui T, Iwashita A, Kuroki M. 2011. Clinical course of colorectal cancer in patients with ulcerative colitis. Anticancer Res. 31(7):2499–2504.

Hnatyszyn A, Hryhorowicz S, Kaczmarek-Ryś M, Lis E, Słomski R, Scott RJ, Pławski A. 2019. Colorectal carcinoma in the course of inflammatory bowel diseases. Hered Cancer Clin Pract. 17(1):18. doi:10.1186/s13053-019-0118-4.

Jeong W-J, Ro EJ, Choi K-Y. 2018. Interaction between Wnt/β-catenin and RAS-ERK pathways and an anti-cancer strategy via degradations of β-catenin and RAS by targeting the Wnt/β-catenin pathway. NPJ Precis Oncol. 2(1):5.

Johnson CM, Wei C, Ensor JE, Smolenski DJ, Amos CI, Levin B, Berry DA. 2013. Meta-analyses of colorectal cancer risk factors. Cancer Causes Control. 24(6):1207–1222. doi:10.1007/s10552-013-0201-5.

Jung B, Staudacher JJ, Beauchamp D. 2017. Transforming growth factor β superfamily signaling in development of colorectal cancer. Gastroenterology. 152(1):36–52.

Kheirelseid EA, Miller N, Chang KH, Curran C, Hennessey E, Sheehan M, Kerin MJ. 2013. Mismatch repair protein expression in colorectal cancer. J Gastrointest Oncol. 4(4):397.

Khlebnikov AI, Schepetkin IA, Domina NG, Kirpotina LN, Quinn MT. 2007. Improved quantitative structure–activity relationship models to predict antioxidant activity of flavonoids in chemical, enzymatic, and cellular systems. Bioorg Med Chem. 15(4):1749–1770.

Koveitypour Z, Panahi F, Vakilian M, Peymani M, Seyed Forootan F, Nasr Esfahani MH, Ghaedi K. 2019. Signaling pathways involved in colorectal cancer progression. Cell Biosci. 9(1):97. doi:10.1186/s13578-019-0361-4.

Kuipers EJ, Rösch T, Bretthauer M. 2013. Colorectal cancer screening—optimizing current strategies and new directions. Nat Rev Clin Oncol. 10(3):130–142.

Kumari S, Badana AK, G MM, G S, Malla R. 2018. Reactive Oxygen Species: A Key Constituent in Cancer Survival. Biomark Insights. 13:1177271918755391. doi:10.1177/1177271918755391.

Laoui D, Van Overmeire E, Movahedi K, Van den Bossche J, Schouppe E, Mommer C, Nikolaou A, Morias Y, De Baetselier P, Van Ginderachter JA. 2011. Mononuclear phagocyte heterogeneity in cancer: different subsets and activation states reaching out at the tumor site. Immunobiology. 216(11):1192–1202.

de Lau W, Barker N, Clevers H. 2007. WNT signaling in the normal intestine and colorectal cancer. Front Biosci. 12(1):471–491.

Levy L, Hill CS. 2005. Smad4 Dependency Defines Two Classes of Transforming Growth Factor β (TGF-β) Target Genes and Distinguishes TGF-β-Induced Epithelial-Mesenchymal Transition from Its Antiproliferative and Migratory Responses. Mol Cell Biol. 25(18):8108–8125. doi:10.1128/MCB.25.18.8108-8125.2005.

Li H, Wu WKK, Li ZJ, Chan KM, Wong CCM, Ye CG, Yu L, Sung JJY, Cho CH, Wang M. 2010. 2,3′,4,4′,5′-Pentamethoxy- *trans* -stilbene, a resveratrol derivative, inhibits colitis-associated colorectal carcinogenesis in mice. Br J Pharmacol. 160(6):1352–1361. doi:10.1111/j.1476-5381.2010.00785.x.

Li N, Lu B, Luo C, Cai J, Lu M, Zhang Y, Chen H, Dai M. 2021. Incidence, mortality, survival, risk factor and screening of colorectal cancer: a comparison among China, Europe, and northern America. Cancer Lett. 522:255–268.

Li W-Y, Chan S-W, Guo D-J, Chung M-K, Leung T-Y, Yu PH-F. 2009. Water extract of Rheum officinale Baill. induces apoptosis in human lung adenocarcinoma A549 and human breast cancer MCF-7 cell lines. J Ethnopharmacol. 124(2):251–256.

Liao W, Li G, You Y, Wan H, Wu Q, Wang C, Lv N. 2018. Antitumor activity of Notch-1 inhibition in human colorectal carcinoma cells. Oncol Rep. 39(3):1063–1071.

Liu W, Zhang J, Yao X, Jiang C, He J, Ni P, Liu J, Chen Q, Li Q, Zang X. 2017. Shenmai injection enhances the cytotoxicity of chemotherapeutic drugs against colorectal cancers via improving their subcellular distribution. Acta Pharmacol Sin. 38(2):264–276.

Liu Z-B, Zhang T, Ye X, Liu Z-Q, Sun X, Zhang L-L, Wu C-J. 2022. Natural substances derived from herbs or plants are promising sources of anticancer agents against colorectal cancer via triggering apoptosis. J Pharm Pharmacol. 74(2):162–178.

Louis P, Hold GL, Flint HJ. 2014. The gut microbiota, bacterial metabolites and colorectal cancer. Nat Rev Microbiol. 12(10):661–672.

Lu M, Chen Q. 1989. Biochemical study of Chinese rhucarb. XXIX. Inhibitory effects of anthraquinone derivatives on P388 leukemia in mice. J China Pharm Univ. 20(3):155–157.

Lv H, Zhen C, Liu J, Yang P, Hu L, Shang P. 2019. Unraveling the Potential Role of Glutathione in Multiple Forms of Cell Death in Cancer Therapy. Oxid Med Cell Longev. 2019:1–16. doi:10.1155/2019/3150145.

Ma Y, Hsiao Y, Lin Ju-Hwa, Hsu S, Chueh F, Weng S, Lai K, Lin Jaung-Geng, Chung J. 2015. Crude extract of *Rheum palmatum L* inhibits migration and invasion of LS1034 human colon cancer cells acts through the inhibition of matrix metalloproteinase-2/-9 by MAPK signaling. Environ Toxicol. 30(7):852–863. doi:10.1002/tox.21962.

Markowitz SD, Bertagnolli MM. 2009. Molecular Basis of Colorectal Cancer. N Engl J Med. 361(25):2449–2460. doi:10.1056/NEJMra0804588.

Marnett LJ. 1999. Lipid peroxidation—DNA damage by malondialdehyde. Mutat Res Mol Mech Mutagen. 424(1–2):83–95.

Martin M, Sun M, Motolani A, Lu T. 2021. The pivotal player: components of NF-κB pathway as promising biomarkers in colorectal cancer. Int J Mol Sci. 22(14):7429.

Martin MS, Martin F, Michiels R, Bastien H, Justrabo E, Bordes M, Viry B. 1973. An Experimental Model for Cancer of the Colon and Rectum: Intestinal Carcinoma Induced in the Rat by 1 2-DimethyIhydrazine. Digestion. 8(1):22–34.

Mishra SK, Tiwari S, Shrivastava A, Srivastava S, Boudh GK, Chourasia SK, Chaturvedi U, Mir SS, Saxena AK, Bhatia G, et al. 2014. Antidyslipidemic effect and antioxidant activity of anthraquinone derivatives from *Rheum emodi* rhizomes in dyslipidemic rats. J Nat Med. 68(2):363–371. doi:10.1007/s11418-013-0810-z.

Morgan E, Arnold M, Gini A, Lorenzoni V, Cabasag CJ, Laversanne M, Vignat J, Ferlay J, Murphy N, Bray F. 2023. Global burden of colorectal cancer in 2020 and 2040: incidence and mortality estimates from GLOBOCAN. Gut. 72(2):338–344.

Mori H, Hata K, Yamada Y, Kuno T, Hara A. 2005. Significance and role of early-lesions in experimental colorectal carcinogenesis. Chem Biol Interact. 155(1–2):1–9.

Murphy N, Norat T, Ferrari P, Jenab M, Bueno-de-Mesquita B, Skeie G, Olsen A, Tjønneland A, Dahm CC, Overvad K. 2013. Consumption of dairy products and colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC). PloS One. 8(9):e72715.

Narayanankutty A. 2019. PI3K/Akt/mTOR pathway as a therapeutic target for colorectal cancer: a review of preclinical and clinical evidence. Curr Drug Targets. 20(12):1217–1226.

Narota A, Kumar S, Kaur R, Kaur S, Aggarwal R, Agnihotri N. 2020. *Althea rosea* seed extract ameliorates 1, 2-dimethylhydrazine induced preneoplastic lesions in mouse model of colon cancer by modulating oxidative stress and inflammation. Pharmacogn Mag. 16(70). [accessed 2025 Feb 24]. https://phcog.com/article/view/2020/16/70/360-370.

Nguyen H, Duong H. 2018 May 9. The molecular characteristics of colorectal cancer: Implications for diagnosis and therapy (Review). Oncol Lett. doi:10.3892/ol.2018.8679. [accessed 2025 Feb 24]. http://www.spandidos-publications.com/10.3892/ol.2018.8679.

Nho KJ, Chun JM, Lee AY, Kim HK. 2015. Anti-metastatic effects of *Rheum Palmatum* L. extract in human MDA-MB-231 breast cancer cells. Environ Toxicol Pharmacol. 40(1):30–38.

Nussbaumer S, Bonnabry P, Veuthey J-L, Fleury-Souverain S. 2011. Analysis of anticancer drugs: a review. Talanta. 85(5):2265–2289.

Pan F-P, Zhou H-K, Bu H-Q, Chen Z-Q, Zhang H, Xu L-P, Tang J, Yu Q-J, Chu Y-Q, Pan J, et al. 2016. Emodin enhances the demethylation by 5-Aza-CdR of pancreatic cancer cell tumor-suppressor genes P16, RASSF1A and ppENK. Oncol Rep. 35(4):1941–1949. doi:10.3892/or.2016.4554.

Pan H, Zhao Z, Deng Y, Zheng Z, Huang Y, Huang S, Chi P. 2022. The global, regional, and national early-onset colorectal cancer burden and trends from 1990 to 2019: results from the Global Burden of Disease Study 2019. BMC Public Health. 22(1):1896. doi:10.1186/s12889-022-14274-7.

Pandey KB, Rizvi SI. 2009. Plant Polyphenols as Dietary Antioxidants in Human Health and Disease. Oxid Med Cell Longev. 2(5):270–278. doi:10.4161/oxim.2.5.9498.

Papanikolaou A, Wang Q-S, Papanikolaou D, Whiteley HE, Rosenberg DW. 2000. Sequential and morphological analyses of aberrant crypt foci formation in mice of differing susceptibility to azoxymethane-induced colon carcinogenesis. Carcinogenesis. 21(8):1567–1572.

Patta A, Fakih M. 2011. First-line cisplatin plus etoposide in high-grade metastatic neuroendocrine tumors of colon and rectum (MCRC NET): review of 8 cases. Anticancer Res. 31(3):975–978.

Pence BC. 1991. Dietary selenium and antioxidant status: toxic effects of 1, 2-dimethylhydrazine in rats. J Nutr. 121(1):138–144.

Picon A, Gold LI, Wang J, Cohen A, Friedman E. 1998. A subset of metastatic human colon cancers expresses elevated levels of transforming growth factor beta1. Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol. 7(6):497–504.

Prakash P, Gnanaprakasam P, Emmanuel R, Arokiyaraj S, Saravanan M. 2013. Green synthesis of silver nanoparticles from leaf extract of *Mimusops elengi*, Linn. for enhanced antibacterial activity against multi drug resistant clinical isolates. Colloids Surf B Biointerfaces. 108:255–259.

Previs RA, Coleman RL, Harris AL, Sood AK. 2015. Molecular pathways: translational and therapeutic implications of the Notch signaling pathway in cancer. Clin Cancer Res. 21(5):955–961.

Rajkumar Venkatadri, Guha G, Ashok Kumar R. 2011. Antioxidant and Anti-Cancer Potentials of *Rheum emodi* Rhizome Extracts. Evid Based Complement Alternat Med. 2011(1):697986. doi:10.1093/ecam/neq048.

Rajkumar V., Guha G, Kumar RA. 2011. Apoptosis induction in MDA-MB-435S, Hep3B and PC-3 cell lines by *Rheum emodi* rhizome extracts. Asian Pac J Cancer Prev. 12(5):1197–1200.

Rani I, Vaiphei K, Agnihotri N. 2014. Supplementation of fish oil augments efficacy and attenuates toxicity of 5-fluorouracil in 1,2-dimethylhydrazine dihydrochloride/dextran sulfate sodium-induced colon carcinogenesis. Cancer Chemother Pharmacol. 74(2):309–322. doi:10.1007/s00280-014-2497-6.

Redondo-Blanco S, Fernández J, Gutiérrez-del-Río I, Villar CJ, Lombó F. 2017. New insights toward colorectal cancer chemotherapy using natural bioactive compounds. Front Pharmacol. 8:109.

Rodrigues MAM, Silva LAG, Salvadori DMF, De Camargo JLV, Montenegro MR. 2002. Aberrant crypt foci and colon cancer: comparison between a short-and medium-term bioassay for colon carcinogenesis using dimethylhydrazine in Wistar rats. Braz J Med Biol Res. 35:351–355.

Schmitt M, Greten FR. 2021. The inflammatory pathogenesis of colorectal cancer. Nat Rev Immunol. 21(10):653–667.

Sever R, Brugge JS. 2015. Signal transduction in cancer. Cold Spring Harb Perspect Med. 5(4):a006098.

Shi Y-Q, Fukai T, Sakagami H, Kuroda J, Miyaoka R, Tamura M, Yoshida N, Nomura T. 2001. Cytotoxic and DNA damage-inducing activities of low molecular weight phenols from rhubarb. Anticancer Res. 21(4A):2847–2853.

Shiezadeh F, Mousavi SH, Amiri MS, Iranshahi M, Tayarani-Najaran Z, Karimi G. 2013. Cytotoxic and apoptotic potential of *rheum turkestanicum* Janisch root extract on human cancer and normal cells. Iran J Pharm Res IJPR. 12(4):811.

Shiono H, Yagi Y, Chikayama Y, Miyazaki S, Nakamura I. 2003. Oxidative damage and phosphatidylserine expression of red blood cells in cattle experimentally infected with *Theileria sergenti*. Parasitol Res. 89(3):228–234. doi:10.1007/s00436-002-0742-0.

Shtutman M, Zhurinsky J, Simcha I, Albanese C, D'Amico M, Pestell R, Ben-Ze'ev A. 1999. The cyclin D1 gene is a target of the β-catenin/LEF-1 pathway. Proc Natl Acad Sci. 96(10):5522–5527. doi:10.1073/pnas.96.10.5522.

Siegel R, Ward E, Brawley O, Jemal A. 2011. The impact of eliminating socioeconomic and racial disparities on premature cancer deaths. Ca- Cancer J Clin. 61(4):212–236.

Singh P, Negi JS, Rawat MSM, Nee Pant GJ. 2010. Quantification of Mineral Elements of *Rheum emodi* Wallr. (Polygonaceae). Biol Trace Elem Res. 138(1–3):293–299. doi:10.1007/s12011-009-8603-7.

Singh SS, Pandey SC, Singh R, Agarwal SK. 2005. 1, 8-Dihydroxyanthraquinone derivatives from rhizomes of *Rheum emodi* Wall. [accessed 2025 Feb 24]. https://nopr.niscpr.res.in/handle/123456789/9126.

Sivaranjani A, Sivagami G, Nalini N. 2016. Chemopreventive effect of carvacrol on 1, 2-dimethylhydrazine induced experimental colon carcinogenesis. J Cancer Res Ther. 12(2):755–762.

Soleimani A, Rahmani F, Ferns GA, Ryzhikov M, Avan A, Hassanian SM. 2020. Role of the NF-κB signaling pathway in the pathogenesis of colorectal cancer. Gene. 726:144132.

Sporn MB, Suh N. 2002. Chemoprevention: an essential approach to controlling cancer. Nat Rev Cancer. 2(7):537–543.

van der Stok EP, Spaander MC, Grünhagen DJ, Verhoef C, Kuipers EJ. 2017. Surveillance after curative treatment for colorectal cancer. Nat Rev Clin Oncol. 14(5):297–315.

Świerczyński M, Szymaszkiewicz A, Fichna J, Zielińska M. 2021. New insights into molecular pathways in colorectal cancer: Adiponectin, interleukin-6 and opioid signaling. Biochim Biophys Acta BBA-Rev Cancer. 1875(1):188460.

Tabin S, Kamili AN, Ganie SA, Zargar O, Sharma V, Gupta RC. 2016. Genetic diversity and population structure of *Rheum* species in Kashmir Himalaya based on ISSR markers. Flora. 223:121–128.

Tan Z-B, Fan H-J, Wu Y-T, Xie L-P, Bi Y-M, Xu H-L, Chen H-M, Li J, Liu B, Zhou Y-C. 2019. *Rheum palmatum* extract exerts anti-hepatocellular carcinoma effects by inhibiting signal transducer and activator of transcription 3 signaling. J Ethnopharmacol. 232:62–72.

Tanaka T. 2009. Colorectal carcinogenesis: Review of human and experimental animal studies. J Carcinog. 8:5.

Tayade A, Dhar P, Ballabh B, Kumar R, Chaurasia OP, Bhatt RP, Srivastava RB. 2012. *Rheum webbianum* royle: A potential medicinal plant from trans-himalayan cold deserts of Ladakh, India. Plant Arch. 12(2):603–606.

Temiz TK, Altun A, Turgut N, Balcı E. 2014. Investigation of the effects of drugs effective on PI3K-AKT signaling pathway in colorectal cancer alone and in combination. Cumhur Med J. 36(2):167–177.

Thangaraj K, Natesan K, Palani M, Vaiyapuri M. 2018. Orientin, a flavanoid, mitigates 1, 2 dimethylhydrazine-induced colorectal lesions in Wistar rats fed a high-fat diet. Toxicol Rep. 5:977–987.

Tiwari A, Saraf S, Verma A, Panda PK, Jain SK. 2018. Novel targeting approaches and signaling pathways of colorectal cancer: An insight. World J Gastroenterol. 24(39):4428.

Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. 2011. Phytochemical screening and extraction: a review. Int Pharm Sci. 1(1):98–106.

Vieira-de-Abreu A, Campbell RA, Weyrich AS, Zimmerman GA. 2012. Platelets: versatile effector cells in hemostasis, inflammation, and the immune continuum. Semin Immunopathol. 34(1):5–30. doi:10.1007/s00281-011-0286-4.

Vinothkumar Rajenderan, Vinothkumar Rajamanickam, Sudha M, Nalini N. 2014. Chemopreventive effect of *zingerone* against colon carcinogenesis induced by 1, 2-dimethylhydrazine in rats. Eur J Cancer Prev. 23(5):361–371.

Wani IA, Verma S, Ahmad P, El-Serehy HA, Hashim MJ. 2022. Reproductive biology of *Rheum webbianum* Royle, a vulnerable medicinal herb from alpines of North-Western Himalaya. Front Plant Sci. 13:699645.

Wei W-T, Chen H, Ni Z-L, Liu H-B, Tong H-F, Fan L, Liu A, Qiu M-X, Liu D-L, Guo H-C. 2011. Antitumor and apoptosis-promoting properties of emodin, an anthraquinone derivative from *Rheum officinale* Baill, against pancreatic cancer in mice via inhibition of Akt activation. Int J Oncol. 39(6):1381–1390.

Wolter S, Frank N. 1982. Metabolism of 1, 2-dimethylhydrazine in isolated perfused rat liver. Chem Biol Interact. 42(3):335–344.

Wong D, Teixeira A, Oikonomopoulos S, Humburg P, Lone IN, Saliba D, Siggers T, Bulyk M, Angelov D, Dimitrov S, et al. 2011. Extensive characterization of NF-κB binding uncovers non-canonical motifs and advances the interpretation of genetic functional traits. Genome Biol. 12(7):R70. doi:10.1186/gb-2011-12-7-r70.

Xi Y, Xu P. 2021. Global colorectal cancer burden in 2020 and projections to 2040. Transl Oncol. 14(10):101174.

Xiang H, Zuo J, Guo F, Dong D. 2020. What we already know about rhubarb: a comprehensive review. Chin Med. 15(1):88. doi:10.1186/s13020-020-00370-6.

Yan H, Talty R, Aladelokun O, Bosenberg M, Johnson CH. 2023. Ferroptosis in colorectal cancer: a future target? Br J Cancer. 128(8):1439–1451.

Zhang Q, Liu J, Li R, Zhao R, Zhang M, Wei S, Ran D, Jin W, Wu C. 2020. A network pharmacology approach to investigate the anticancer mechanism and potential active ingredients of *Rheum palmatum* L. against lung cancer via induction of apoptosis. Front Pharmacol. 11:528308.

Zhou G, Peng F, Zhong Y, Chen Y, Tang M, Li D. 2017. Rhein suppresses matrix metalloproteinase production by regulating the Rac1/ROS/MAPK/AP-1 pathway in human ovarian carcinoma cells. Int J Oncol. 50(3):933–941. doi:10.3892/ijo.2017.3853.

APPENDIX

10.List of publications

- **Umer Majeed Khaja ,** Farhat Jabeen, Asma Rafiq, Chirag Chopra, Reena Singh, Showkat Ahmad Ganie. *Studies on the Ameliorative Potential of Rheum Webbianum Rhizome Extracts on 1,2-Dimethylhydrazine (DMH) Induced Colorectal Cancer and Associated Hepatic and Haematological Abnormalities in Swiss Albino Rats.* **Journal of Ethnopharmacology** 335 (**2024**) 118652 **(Impact Factor 5.4).** https://doi.org/10.1016/j.jep.2024.118652

- **Umer Majeed Khaja**, Chirag Chopra, Amit Sehgal, Reena Singh, Showkat Ahmad Ganie, *Unveiling the Cancer-Fighting Potential of Rheum Species (Rhubarb): Phytochemistry, Ethnopharmacology, and Mechanistic Insights into the Anticancer Effects of Key Anthraquinones,* **Phytomedicine Plus (2025)**. **(Impact Factor 3)** https://doi.org/10.1016/j.phyplu.2025.100831

- Khalid Bashir Dar, Ruhban Ansar Parry, Aashiq Hussain Bhat, Afaq Hameed Beigh, Maroof Ahmed, **Umer Majeed Khaja**, Aijaz Hassan Ganie, Manzoor Ahmad Mir, Bilal Ahmad Reshi, Ishfaq Shafi Khan and Showkat Ahmad Ganie. *Immunomodulatory efficacy of Cousinia thomsonii C.B. Clarke in ameliorating inflammatory cascade expressions.* **Journal of Ethnopharmacology. 2022**, 300: 1-12. **(Impact Factor 5.4)** https://doi.org/10.1016/j.jep.2022.115727

- **Umer Majeed Khaja**, Aashiq Hussain Bhat, Maroof Ahmed, Aarif Ali, Showkat Ahmad Ganie. *Pharmacogenomics in viral diseases.* Pharmacogenomics: From Discovery to Clinical Implementation. Pp 247-26. **Elsevier. ISBN: 978-0-443-15336-5. (2023).** https://doi.org/10.1016/B978-0-443-15336-5.00006-3

- Aashiq Hussain Bhat, **Umer Majeed Khaja**, Maroof Ahmed, Waseem Younis Khan, Showkat Ahmad Ganie. *Pharmacogenomics in cancer.* Pharmacogenomics: From Discovery to Clinical Implementation. Pp 195-221. **Elsevier. ISBN: 978-0-443-15336-5. (2023).** https://doi.org/10.1016/B978-0-443-15336-5.00001-4

- Maroof Ahmed, Bashir Ahmad Malla, **Umer Majeed Khaja**, Aashiq Hussain Bhat, Aarif Ali, Showkat Ahmad Ganie, Muneeb U. Rehman, Zuha Imtiyaz. *Pharmacogenomics in cardiovascular diseases.* Pharmacogenomics: From Discovery to Clinical Implementation. Pp 137-168. **Elsevier. ISBN: 978-0-443-15336-5. (2023).** https://doi.org/10.1016/B978-0-443-15336-5.00009-9

- Gani, Showkat and Jabeen, Farhat and Khan, Waseem Younis and Rashid, Safeena and Parry, Ruhban Ansar and Manzoor, Syed ifra and **Khaja, Umer Majeed** and Khursheed, Nuzhat and Farooq, Ambreen and Amin, Shajrul, *Exploring the Therapeutic Potential of Inula Obtusifolia: In-Vitro and In-Vivo Insights into its Antioxidant, Anti-Inflammatory and Antidiabetic Effects Against Alloxan-Induced Oxidative Stress in Diabetic Rats.* (Pre Print, 2025) SSRN: http://dx.doi.org/10.2139/ssrn.5193250

- **Umer Majeed Khaja,** Reena Singh, Showkat Ganie, Rauf Ahmad Wani, Chirag Chopra. *Advancements in Understanding Colorectal Cancer: Microenvironment Insights, Novel Biomarkers, and Therapeutic Innovations*.

- **Abstracts published**

- **Umer Majeed Khaja**, Reena Singh, Showkat Ahmad Ganie; Abstract 6330: *Unveiling the anticancer potential of Rheum webbianum in colorectal cancer defense through modulation of key wnt/β-catenin and TGF-β signaling pathways: An in-vitro and in-vivo investigation.* **AACR Annual Meeting 2025 held in Chicago, USA,** April 25th–30th, 2025. ***Cancer Research,*** 2025; 85 (8_Supplement_1): 6330 (**Impact Factor 16.6).** https://doi.org/10.1158/1538-7445.AM2025-6330

- **Umer Majeed Khaja**, Reena Sing, Showkat Ahmad Ganie. *Studies on the Potential of Rheum Webbianum Rhizome Extracts on the Regression of 1,2-Dimethylhydrazine-Induced Colorectal Cancer In Swiss Albino Rats.* **"JK-AGRI-MED Science Congress 2023-2024"**: 27th -29th Feb, 2024.

- **Umer Majeed Khaja**, Amit Sehgal, Showkat Ahmad Ganie, Ab Ahad Bhat. *Bioactive metabolites of medicinal plants in cancer prevention and treatment: A Review.* **International conference on Nanotechnology for better living**. **2021**: 7th-11th September.

- **Umer Majeed Khaja,** Amit Sehgal, Showkat Ahmad Ganie. *Phytoconstituents and Potential of Rheum species (Rhubarb) for prevention and treatment of oncologic diseases: A Comprehensive Review.* **International conference on "Recent advances in Biomedical Sciences and Regenerative Medicine RABSRM 2022"**: 06th-07th May 2022.

11.List of conferences

- Participated in the AACR Annual Meeting 2025 held in Chicago, USA, as a recipient of the prestigious **Global Scholar-in-Training Award (GSITA)** from the **American Association for Cancer Research (AACR)**, from April 25th–30th, 2025.

- Participated in the **JK-AGRI-MED Science Congress 2023-2024** on "Reshaping Bio-economy Towards One Health" Organized by SKUAST Kashmir in collaboration with J&K Science Technology and Innovation Council, Department of Science and Technology, Government of J&K, held on 27th-29th Feb, 2024.

- Participated in the **International conference** on "Recent advances in Biomedical Sciences and Regenerative Medicine RABSRM 2022" Organized by Department of Science and Technology, SKUAST and Kashmir University, held on 6th-7th May 2022.

- Participated in the **International Conference** on "Nanotechnology for Better Living" NBL-2021, jointly organized by NIT Srinagar, IIT Delhi in association with prestigious Universities from 7th-11th September, 2021.

- Attended **Animal Science Congress**: Horizons in Zoological Studies, organized by Department of Zoology, University of Kashmir in collaboration with Zoological Society of India and J&K Academy Of Sciences from 4th-6th August, 2018.

Awards and Achievements

- **Global Scholar-in-Training Award (GSITA) 2025 by the American Association for Cancer Research (AACR), USA.**
- **INSC Young Researcher Award 2024 by Institute Of Scholars (INSC).**
- **Development of the first chemically induced *in-vivo* colorectal cancer (CRC) rat model, marking the first of its kind in the history of Lovely Professional University and the University of Kashmir.**

Ivory Tower: LPU, Kashmir varsity achieve milestone in colorectal cancer research

Researchers at Lovely Professional University (LPU), in collaboration with the University of Kashmir, have announced achieving a milestone in cancer research by developing an in-vivo colorectal cancer (CRC) rat model.

The model provides a robust platform for testing new chemopreventive agents, understanding the molecular basis of CRC, and exploring potential therapeutic interventions.

"The development of the CRC rat model is a pioneering effort that has opened new avenues for the cancer research and will allow in-depth studies on the mechanisms of colorectal cancer development, progression and metastasis, which were previously not possible within our facilities," the research team said.

The implications of the development are profound as it sets the stage for future research projects that can lead to significant advancements in cancer treatment and prevention.

Umer Majeed Khaja, a research scholar at the Department of Zoology, LPU, carried out the pioneering work under the supervision of Dr Showkat Ahmad Ganie, Senior Assistant Professor, Department of Clinical Biochemistry, University of Kashmir; and Dr Reena Singh, Associate Professor, LPU.

The research titled, 'Studies on the Ameliorative Potential of Rheum Webbianum Rhizome Extracts on 1,2-Dimethylhydrazine (DMH)-Induced Colorectal Cancer and Associated Hepatic and Haematological Abnormalities in Swiss Albino Rats', has made noteworthy contributions to the field of cancer research.

DMH is a well-established colon carcinogen.

The work has received international recognition and has been accepted for publication in the prestigious Journal of Ethnopharmacology, Elsevier.

Several experts have appreciated the research as a valuable contribution to ethnopharmacology and cancer research, highlighting its comprehensive investigation, use of both in-vitro and in-vivo CRC models. They have also appreciated promising findings on the chemopreventive potential of Rheum webbianum (RW) extracts. The study investigates the chemopreventive potential of RW rhizome extracts on DMH-induced colorectal cancer in Swiss albino rats.

The findings demonstrated the ameliorative potential of RW rhizome extracts on colorectal cancer and associated hepatic and haematological abnormalities, highlighting its potential as a natural chemopreventive agent, the statement added.

Adhering to the guidelines of the Institutional Animal Ethics Committee, Umer's work has been supported by the Department of Zoology and faculty members such as Prof Fayaz Ahmad.

The teams have acknowledged the efforts of Dr Chirag Chopra, Assistant Professor at the School of Bioengineering and Biosciences, LPU, for his inputs and support during the research work.

Both LPU and University of Kashmir have termed it a significant step in cancer research and treatment innovation.

Umer Majeed Khaja, research scholar at LPU Zoology Dept.

BREAKTHROUGH WINS ACCLAIM

- Researchers have developed an in-vivo colorectal cancer rat model, which provides a robust platform for testing new chemopreventive agents.
- Study investigated chemopreventive potential of Rheum webbianum (RW) rhizome extracts on colorectal cancer induced by Dimethylhydrazine (DMH) – a well-established colon carcinogen – in Swiss albino rats.
- Findings demonstrated the ameliorative potential of RW rhizome extracts on colorectal cancer and associated abnormalities, highlighting its potential as a natural chemopreventive agent.
- Experts have appreciated the research as a valuable contribution to ethnopharmacology and cancer research.
- The work has received international recognition and has been accepted for publication in prestigious Journal of Ethnopharmacology, Elsevier.

The Tribune | Trending | Videos | India | Opinion | Sports | State | City | World | Diaspora

Ivory Tower: LPU, Kashmir varsity achieve milestone in colorectal cancer research

Researchers at Lovely Professional University (LPU), in collaboration with the University of Kashmir, have announced achieving a milestone in cancer research by developing an in-vivo colorectal cancer (CRC) rat model. The model provides a robust platform for testing new chemopreventive...

TRIBUNE NEWS SERVICE
Updated At : 06:47 AM Apr 08, 2025 IST

International Recognition

myAACR

AACR American Association for Cancer Research

Search DONATE TODAY

CANCER RESEARCHERS / OTHER HEALTH CARE PROFESSIONALS | PATIENTS, CAREGIVERS, AND ADVOCATES | GET INVOLVED | WAYS TO GIVE | ABOUT THE AACR

UMER MAJEED KHAJA, PHD

Lovely Professional University and University of Kashmir, India

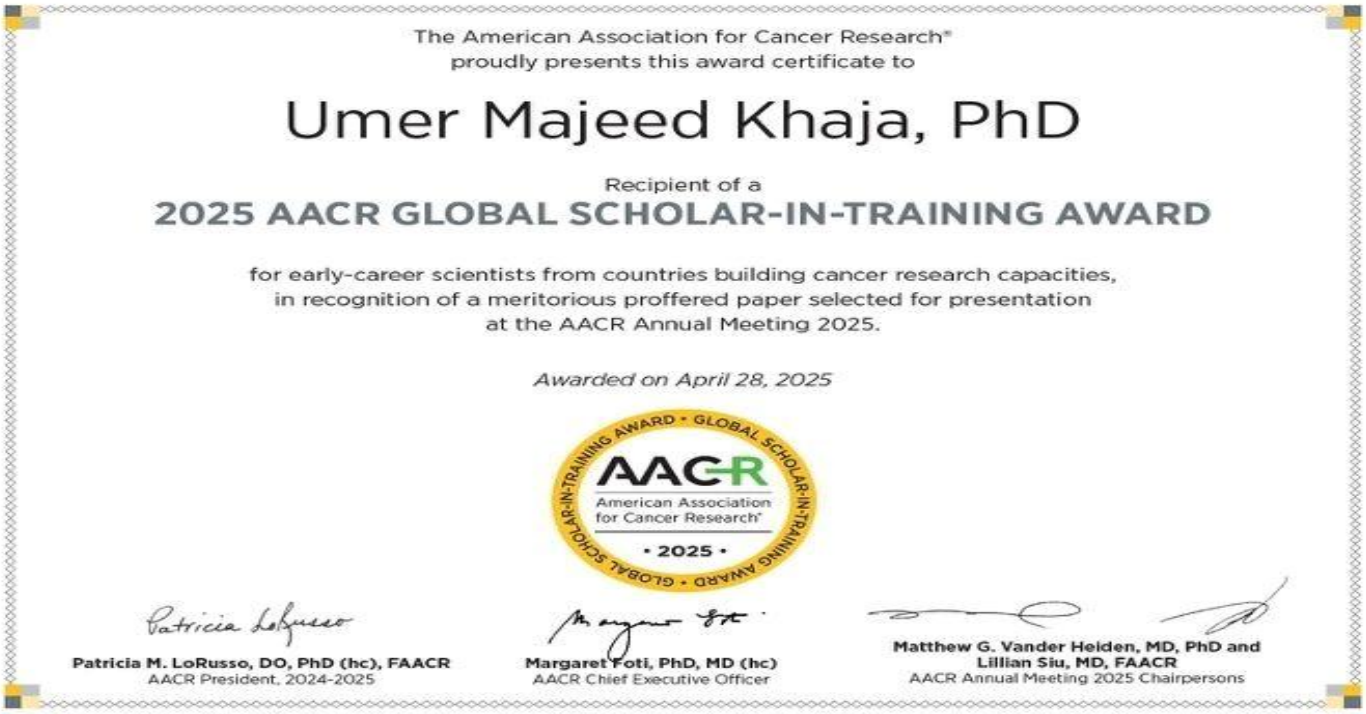
Abstract 3622: Unveiling the anticancer potential of *Rheum webbianum* in colorectal cancer defense through modulation of key Wnt/β-catenin and TGF-β signaling pathways: An in-vitro and in-vivo investigation

HOME WORLD NATIONAL SPORTS JAMMU KASHMIR LADAKH HEALTH

American Association for Cancer Research Recognises Kashmiri Researcher with Prestigious Global Scholar-in-Training Award

BY NEWS DESK FEBRUARY 13, 2025 0

Umer Majeed Khaja, a dedicated researcher from Jammu and Kashmir, has achieved a significant milestone in his academic journey. Affiliated...



The American Association for Cancer Research®
proudly presents this award certificate to

Umer Majeed Khaja, PhD

Recipient of a

2025 AACR GLOBAL SCHOLAR-IN-TRAINING AWARD

for early-career scientists from countries building cancer research capacities,
in recognition of a meritorious proffered paper selected for presentation
at the AACR Annual Meeting 2025.

Awarded on April 28, 2025

GLOBAL SCHOLAR-IN-TRAINING AWARD • GLOBAL SCHOLAR-IN-TRAINING AWARD • AACR American Association for Cancer Research • 2025 •

Patricia M. LoRusso, DO, PhD (hc), FAACR
AACR President, 2024-2025

Margaret Foti, PhD, MD (hc)
AACR Chief Executive Officer

Matthew G. Vander Heiden, MD, PhD and Lillian Siu, MD, FAACR
AACR Annual Meeting 2025 Chairpersons

the northlines

KU researcher wins prestigious award for Cancer Research

Science · Cancer · Medicine · Health Conditions

11 Mar 2025 +1 more NL CORRESPONDENT

SRINAGAR, MARCH 10 In a remarkable achievement for the University of Kashmir, (KU) Umer Majeed Khaja, a researcher from the Department of Clinical Biochemistry, has been awarded the prestigious Global Scholarin-training Award (GSITA) 2025 by the American Association for Cancer Research (AACR).

The highly competitive GSITA award, granted to only 15 researchers worldwide, recognizes Umer's outstanding contributions to colorectal cancer research. He is among just two researchers from India selected this year, the other being from AIIMS, New Delhi.

The award provides full sponsorship for Umer to attend the AACR Annual Meeting 2025 in Chicago, USA (April 25– 30), where he will present his research, engage with leading cancer

KU researcher wins prestigious global award for cancer research

Srinagar, March 10: In a remarkable achievement for the University of Kashmir, (KU) Umer Majeed Khaja, a researcher from the department of Clinical Biochemistry, has been awarded the prestigious Global Scholar-in-Training Award (GSITA) 2025 by the American Association for Cancer Research (AACR). The highly competitive GSITA award, granted to only 15 researchers worldwide, recognizes Umer's outstanding contributions to colorectal cancer research. He is among just two researchers from India selected this year, the other being from AIIMS, New Delhi.

The award provides full sponsorship for Umer to attend the AACR Annual Meeting 2025 in Chicago, USA (April 25–30), where he will present his research, engage with leading cancer scientists, and explore advancements in cancer research. Vice-Chancellor KU Prof. Nilofer Khan congratulated Umer on this international recognition, stating, that, "This prestigious recognition reflects the high-quality research emerging from the University of Kashmir in collaboration with reputed institutions. Umer's success is a proud moment for the university and will inspire future scholars to aim for international recognition in their respective fields."

Prof. Mohamad Sultan Bhat, Dean Research, KU also lauded Umer's achievement, emphasizing "This accomplishment highlights the significance of collaborative research in addressing global health challenges. Umer's work in colorectal cancer research is commendable, and his recognition at an international platform like AACR speaks volumes about the caliber of research conducted at our institution." Umer's PhD research, conducted under the supervision of Dr. Reena Singh (Lovely Professional University, Punjab) and Dr. Showkat Ahmad Ganie (University of Kashmir), focuses on colorectal cancer. His work investigates the chemopreventive potential of Rheum webbianum rhizome extracts against colorectal carcinogenesis, offering valuable insights into cancer biology and therapeutic strategies. Beyond his research, Umer has published extensively in reputed international journals, serves as a peer reviewer for leading scientific publications, and actively raises cancer awareness in Kashmir. His success is expected to strengthen the research ecosystem in Jammu and Kashmir, encouraging greater academic collaborations and motivating young scholars to contribute to impactful scientific studies. As he prepares to represent India on a global platform, his journey stands as a testament to the power of perseverance, dedication, and scientific excellence.

Recognition at an int'l platform speaks volumes of Umer's calibre: Sultan Bhat

BK NEWS SERVICE

Srinagar, Mar 10: In a remarkable achievement for the University of Kashmir, (KU) Umer Majeed Khaja, a researcher from the Department of Clinical Biochemistry, has been awarded the prestigious Global Scholar-in-Training Award (GSITA) 2025 by the American Association for Cancer Research (AACR).

The highly competitive GSITA award, granted to only 15 researchers worldwide, recognizes Umer's outstanding contributions to colorectal cancer research. He is among just two researchers from India selected this year, the other being from AIIMS, New Delhi.

The award provides full sponsorship for Umer to attend the AACR Annual Meeting 2025 in Chicago, USA (April 25–30), where he will present his research, engage with leading cancer scientists, and explore advancements in cancer research.

Vice-Chancellor KU Prof. Nilofer Khan congratulated Umer on this international recognition, stating

"This prestigious recognition reflects the high-quality research emerging from the University of Kashmir in collaboration with reputed institutions. Umer's success is a proud moment for the university and will inspire future scholars to aim for international recognition in their respective fields."

Prof. Mohamad Sultan Bhat, Dean Research, KU also lauded Umer's achievement, emphasizing "This accomplishment highlights the significance of collaborative research in addressing global health challenges. Umer's work in colorectal cancer research is commendable, and his recognition at an international platform like AACR speaks volumes about the caliber of research conducted at our institution."

Umer's PhD research, conducted under the supervision of Dr. Reena Singh (Lovely Professional University, Punjab) and Dr. Showkat Ahmad Ganie (University of Kashmir), focuses on colorectal cancer. His work investigates the chemopreventive potential of Rheum webbianum rhizome extracts against colorectal carcinogenesis, offering valuable insights into cancer biology and therapeutic strategies.

Beyond his research, Umer has published extensively in reputed international journals, serves as a peer reviewer for leading scientific publications, and actively raises cancer awareness in Kashmir.

KU researcher wins prestigious global award for Cancer Research

GK News Service
Srinagar, Mar 10

In a remarkable achievement for the University of Kashmir, (KU) Umer Majeed Khaja, a researcher from the Department of Clinical Biochemistry, has been awarded the prestigious Global Scholar-in-Training Award (GSITA) 2025 by the American Association for Cancer Research (AACR).

The highly competitive GSITA award, granted to only 15 researchers worldwide, recognizes Umer's outstanding contributions to colorectal cancer research. He is among just two researchers from India selected this year, the other being from AIIMS, New Delhi. The award provides full sponsorship for Umer to attend the AACR Annual Meeting 2025 in Chicago, USA (April 25–30), where he will present his research, engage with leading cancer scientists, and explore advancements in cancer research.

Vice-Chancellor KU Prof. Nilofer Khan congratulated Umer on this international recognition, stating

"This prestigious recognition reflects the high-quality research emerging from the University of Kashmir in collaboration with reputed institutions. Umer's success is a proud moment for the university and will inspire future scholars to aim for international recognition in their respective fields."

Full text on greaterkashmir.com

کینسر تحقیق: کشمیر یونیورسٹی کے محقق کو ممتاز عالمی اعزاز عطا

سرینگر (کے ایم) نیوز سروس/ کشمیر یونیورسٹی کے لیے ایک قابل ذکر کارنامے میں، شعبہ کیمسٹری کے ایک محقق عمر مجید خواجہ کو امریکن ایسوسی ایشن فار کینسر ریسرچ (AACR) کی جانب سے ممتاز گلوبل سکالر-ان-ٹریننگ ایوارڈ (GSITA) 2025 سے نوازا گیا ہے۔ انتہائی مسابقتی GSITA ایوارڈ، جو دنیا بھر میں صرف 15 محققین کو دیا جاتا ہے، کولوریکٹل کینسر کی تحقیق میں عمر کی شاندار شراکت کو تسلیم کرتا ہے۔ وہ اس سال ہندوستان کے صرف دو محققین میں شامل ہیں، دوسرے ایمس، نئی دہلی سے ہیں۔ یہ ایوارڈ عمر کو شکاگو USA (اپریل 25-30) میں ہونے والی AACR سالانہ میٹنگ 2025 میں شرکت کے لیے مکمل کفالت فراہم کرتا ہے، جہاں وہ اپنی تحقیق پیش کریں گے، دنیا بھر کے سائنسدانوں کے ساتھ مشغول ہوں گے اور کینسر کی تحقیق میں جدید پیشرفت کو دریافت کریں گے۔ وائس چانسلر کے یو پروفیسر نیلوفر خان نے عمر کو اس بین الاقوامی پہچان پر مبارکباد دیتے ہوئے کہا، "یہ باوقار شناخت معروف اداروں کے تعاون سے کشمیر یونیورسٹی کی جانب سے ابھرنے والی اعلیٰ معیاری تحقیق کی عکاسی کرتی ہے۔ عمر کی کامیابی یونیورسٹی کے لیے ایک قابل فخر لمحہ ہے اور مستقبل کے اسکالرز کو ان کے متعلقہ شعبوں میں بین الاقوامی سطح پر شناخت حاصل کرنے کی ترغیب دے گی۔" پروفیسر محمد سلطان بھٹ، ڈین ریسرچ، نے بھی عمر کے کارنامے کو سراہتے ہوئے اس بات پر زور دیا کہ یہ کامیابی عالمی سحت کے چیلنجوں سے نمٹنے کے لیے مشترکہ تحقیق کی اہمیت کو اجاگر کرتی ہے۔ "یہ بڑی آفت کے کینسر کی تحقیق میں عمر کا کام قابل ستائش ہے۔ او AACR جیسے بین الاقوامی پلیٹ فارم پر ان کی پہچان ہمارے AACR کے یقینی ادارے کے بارے میں بات کرتی ہے۔ اپنی تحقیق کے علاوہ، عمر نے معروف بین الاقوامی جرائد میں بڑے پیمانے پر اپنے مضامین شائع کیے۔ معروف سائنسی اشاعتوں کے لیے ہم مرتبہ جائزہ نگار کے طور پر کام کیا ہے۔ اور کشمیر میں کینسر کے بارے میں آگاہی کو فعال طور پر بڑھایا ہے۔

Journal of Ethnopharmacology 335 (2024) 118652

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Studies on the ameliorative potential of *Rheum webbianum* rhizome extracts on 1,2-dimethylhydrazine (DMH) induced colorectal cancer and associated hepatic and haematological abnormalities in swiss albino rats

Umer Majeed Khaja [a,b], Farhat Jabeen [b], Maroof Ahmed [b], Asma Rafiq [c], Ruhban Ansar [b], Khushboo Javaid [b], Chirag Chopra [a], Reena Singh [a,*], Showkat Ahmad Ganie [b,**]

[a] School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India
[b] Department of Clinical Biochemistry, University of Kashmir, J&K, India
[c] Department of Nanotechnology, University of Kashmir, J&K, India

ARTICLE INFO

Handling Editor: Thomas Efferth

Keywords:
Rheum webbianum
Colorectal cancer
Phytochemicals
Anticancer agents
Dimethylhydrazine

ABSTRACT

Ethnopharmacological relevance: *Rheum webbianum* Royle (RW) holds significant ethnopharmacological importance owing to its 5000-year history of cultivation for medicinal and culinary purposes. Demonstrating therapeutic advantages in traditional and contemporary medical practices, RW exhibits key pharmacological effects including anticancer activity, gastrointestinal control, anti-inflammatory properties, and suppression of fibrosis. Despite its recognized vast bioactivities in ethnopharmacology, its efficacy against the colorectal cancer (CRC) remains incompletely understood.

Aim of the study: This study for the first time aims to investigate the chemo-preventive capabilities of various extracts derived from RW rhizomes against CRC development.

Materials and methods: Four types of RW extracts were prepared by using different solvents viz: Hexane, Ethyacetate, Ethanol and Methanol. All the four extracts were evaluated for cytotoxicity on HCT-116 human CRC cells. Promising extracts were further investigated *in-vivo* at varying doses using 1,2-dimethylhydrazine (DMH) induced rat CRC model to assess the anti-oxidant and anticancer properties as well as their effects on the associated hepatic deterioration and hematological alterations.

Results: Cell viability: *In-vitro* assessments demonstrated a dose and time-dependent reduction in HCT-116 cell viability following treatment with methanolic and ethanolic extracts of RW, reducing viability by up to 85% and 90%, respectively, at 200 μg/ml. Histopathology: Histopathological analyses revealed significant improvements in colon tissue morphology in RW extract-treated groups compared to DMH-only treated animals. RW-treated groups showed reduced structural abnormalities, congestion, inflammatory cell infiltration, crypt abscess formation, and dysplasia. In contrast, the DMH-only group exhibited irregular glandular structure, mucosal destruction, extensive inflammatory cell infiltration, crypt abscess formation, and dysplasia. These results highlight the potential of RW methanolic and ethanolic extracts in mitigating colon cancer-related histopathological alterations. Haematological, and hepatic parameters: In the DMH-induced colorectal cancer rat model, significant hematological imbalances were evident, including a 49.13% decrease in erythrocytes, 32.18% in hemoglobin, and 26.79% in hematocrit, along with a 79.62% increase in white blood cells and 68.96% rise in platelets. Administration of RW rhizome extracts effectively restored these hematological parameters to levels comparable to those in the control group. Furthermore, RW treatment significantly reduced serum ALT and AST levels, which had increased by 36.78% and 33.12%, respectively, due to DMH exposure. RW intervention also mitigated the onset of atherosclerosis, evidenced by notable reductions in serum total cholesterol and triglyceride levels. Comparative analysis indicated that RW-treated DMH groups effectively restored lipid profiles,

Abbreviations: RW, *Rheum webbianum*; CRC, colorectal cancer; DMH, 1,2-Dimethylhydrazine; GSH, reduced glutathione; GR, glutathione reductase; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; RBC, red blood cell; Hb, hemoglobin; HCT, hematocrit; WBC, white blood cell count; Plt, platelet; EDTA, ethylenediaminetetraacetic acid.

* Corresponding author.
** Corresponding author.
E-mail addresses: reena.19408@lpu.co.in (R. Singh), showkatganie@kashmiruniversity.ac.in (S.A. Ganie).

https://doi.org/10.1016/j.jep.2024.118652
Received 28 May 2024; Received in revised form 15 July 2024; Accepted 30 July 2024
Available online 2 August 2024

Phytomedicine Plus 5 (2025) 100831

Contents lists available at ScienceDirect

Phytomedicine Plus

journal homepage: www.sciencedirect.com/journal/phytomedicine-plus

Unveiling the cancer-fighting potential of Rheum species (Rhubarb): Phytochemistry, ethnopharmacology, and mechanistic insights into the anticancer effects of key anthraquinones

Umer Majeed Khaja [a,c], Chirag Chopra [a], Amit Sehgal [b], Reena Singh [a,**], Showkat Ahmad Ganie [c,*]

[a] School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, 144411, India
[b] Choudhary Bansilal Government College for Women, Bhiwani, Haryana
[c] Department of Clinical Biochemistry, University of Kashmir, Hazratbal, Srinagar, 190006, J&K, India

ARTICLE INFO

Keywords:
Cancer
Herbal medicine
Rhubarb
Medicinal plant research
Phyto-constituents
Cancer prevention

ABSTRACT

Background: Rhubarb (*Rheum* spp.) has been cultivated for over 5,000 years for both medicinal and culinary purposes. Renowned in traditional and modern medicine, it offers a range of therapeutic benefits. The rhizome of rhubarb, recognized as a significant medicinal plant, was first documented as early as 270 BC in the ancient Chinese text "Shen Nong Ben Cao Jing". Notably, rhubarb is celebrated for its anti-cancer properties, gastrointestinal regulation, anti-inflammatory effects, and ability to inhibit fibrosis. Cancer, a leading cause of global morbidity and mortality, underscores the urgency to identify new therapeutic agents. Despite rhubarb's extensive history of use, there is a pressing need for a comprehensive review that examines its anti-cancer properties and underlying mechanisms.

Purpose: This review aims to examine the phytochemistry, ethno-medicinal applications, and anti-cancer capabilities of Rhubarb. Additionally, it will explore the underlying mechanisms of the anti-neoplastic activity of the most prevalent anthraquinones found in *Rheum* species.

Methods: A comprehensive search was conducted for randomized controlled trials on the benefits of *Rheum* species, using databases such as PubMed, Elsevier, SCOPUS, and the Cochrane Database for Systematic Review.

Results: Rhubarb exhibits diverse biological effects through its phytoconstituents, making it effective in preventing and treating various diseases, including cancer. Key anthraquinones in rhubarb, such as emodin and aloe-emodin, have demonstrated the ability to inhibit cellular proliferation, induce apoptosis, and suppress metastasis in cancers of the breast, colon, lung, liver, blood, pancreas, stomach, and oral cavity. The chemopreventive and anti-carcinogenic potential of *Rheum* species stems from their modulation of critical molecular mechanisms and signaling pathways including NF-kappa B (NF-κB), Tumor Suppressor Gene (p53), tyrosine kinases, phosphoinositol 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), protein kinase C (PKC) involved in various anti-cancer activities. The review findings also elucidate the potent anti-inflammatory activity of rhubarb, supported by various mechanisms including inhibition of lipoxygenase (LOX), cyclooxygenase (COX) and hyaluronoglucosaminidase (HYAL) enzymes, reduction of pro-inflammatory responses, and modulation of inflammatory signaling pathways. These findings highlight the therapeutic potential of rhubarb's bioactive anthraquinones that show a great potential in fighting cancer and could be used for various therapeutic applications.

Conclusions: Rhubarb contains herbal remedies that have the potential to prevent and treat a variety of human malignancies. Many chemical constituents of rhubarb, especially anthraquinones may be the cause of its therapeutic properties. The preclinical research discussed in this review strongly implies that *Rheum* species have a significant ability to prevent and treat human malignancies.

Abbreviations: AE, Aloe Emodin; ER, Estrogen receptor; MMP, Matrix metalloproteinases; PAI, Plasminogen activator inhibitor-1; R, Rheum; RCTs, Randomised controlled trials; RPE, Rheum palmatum ethanol extract; uPA, Urokinase-Plasminogen Activator (uPA); uPAR, Urokinase-Plasminogen Activator Receptor (uPAR).

* Corresponding author at: Department of Clinical Biochemistry, University of Kashmir, Hazratbal Srinagar 190006, J&K, India.
** Co-corresponding author at: School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, 144411, India.
E-mail addresses: reena.19408@lpu.co.in (R. Singh), showkatganie@kashmiruniversity.ac.in (S.A. Ganie).

https://doi.org/10.1016/j.phyplu.2025.100831

Available online 30 May 2025

Journal of Ethnopharmacology 300 (2023) 115727

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Immunomodulatory efficacy of *Cousinia thomsonii* C.B. Clarke in ameliorating inflammatory cascade expressions

Khalid Bashir Dar [a,1], Ruhban Ansar Parry [a,1], Aashiq Hussain Bhat [a], Afaq Hameed Beigh [b], Maroof Ahmed [a], Umer Majeed Khaja [a,f], Aijaz Hassan Ganie [c], Manzoor Ahmad Mir [d], Bilal Ahmad Reshi [e], Ishfaq Shafi Khan [g], Showkat Ahmad Ganie [a,*]

[a] Department of Clinical Biochemistry, University of Kashmir, Srinagar, J&K, India
[b] Department of Immunology and Molecular Medicine SKIMS, Srinagar, J&K, India
[c] Department of Botany, University of Kashmir, Srinagar, J&K, India
[d] Department of Bioresource, University of Kashmir, Srinagar, J&K, India
[e] Department of Biotechnology, University of Kashmir, Srinagar, India
[f] Department of Zoology, Lovely Professional University, Punjab, India
[g] Centre of Research for Development, University of Kashmir, Srinagar, J&K, India

ARTICLE INFO

Keywords:
Immunomodulatory
Lipopolysaccharide
Rat model
Methanol and aqueous extracts
Molecular docking

ABSTRACT

Ethnopharmacological relevance: Cousinia thomsonii is traditionally known for treating various diseases including joint pain, swelling, body ache, asthma, dermatitis, cough and arthritis.

Aim of the study: This study employs lipopolysaccharide induced inflammatory wistar-rat model to evaluate efficacy of *Cousinia thomsonii* active-extracts on the expression of crucial inflammatory markers viz. iNOS, PPAR-γ, Rel-A, COX-2 and serum analysis of CRP.

Materials and methods: Methanol and aqueous extracts were administered orally at 25, 50, 100 mg/kg doses for 21 days. Serum was collected on 22nd day and rats were sacrificed to extract paw tissues. Dexamethasone (0.5 mg/kg) served as positive control. Immunoblotting and qPCR was used for expression analysis of iNOS, PPAR-γ, Rel-A, COX-2 respectively. ELISA was employed for evaluating CRP levels. Discovery-studio and Auto-Dock-Vina were used to check docking interactions of various identified compounds.

Results: Both extracts caused dose-dependent decline in iNOS, Rel-A, COX-2 and CRP levels, while there was a dose-dependent increase in PPAR-γ expression. Methanol extract dominated immunomodulatory potential as compared with the aqueous extract. The results of the GCMS revealed the presence of ten compounds. Some of these compounds include 1-Octacosanol, Ethyl Linoleate, 1-Heptacosanol, 1-Hexadecanol, 1-Dodecanol and Behenic alcohol having strong anti-inflammatory, antimicrobial, anti-acne and anti-viral activities. Molecular Docking scores were calculated between each target protein and selected compounds. The best affinity/interactions were observed between 1-Octacosanol towards iNOS, PPAR-γ, Rel-A, COX-2 and CRP with binding energy of −10.4, −11.1, −8.6, −9.9 and −7.9 (kcal/mol) respectively. These compounds may act as strong inhibitors for iNOS, Rel-A, COX-2 and CRP or as agonists for PPAR-γ; thereby inducing anti-inflammatory/immuno-modulatory activities.

Conclusions: The results indicate that *Cousinia thomsonii* contains therapeutically active compounds and thus could serve as potential therapeutic regimen against diverse inflammatory diseases.

1. Introduction

Inflammation is an important biochemical process triggered by numerous agents like microbes, impaired cells, ultraviolet (UV)-irradiation, toxins and injuries (Chen et al., 2018; Sreena and Nair, 2016). It has deeper implications in regulating immunity and human health. Deregulated form of inflammation often results in multiple human pathologies like in general, there are two types of inflammatory

* Corresponding author.
E-mail address: showkatganie@kashmiruniversity.ac.in (S.A. Ganie).
[1] Contributed equally and hold first authorship.

https://doi.org/10.1016/j.jep.2022.115727
Received 12 July 2022; Received in revised form 12 September 2022; Accepted 12 September 2022
Available online 16 September 2022

ScienceDirect

Pharmacogenomics
From Discovery to Clinical Implementation

2023, Pages 195-221

Chapter 8 - Pharmacogenomics in cancer

Aashiq Hussain Bhat [a], Umer Majeed Khaja [a b], Maroof Ahmed [a], Waseem Younis Khan [a], Showkat Ahmad Ganie [a]

[a] Department of Clinical Biochemistry, School of Biological Sciences, University of Kashmir, Srinagar, Jammu and Kashmir, India
[b] Department of Zoology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India

Available online 23 June 2023, Version of Record 23 June 2023.

https://doi.org/10.1016/B978-0-443-15336-5.00001-4

Abstract

The word "pharmacogenetics" refers to the study of drug response variability caused by inheritance. It has to do with "gene–drug interactions." It means all the genes that could potentially influence the response to the medication. Due to the limited therapeutic range of chemotherapeutic drugs and the potential for potentially fatal adverse, pharmacogenetics plays a role in oncology. Much cancer genomics research has focused on somatic mutations that are acquired, although mounting evidence suggests that inherited germline genetic changes have a significant influence on cancer threat and treatment results. Tumors may include disease-defining mutations in the setting of cancer, but a patient's germline genetic diversity will too impact medication response (both toxicity and efficacy). Cancer pharmacogenetics enables us to pinpoint patients who are at risk for serious toxicity or who are most likely to benefit from a certain drug, bringing us one step closer to our goal of personalized cancer therapy. Finding variants connected to therapy response has been made possible by advances in sequencing technology, clinical trial designs, and statistical genetic analysis methods. In this chapter, we have addressed how germline genetics analytic approaches may be used in pharmacogenomics research on cancer, with a focus on the particular study design concerns. Moreover, a comprehensive study on candidate genes, pharmacogenomic discovery methods, challenges, promises, and application of pharmacogenomics in cancer has been discussed extensively in this chapter.

ScienceDirect

Pharmacogenomics
From Discovery to Clinical Implementation

2023, Pages 247-269

Chapter 10 - Pharmacogenomics in viral diseases

Umer Majeed Khaja [a] [b], Aashiq Hussain Bhat [b], Maroof Ahmed [b], Aarif Ali [b], Showkat Ahmad Ganie [b]

[a] Department of Zoology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India
[b] Department of Clinical Biochemistry, School of Biological Sciences, University of Kashmir, Srinagar, Jammu and Kashmir, India

Available online 23 June 2023, Version of Record 23 June 2023.

https://doi.org/10.1016/B978-0-443-15336-5.00006-3

Abstract

Viral diseases are the prominent infectious diseases that constitute the leading cause of death globally. Among the viral diseases, the commonly found diseases in humans include common cold, flu, hepatitis, chickenpox, Ebola, COVID-19, and AIDS. Hepatitis A virus affects more than 80% of the population, incuding hepatitis B (360 million), hepatitis C virus (123 million), and HIV (30 million) worldwide. As a result, millions of people around the world are still battling for their survival. Smallpox, chickenpox, dengue, and other lethal diseases have been conquered thanks to advancements in medical research, but unexpectedly, hepatitis, influenza, and HIV viruses have posed new challenges. The absence of vaccinations, harmful drug reactions, the occurrence of antibiotic resistance variants of common organismsas a result of antibiotic abuse, and susceptibility issues are few of the therapy's problems and roadblocks. The human genome project's finding has created new avenues for research into how genetic makeup affects disease development and progression, as well as therapeutic options for numerous viral diseases. New antiviral therapeutic medicines have significantly improved survival rates overall, but this success has fallen short of expectations because some people have experienced negative side effects from these medications. The current burden of viral diseases may be reduced with the help of pharmacogenomics research and knowledge development. As a result, choosing the best treatment agents, dosages, and drug response for each person will undoubtedly be made easier. Hence, it will become possible to translate the lab studies to clinical bench side, which will aid in gaining proper knowledge of etiology of various diseases of virus with

ScienceDirect

Pharmacogenomics
From Discovery to Clinical Implementation

2023, Pages 137-168

Chapter 6 - Pharmacogenomics in cardiovascular diseases

Maroof Ahmed [a], Bashir Ahmad Malla [b], Umer Majeed Khaja [c], Aashiq Hussain Bhat [a], Aarif Ali [a], Showkat Ahmad Ganie [a], Muneeb U. Rehman [d], Zuha Imtiyaz [e]

[a] Department of Clinical Biochemistry, School of Biological Sciences, University of Kashmir, Srinagar, Jammu and Kashmir, India
[b] Department of Biochemistry, School of Biological Sciences, University of Kashmir, Srinagar, Jammu and Kashmir, India
[c] Department of Zoology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India
[d] Department of Clinical Pharmacy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia
[e] Department of Pathology, University Maryland School of Medicine, University of Maryland, Baltimore, MD, United States

Available online 23 June 2023, Version of Record 23 June 2023.

https://doi.org/10.1016/B978-0-443-15336-5.00009-9
Get rights and content

Abstract

Cardiovascular disease (CVD) is multifactorial, complex, and the foremost cause of death globally. CVD is responsible for about one in every four fatalities globally. CVD in general refers to a collection of overlying anatomical and persistent defects in the blood arteries and heart that comprise the cardiovascular system. As a result, the appropriate medications can cure or prevent these overlapping disorders. Since one medicine alone cannot address a medical issue, many medications are frequently given. Because of the high mortality and morbidity associated with CVD, physicians are compelled to treat it aggressively, which might result in severe medication responses. Even when the highest suggested medicine dosage is used, some individuals may not respond well. CVD research has found a relationship between variation in genes and the severity of disorders such as blood cholesterol levels and blood pressure. Similarly, research conducted over the last four decades has proven a relationship between genetic diversity and the efficiency of CVD therapy. The field of pharmacogenomics aims to reveal how individual genome variability affects drug toxicity and

प्रमाणपत्र सं. / Certificate No.:
LD-20250165824

प्रतिलिप्याधिकार कार्यालय, भारत सरकार | Copyright Office, Government Of India

प्रतिलिप्यधिकार प्रमाणपत्र | Copyright Certificate

प्रतिलिप्यधिकार नियम का नियम 70 | Rule 70 of The Copyright Rules

प्रतिलिप्यधिकार रजिस्टर से उद्धरण | Extracts from the Register of Copyrights

आवेदन सं. / Application No.: 41106/2024-CO/L **दाखिल करने की तिथि/ Date of Filing:** 25/12/2024

एतद्द्वारा यह प्रमाणित किया जाता है कि प्रतिलिप्यधिकार अधिनियम, 1957 के प्रावधानों के अनुसार, उपर्युक्त आवेदन में प्रकट किए गए "Studies On The Potential Of Rheum Webbianum Extracts On The Regression Of 1 2-dimethylhydrazine-induced Tumors In Swiss Albino Rats" नामक कार्य के लिए प्रतिलिप्यधिकार प्रदान किया गया है।

This is to certify that a copyright has been registered for the work titled "Studies On The Potential Of Rheum Webbianum Extracts On The Regression Of 1 2-dimethylhydrazine-induced Tumors In Swiss Albino Rats" as disclosed in the below mentioned application in accordance with the provisions of the Copyright Act,1957.

रचयिता / Authors:

सं. / SNO.	नाम / Name	पता / Address
1	Umer Majeed Khaja	Lovely Professional University Jalandhar Delhi-Gt Road Phagwara Punjab-144411
2	Dr. Reena Singh	Lovely Professional University Jalandhar Delhi-Gt Road Phagwara Punjab-144411
3	Chirag Chopra	Lovely Professional University Jalandhar Delhi-Gt Road Phagwara Punjab-144411
4	Dr. Showkat Ahmad Ganie	University Of Kashmir Srinagar Jammu And Kashmir-190006

स्वामी / Owners:

सं. / SNO.	नाम / Name	पता / Address
1	Lovely Professional University	Lovely Professional University Jalandhar Delhi-Gt Road Phagwara Punjab-144411

प्रकाशक विवरण / Publisher Details: N.A.

कार्य की भाषा / Language of the Work: English

कार्य का विवरण / Description of the Work: N.A.

(शर्तें / टिप्पणियाँ, यदि कोई हो) / (Conditions/ Remarks, if any): N.A.

आर. ओ. सी. जारी होने की तिथि / Date of ROC: 29/05/2025

Registrar of Copyrights