Toxicological Impacts of Deltamethrin Pesticide on Immune-compromised Mice Model and Related Immunomodulation Studies

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ANUPAM KUMAR

(Registration Number: 41300111)

Supervised By

Dr. Anand Mohan

Co-Supervised by

Dr. Neeta Raj Sharma

Dr. Hasibur Rehman

LOVELY FACULTY OF TECHNOLOGY AND SCIENCES

LOVELY PROFESSIONAL UNIVERSITY

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DECLARATION

I declare that the thesis entitled "Toxicological Impacts of Deltamethrin Pesticide on Immune-compromised Mice Model and Related Immunomodulation Studies" has been prepared by me under the guidance of Dr. Anand Mohan, Associate Professor, Department of Biotechnology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

Anupam Kumar (Reg No.- 41300111)

Department of Biotechnology

School of Bioengineering and Biosciences

Lovely Professional University

Jalandhar-Delhi GT Road (NH-1), Phagwara

Punjab, India - 144411

Place: Phagwara

Date:

CERTIFICATE

This is to certify that **Mr. Anupam Kumar** has completed the Ph.D. thesis titled **"Toxicological Impacts of Deltamethrin Pesticide on Immune-compromised Mice Model and Related Immunomodulation Studies"** under my guidance and supervision. To the best of my knowledge, the present work is the result of his original investigation study. No part of this thesis has ever been submitted for any other degree or diploma.

The thesis is eloquently fit for the submission for the partial fulfilment of the condition for the award of the degree of Ph.D in Biotechnology.

(Supervisor)

Dr. Anand Mohan

Associate Professor

School of Bioengg & Biosciences

Lovely Professional University

Phagwara, Punjab, India

(Co-Supervisor)

Dr. Neeta Raj Sharma

Professor and Head of School

School of Bioengg & Biosciences

Lovely Professional University

Phagwara, Punjab, India

Dr. Hasibur Rehman

Scientist, School of Medicine

University of Alabama

Birmingham, USA

ABSTRACT

The synthetic pesticides have been employed to increase the agrarian produce to meet the food demands of up surging population. Various types of pesticides are also subjugated for numerous pest control programmes like; pest and insects associated to food crop, several vectors borne diseases in humans and other animals. In India GDP share from agriculture sector is 23 % and provides 59% of employment of total workforce of the country and to meet the GDP demand from agriculture sector India is producing and importing huge amount of pesticides. Extensive applications of pesticides in agriculture sector, hospitals, homes, and at work places resulting to various ailments in humans and other animals. The chemically synthesized pesticides are broadly classifieds as organophosphorus insecticide, organochlorines insecticides, carbamates, pyrethrin and pyrethroids. These insecticides show different level of toxicity to specific group of organisms due to different functional group. Pyrethroids were synthesized to replace organophosphorus, organochlorine and carbamates because these were showing more adverse effects on environment and targeting wide group of organisms, but due to it humans became more exposed towards pyrethroids and leading to develop immune suppression state. The entry of these pesticides in their body systems through ingestion, inhalation, direct contact or skin absorption causes the many immune challenging state by weakening the immune system.

To minimize and neutralize the toxic effects of deltamethrin in *in-vitro* and *in-vivo* conditions various research studies have been conducted by using established synthetic antioxidants, plant derived antioxidants or plant extracts. In a research study, synergistic and defensive effects of ceftriaxone and ascorbic acid an antioxidant in distinction to subacute dose of deltamethrin-induced nephrotoxicity in rats was established. Lycopene a tomato derived antioxidant when co-administered with deltamethrin in rats it displayed significant recovery and restoration of damaged kidney tissues and decreased level of tumor necrosis factor-α (TNF-α) in serum. L-glutamine, an organic compound had shown hepatoprotective effects in deltamethrin treated rats and conjointly shown decrease in TNF-α and Interleukin-6 (IL-6) levels when compared to exclusively deltamethrin induced rats. In current research study, keeping all above

in mind, the immunomodulatory effects of deltamethrin, antimicrobial, antioxidant and protective effects of seed extracts derived from *Syzygium cumini* and *Nigella sativa* was evaluated in in-vitro and in-vivo condition, beside these, the known bioactive compounds from seeds of *Syzygium cumini* and *Nigella sativa* have been evaluated for immunomodulatory effects in in-silico condition.

The results of cytotoxicity effects on three different cell lines were evaluated, maximum cytotoxicity effects were observed in methanolic extracts of *N. sativa* for all three cell lines SW480, A549 and HeLa while ethanolic extracts shown less potential cytotoxicity effects cell lines. Moreover, *S. cumini* ethanolic as well as methanolic extracts have shown feebler antiproliferative and cytotoxic effects against all three cell lines. While deltamethrin alone with dose 100 µg/ml and 200 µg/ml shown the antiproliferative effects and cytotoxic effects against HeLa, SW480 and A549 cell line, but it has shown low cytotoxic effects against HeLa cell lines. In combinatorial effects of NSM and DM has shown effects against all three cell lines used in study, while NSE+DM has low cytotoxic effects against all three cell lines. SCM and DM combination shown the decent effects against two cell lines while against HeLa cell line cytotoxic effects were noted deprived. SCE and DM in combination shown effects against all three cell lines. DM, SCE and NSE combination shown against all three cell lines but least was recorded for HeLa cell lines.

The results of in in-vivo study were also recorded promising, deltamethrin exposure causes the reduction in body weight and organ weight while the protective effects of seed extracts of *N. sativa* and *S. cumini* was observed. Hematological and biochemical parameters of different treated groups also shown the significant difference in comparison to control group. The RBC value was increased by 12.52% in DM group than control group, the increased value of RBC depicts the oxidative stress condition in mice of DM group. The WBC value also increased by 38.97% in DM group than control group. The increase in lymphocytes was also observed by 16.94% in DM group than control group. Neutrophil count was reduced by 10.74% in DM group and hemoglobin was reduced by 12.27%. The TLC value was increased by 18.21 %, while Hematocrit, MCV, MCH and PCV values were reduced by 22.06%, 10.19%, 14.74% and 9.5% respectively. The changes in the parameters depict the severe metabolic and biochemical changes due to exposure of deltamethrin pesticides. The various biochemical parameters of blood reflect the changes due to deltamethrin pesticides and its effects on oxidative stress in organs actively

involved in metabolism. All biochemical parameters of lipid profile of blood serum shown significance difference in DM group than control group. There was an increase in DM group was observed in lipid profile, 76.68% in cholesterol, 91.78% increase in triglyceride, HDL was increased by 38.11%, LDL was increased by 99.31% and VLDL was increased by 114.29%. The overexpression of oxidative marker enzymes were observed in DM treated group than control group, the ALKP expression was increased by 97.88%, AST was increased by 645.67% and ALT by 161.72%. The biochemical parameters of different tissues also shown significant differences in treatment groups than control group.

Results of immunological parameters, there was significant decrease in parameters in DM group relative to control group, PFC by 77.04%, DTH by 62.34%, Hemagglutinin titer by 88.54%, oxidative burst assay value by 47.83%, macrophage percentage by 63.19%, and Interleukin-6 value was recorded to decrease by 60.28%. There was elevation in TNF-α expression in DM group by 673.36% than control group. Histopathological evaluation also revealed that damage of hepatic tissues, nephritic tissues, cardiac tissues, splenic tissues and lungs tissues due to defined dose of deltamethrin while seed extracts shown the protective effects in deltamethrin challenge group alone as well as synergistically. In silico studies also shown the immunomodulatory effects of deltamethrin and bioactive compounds from seeds of *N. sativa* and *S. cumini.* The seed extract of *N. sativa* and *S. cumini* has intensely shown the antioxidant properties, antibacterial effects, antifungal effects, and protective effects in in-vitro as well as significant difference observed *in-vivo* condition in deltamethrin induced toxicity.

Key Words: Deltamethrin, Immunomodulation, Oxidative stress, *Nigella sativa*, *Syzygium cumini*, Hepatotoxicity, Nephrotoxicity, Immunotoxicity

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ABBREVIATIONS

AAT	:	Alanine aminotransferase
ACh	:	Acetylcholine
AChE	:	Acetylcholinesterase
ACP	:	Acid phosphatases
AKLP	:	Alkaline phosphatase
ANOVA	:	Analysis of Variance
ApoE	:	Apolipoprotein E
AST	:	Aspartate amino transferase
ATP	:	Adenosine triphosphate
BSA	:	Bovine serum albumin
Bw	:	Body weight
Ca^{2+}	:	Calcium ion
$CaCl_2$	:	Calcium chloride
CAT	:	Catalase
CEC	:	Commission of European Communities
cGMP	:	Cyclic guanosine monophosphate
CH_3OH	:	Methyl Alcohol
Cl^-	:	Chloride ion
CNS	:	Central nervous system
COX	:	Cyclooxygenase
CRP	:	C-reactive proteins
Cu	:	Copper
CVD	:	Cardiovascular diseases
DAT	:	Dopamine transporter
DM	:	Deltamethrin
DPPH	:	2, 2-diphenyl-1-picrylhydrazyl
EDTA	:	Ethylene diamine tetra acetic acid
eNOS	:	endothelial nitric oxide synthase
$FeCl_3$	:	Ferric chloride

FSH	:	Follicle stimulating hormone
FTIR	:	Fourier Transformer Infrared
GDP	:	Gross domestic product
GGT	:	Gamma glutamyl transferase
GS	:	Guggulsterone
GSH	:	Glutathione
GSH	:	Reduced glutathione
GSSG	:	Oxidized glutathione
GST	:	Glutathione S-transferase
H^+	:	Hydrogen ion
H_2O	:	Water
H_2O_2	:	Hydrogen peroxide
H_2SO_4	:	Sulphuric Acid
H_3PO_4	:	Phosphoric acid
HCl	:	Hydrogen chloride
HDL-C	:	High density lipoprotein-Cholesterol
HMG Co.A	:	3-hydroxyl 3-methyl glutaryl co.enzyme A
HPC	:	Hypercholesterolemia
HPLC	:	High performance liquid chromatography
HT	:	Haemagglutinin titre
IL	:	Interleukin
IPCS	:	International Programmes on Chemical Safety
K^+	:	Potassium ion
KCl	:	Potassium chloride
LC_{50}	:	Lethal concentration at which 50% organisms killed
LD_{50}	:	Lethal dose at which 50% organisms dies
LDH	:	Lactate dehydrogenase
LDL-C	:	Low density lipoprotein-Cholesterol
LH	:	Luteinizing hormone
LPO	:	Lipid peroxidation
LPO	:	Lipid peroxidase
M	:	Molar

MDA	:	Malondialdehyde
mg	:	milligram
$MgCl_2$	:	Magnesium Chloride
MHC	:	Major histocompatibility complex
min	:	minute
ml	:	milliliter
mm	:	millimeter
mM	:	millimolar
MW	:	molecular weight
Na^+	:	Sodium ion
NaCl	:	Sodium Chloride
NADH	:	Nicotinamide adenine dinucleotide (NAD) + hydrogen (H)
NaOH	:	Sodium Hydroxide
NBT	:	Nitro blue tetrazolium
nM	:	Nanomolar
NMR	:	Nuclear magnetic resonance
NO	:	Nitric oxide
NO_2^-	:	Nitrite ion
NO_3	:	Nitrate
NOS	:	Nitric oxide synthase
$O_2^{\cdot -}$	:	Superoxide radical
OECD	:	Organization for economic co-operation and development
OH^-	:	Hydroxyl radical
Ops	:	Organophosphorus pesticides
PBS	:	Phosphate buffer saline
PBS	:	Phosphate buffered saline
PFC	:	Plaque forming cells
PG	:	Prostaglandins
ROS	:	Reactive Oxygen Species
RT	:	Room temperature
S.E.M.	:	Standard Error of the Mean
SDS	:	Sodium dodecyl sulphate

SGOT	:	Serum Glutamate oxaloacetate transaminase
SGPT	:	Serum Glutamate pyruvate transaminase
SOD	:	Super oxide dismutase
TC	:	Total Cholesterol
TDW	:	Triple Distilled Water
TG	:	Total glycerides
TNF	:	Tumour necrosis factor
TP	:	Total proteins
TPSA	:	Topological polar surface area
U	:	Units
VLDL-C	:	Very low density lipoprotein-Cholesterol
WHO	:	World health organization
wt	:	weight
XOD	:	Xanthine oxidase
ZN	:	Zinc
μ	:	Micro
μg	:	Microgram
μM	:	Micromolar

CHAPTERS

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INTRODUCTION

1.1: INTRODUCTION

During the last few decades, extensive amount of pesticides has been employed to boost the agricultural yield to meet the food demands of up surging population. Pesticides are also exploited for various pest control programmes like, pest and insects related to food crop, various vector borne diseases in humans and other animals (Naik and Prasad 2006). In most of the developing countries, agriculture is the main source of gross domestic product (GDP) and to increase the agriculture production, like crop production, horticulture, milk and milk products, animal farming, pisciculture, production of silk and rearing of silk worm, breeding and rearing of birds, growing and conserving forests and related activities. In India GDP share from agriculture sector is 23 % and provides 59% of employment of total workforce of the country (Benchmark Report 2017 – India). To meet the GDP demand from agriculture sector India is producing and importing huge amount of pesticides. There is huge amount of pesticide production (in thousand metric tons) during last five fiscal year in India, in 2013-155, in 2014- 179, 2015-186, 2016- 188, 2017-214, 2018-213 and 2019-217 (CAGR report 2012-2013 and Statista Research Department 2013-2019). Extensive applications of pesticides in agriculture sector, hospitals, homes, and at work places, humans are exposed to pesticides and allow entry of these pesticides in their body systems through diet, inhalation or skin absorption.

There are different classes of pesticides on the basis of targets such as insecticides (insects), fungicides (fungi), herbicides (unwanted herbs), rodenticides (rodents), nematicides (nematodes), chemosterilants, molluscicides (molluscs), plant growth regulators (weeds), Defoliants and Desiccants (Aktar *et al*. 2009). Out of these classes of pesticides, one of the class insecticides are persistent to environment and cause adverse effects to various living organisms including human beings. Based on various chemical composition insecticides are further classified in organophosphorus insecticide, organochlorines insecticides, carbamates, pyrethrin and pyrethroids. These insecticides show different level of toxicity to specific group of organisms due to different functional group (Corsini *et al* 2013). Pyrethroids are synthetic compounds, more thermostable and sustain the sunlight exposure for longer time and commonly implemented to

control insects and pests of crop (Sudakin 2006). Pyrethroids were synthesized to replace organophosphorus, organochlorine and carbamates because these were showing more adverse effects on environment and harming wide group of organisms, but due to it humans became more exposed towards pyrethroids and leading to develop immune suppression state (Khan *et al* 2008).

Pyrethroids are artificial organic compounds synthesized from chrysanthemum flowers that are used extensively as house, social unit and industrial pesticides. The keto-alocoholic esters of chrysanthemic and pyrethroic acid being lipotropic are liable for its insecticidal properties. Pyrethroids are mainly classified into first and second generation pyrethroids. The primary generation (Type 1) pyrethroids are less harmful and toxicant to mammals than the second generation (Type II) pyrethroids. Primarily class II pyrethroids routinely cause burning or prickling sensation and cause symptoms, that is characterised by transient burning/tingling/numbness/itching sensation of the exposed skin of hands, arms, or legs. The class II pyrethroids has been suggested that a number of them are root of biological process neurotoxicity, however existing resources aren't to be equal to acclaim biological process neurotoxicity (Rehman *et al*., 2014). Pyrethroids are progressively being employed for public health and farming purposes and are claimed to create comparatively low human toxicity. Pyrethroids are unlikely to be of acute toxicity for occupationally exposed subjects using smart and sensible work practices and safety precautions (He *et al*., 1989).

Deltamethrin is an alpha-cyano type II, fourth generation artificial, synthetic ester compound, which is most commonly used in household and agricultural insects in various crops and pest management programmes related to vector borne diseases (Leahey, 1985). Implementation of deltamethrin to prevent tick-infested prairie dogs, spiders, ticks, carpenter ants, fleas, bees, cockroaches, bedbugs, aphids, white flies, caterpillars on pears, apples and hops etc. (ETN, 1995). Deltamethrin is used in various formulations for pest control programme, like in ant chalk, mosquito repellent, crop pest management etc. which kills insects when insects encounter it and eaten orally. Deltamethrin affects the nervous system of vertebrate as well as invertebrate followed by paralysing and making a quick knock-down effect. Due to over exploitation of deltamethrin it became the most common xenobiotic compound present in environment and in food resulting into impending effects which is harmful to the immune system and make human beings and other animals susceptible to various dysfunctions and diseases.

In 1978, when industrial production of deltamethrin started at that time it was considered as innocuous but later it was reported as enormously toxic to fishes, other aquatic animals, various insects and human beings (Eells *et al*., 1993; Balint *et al*., 1995; Delistraty, 2000; Viran *et al*., 2003; Datta and Kaviraj, 2003; Svobodova *et al*., 2003).. Some of the insecticidal toxic effects of deltamethrin include neurotoxicity, cardiotoxicity, reproductive toxicity, genotoxicity, etc (WHO, 1989). As per earlier research findings it has been shown that deltamethrin exhibits the immunosuppressive effects on immune-compromised hosts. Deltamethrin shows neurotoxic effects on human beings and especially passes through skin in woman and reach to breast milk and causing damage in neuro-system of infants (Bouwman *et al.*, 2006). Due to its high hydrophobicity, deltamethrin may easily enters inside cells or tissues and accumulate in body systems and increases reactive oxygen species production and leading to oxidative stress induced toxicity (Michelangeli *et al.*,1990).

Deltamethrin causes diverse signs of toxicity in humans, symptoms are related to respiratory tract disorders, gastro-intestinal, and kidney breakdowns. Some common symptoms are dermatitis, headache, irritability, edema, diarrhoea, fever, vomiting, ataxia, and paralysis. When, some mammals were exposed with deltamethrin orally or any other route of administration had shown typical type II motor signs, which comprise a writhing syndrome and abundant salivation in rodents due to endocrine disruption (Garey and Wolff, 1998). In South Africa during malaria control programme some cases have been reported that DDT and deltamethrin were found in breast milk (Bouwman *et al*., 2006). There are only three death reports of human beings due to deltamethrin poisoning due to ingestion or inhalation out of total ten deaths due to pyrethroids (He *et al.,* 1989; Robinson, 1996).

In some earlier studies on animal system it's been rendering that deltamethrin isn't showing any toxicant effects on non-aquatic vertebrates (Pham *et al*., 1984). However, various studies had been conducted and reported that deltamethrin displays cellular toxicity in in-vitro conditions (Romero *et al* 2012; Ismail *et al* 2013), while, in animal model studies it had been reported neurotoxicity (Song *et al.*, 1996; Shafer *et al*., 2004), hepatotoxicity (Catinot *et al*., 1989; Anadón *et al.*, 1991; Anadón *et al.,* 1996), nephrotoxicity (Shona *et al*., 2010), generative toxicity like, scale back gonad, prostate, and cyst weights and reduce spermatozoon counts and reduced testosterone levels (Abd *et al*., 1994), immunotoxicity by suppressing the immune system and

build a prone towards microbial infections (Yarsan *et al*., 2002; Rehman *et al*., 2011; Kumar *et al*., 2015) and additionally causes foundation of reactive oxygen species (ROS) generation that are responsible to impairment of varied tissues and organs (Manna *et al*., 2005).

The various studies have been conducted on different cell lines to evaluate the cytotoxic effects of deltamethrin in *in-vitro* conditions, like, change in cellular morphology, changes in nuclear genetic material and increase in ROS level. Various established antioxidants and plant extracts have been implemented to minimize the toxic properties of deltamethrin in different cell lines. The cytotoxic effects of deltamethrin is reported in SH-SY5Y cell lines and which can be reduced by using selected antioxidants like, melatonin, Trolox and N-acetylcysteine. These antioxidants had shown the protective role in deltamethrin treated SH-SY5Y cell lines by decreasing the levels of nitrogen oxides (NO) and MDA (Romero *et al.*, 2012). The cytotoxic effects of Deltamethrin, DECIS and DECIS FLO from 5×10^{-5} M concentration affected the growth of fibroblast cell lines and plant algae in cell culture *in-vitro* conditions (Armella *et al.*, 1987).

To minimize and neutralize the toxic effects of deltamethrin in *in-vitro* and *in-vivo* conditions various research studies have been conducted by using established synthetic antioxidants, plant derived antioxidants or plant extracts. In a research study, synergistic and defensive effects of ceftriaxone and ascorbic acid an antioxidant in distinction to subacute dose of deltamethrin-induced nephrotoxicity in rats was established (Adel-Daim and El-Ghoneimy, 2015). Spirulina supplement has shown overwhelmed deltamethrin persuaded hepatotoxicty, nephrotoxicity and neurotoxicity by eliminating oxidative tissue injuries and reducing reactive oxygen species (Adel-Daim *et al*, 2016). Lycopene a tomato derived antioxidant when co-administered with deltamethrin in rats it displayed significant recovery and restoration of damaged kidney tissues and decreased level of tumor necrosis factor-α (TNF-α) in serum (El-Gerbed, 2014). L-glutamine, an organic compound had shown hepatoprotective effects in deltamethrin treated rats and conjointly shown decrease in TNF-α and Interleukin-6 (IL-6) levels when compared to solely deltamethrin induced rats (Gündüz *et al.*, 2015). Allicin, a bioactive compound in clove of garlic has been reported in preventing aerophilic stress damages in a fish found in Nile River, *Nile tilapia* by reducing the amount of total protein and albumin in serum as well as reduction in superoxide dismutase enzyme (SOD), catalase (CAT), reduced glutathione (GSH), and antioxidant

glutathione peroxidase (GSH-Px) due to exposure of deltamethrin in fishes (Adel-Daim *et al.*, 2016). *Globularia alypum* leaves extracts have been reported to prevent nephrotoxicity in rats induced by chronic dose of deltamethrin by decreasing the level of plasma creatinine, urea, uric acid, MDA levels and restored kidney tissues by decreasing the necrosis of proximal tubule in kidney (Feriani *et al.,* 2017). The Aqueous seed extract of *Trigonella foenum graecum* (fenugreek) has been reported to prevent cypermethrin (A type II pyrethroids) induced hepatotoxicity and nephrotoxicity in male Wistar rats by decreasing the levels of thiobarbituric acid reactive substances (TBARS), GSH, SOD, CAT, GPx, and glutathione-S-transferase (GST) (Sushma and Devasena, 2010). The leaves extract of *Ocimum basilicum* also shown significant effects to reduce the oxidative stress and nephrotoxicity in deltamethrin induced rats by decreasing the MDA level and increasing the SOD and CAT level in renal tissues (Sakr and Al-Amoudi, 2012). Olive fruit extracts and phenolics oleuropein has also been reported to attenuate the hepatotoxicity and nephrotoxicity in deltamethrin induced rats by improving oxidative stress conditions, decreasing MDA and significant improvement in SOD and CAT levels in renal and hepatic tissues (Maalej *et al.,* 2017). Virgin olive oil had been reported also to ameliorates the nephrotoxicity and immunotoxicity in deltamethrin induced rats by reducing the oxidative stress marker enzymes level especially; MDA, GSH, CAT, caspase-3, cyclooxygenase-2 (cox-2) and poly-ADP-ribose polymerase (PARP) and also recoverd the renal tissues damages (Khalatbary *et al.*, 2016). Curcumin and quercetin have been reported to reduce the reproductive toxicity due to cypermethrin and deltamethrin by improving the activity of pituitary-gonadal hormones and steroidogenic enzymes (Sharma *et al.*,2018). The established antioxidant molecule piperine and curcumin have been reported to show the protective effects against deltamethrin persuaded thymic and splenic toxicity effects in mice by decreasing the oxidative stress level in both thymus and spleen, spleen has shown more adverse damages than thymus due to deltamethrin exposure (Kumar *et al.*,2018).

There are very few inputs had been reported on deltamethrin induced immunotoxicity, moreover, no input is available on immunotoxicity due to high dose of deltamethrin. Pesticides cause pollution in atmosphere and causes intensive health challenges for humans and different organisms even at low-dose exposure to those are bit by bit fixed to disorders of respiratory system, reproductive anomalies, hormonal distraction, generative anomalies, secretion distraction, immunosupression and rising cancer frequencies (Zhang *et al*., 2002; Gupta 2004). Deltamethrin

overwhelmed the immune system in Balb/c mice by modulating the cytokines like toll like receptors (Lukowicz and Krechniak, 1992). It's cytotoxic effect on various types of immune system cells are also not known. Studies on the evaluation of immunotoxicity potential may thus prove to be rewarding as deltamethrin is being used in various vector control programs in India. In the proposed work an attempt has been made to study as high dose deltamethrin induced cytotoxicity in *in-vitro* and immunotoxicity in mice. The immunomodulation effects of medicinal plants produce positive responses, by employing a probable healing live has occurred within the past sacred text scripture, the Ayurveda, and has been intimate with in Indian ancient medication and medication practices for many centuries (Agarwal and Singh, 1999). Medicinally vital plants are an amusing supply of ingredients, which are acclaimed to steer specific and non-specific immune functions and ends up in healthy lifetime of a private, particularly at cellular basis the modulation of effectiveness of granulocytes, macrophages, natural killer cells and complement roles are the main end-points of immunomodulation. Agarwal & Singh (1999) have reviewed Indian medicative plants; which have immunomodulatory properties and later explored for analysis of immunomodulation effects and several other plants which can be probable candidates to own antioxidant ingredients. Therefore, it would be ideal to select a plant with dual activities of immunomodulation and antioxidant response for evaluation of its efficacy to protect the host from toxic (neurotoxic/hepatotoxic/nephrotoxic/immunotoxic) effects of chemicals and pesticides.

Since ancient time, the plants are the companion of man and gave evidences of numerous valuable drugs for the treatment of range of ailments. Therefore, discovery of new effective therapeutic mediator/s is/are immediately required, specifically to overwhelmed deltamethrin induced malfunctions in the human beings and to remain disease free. There are wide variety of herbal plants listed in various medicinal journals and papers which are known to exert their immuno-modulatory effects in response to various oxidative stress and immune toxicological challenges. *Nigella sativa* is an annual herb plant belongs Ranunculaceae family, the seeds obtained from *N. sativa* used by people for cooking and culinary purposes, to cure digestive disorders, recuperate joint aches, obesity, cold, headache, asthma, rheumatic diseases etc in India, Pakistan, Bangladesh, China and Middle East countries (Naz, 2011). *N. sativa* containing bioactive compounds like; nigellone, dithymoquinone, thymoquinone, thymol and thymohydroquinone and have potent as antimicrobial agents (Forouzanfar *et al.,* 2014). These bioactive compounds are considered as responsible for antioxidant activities, antimicrobial activities and responsible for

antitumor as well as anti-cancerous activity (Salomi *et al.,* 1991; Arora and Kaur, 1999; Morsi, 2000; Thabrew *et al.,* 2005; Halawani, 2009; Khan *et.al*., 2016). Bioactive compounds or extracts of *Nigella sativa L.* have been reported for antiproliferative and anticancerous effects on various cell lines (Badary and Gamal, 2001; Farah and Begum, 2003; Salim and Fukushima 2003; Gali-Muhtasib *et al.* 2004; Al-Sheddi *et al.* 2014). *Nigella sativa* L. seeds are known for their immuno-modulatory activity against fungal pathogens and have antioxidant activity as well (Rogozhin 2010). Similarly, *Syzygium cumini*, dicot genus, ordinarily referred to as Jamun, a tropical tree, a perineal plant, belongs to family Myrtaceae that produces purple ovoid fleshy fruit and has sturdy antioxidant activity (Benherlal and Arumughan, 2007). There are so many medicinally important plants and their role to cure numerous diseases have been reported earlier and one of the most significant plants of interest for people is *Syzygium cumini* L. Since the history of Indian medicinal science, *S. cumini* had been reported as to cure many diseases due to its antibacterial, antifungal, antioxidant, anti-inflammatory, hypoglycaemic, hypolipidemic effects (Stanely *et al,* 1997; Sharma *et al.*, 2003; Ruan *et al.*, 2008; Kumar *et al.,* 2017). *Syzygium cumini* L. is found mainly in India, Pakistan, Bangladesh, Sri Lanka, China, Middle East, Malaysia, and Australia. All plant parts of *S. cumini* L. has significant medicinal properties; it has been employed by folks to treat numerous diseases like- anti-inflammatory (Modi *et al,* 2010; Roy *et al*, 2011), antidiabetic (Stanely *et al,* 1997; Sharma *et al,* 2003), anti-diarrheal, antifungal and antibacterial effects (Bhuiyan *et al,* 1996; Kumar *et al*, 2006, Kumar *et al* 2017). The seed extracts of *S. cumini L.* contains so many bioactive compounds like; quercetin, isoquercitin, myricetin, ellagic acid, gallic acid, ferulic acid, kaempferol etc. These bioactive compounds are mainly responsible for antioxidant, antimicrobial, antidiabetic, anti-inflammatory and anti-cancerous activities (Tanaka *et al*., 1996).

So, keeping all above effects in attention, the present research study has been designed to establish the hazardous effects of deltamethrin pyrethroids-II pesticides in cellular toxicity, nephrotoxicity, hepatotoxicity, cardiotoxicity, splenic toxicity, lung toxicity and immunotoxicity. The immunomodulation studies of deltamethrin and seed extracts of *N. sativa* and *S. cumini L.* is included first time in any research design. In this research design, phytochemical analysis, antioxidant potential, antibacterial activity and antifungal activity of seed extracts of *N. sativa* and *S. cumini L.* have been performed. In the present research study, Patch Dock method is used to establish the interaction of bioactive molecules derived from *N. sativa* and *S. cumini L.* and

deltamethrin with MHC I and MHC II of human and mice origin for potential immunomodulation analysis.

The outcome of this research design established the effects of bioactive molecules present in seed extracts *N. sativa* and *S. cumini L.* to encounter and neutralize the cytotoxicity, immunotoxicity, hepatotoxicity, nephrotoxicity, splenic toxicity and lung toxicity due to the exposure of deltamethrin pesticide. This research study outcomes created the awareness in society about the pesticide usages and practices. As we know that farmers, labourers who work in the field and people who work in pest management programme are at high risks due to exposure of pesticides and may develop immune suppress challenges and become more prone towards bacterial, fungal and other infections. So, addition of bioactive compounds in diet or as nutraceuticals derived from seed extracts of *N. sativa* and *S. cumini L.* can minimize the adverse effects of deltamethrin toxicity.

REVIEW OF LITERATURE

2.1: Timeline of pesticides uses

Since early days of civilization there is battle against pests and insects for survival and healthy life of human beings. There are many examples from history when pests have play influential role in human beings' survival and deaths, like during 14th century one third of European population suffered and died from a mysterious disease "Black Plague". In 19th century there was elimination of staple food potato from Ireland due to "Late blight" disease in potato plants and leading to starvation and deaths in this European country followed by migration in United states of America. Pagan priests were using brimstone 2000 years earlier than the birth of Jesus Christ for religious aspects later this was used as fumigants, bleaching agents, incense and to purify the sick room by pre-Roman and Roman civilized people. Similar uses of sulfur were delineated by Homer in Odyssey one thousand years BC (Fishel, 2013). Plant derived insecticides have been identified and applied against various pest control, like, body lice can be controlled by hellebore, aphids can be controlled by nicotine (tobacco plant derived metabolite) and pyrethrin derived from *Chrysanthemum cinerariifolium* is applied to control various groups of insects (Fishel, 2013). During sixteenth century, Chinese utilized reasonable quantities of arsenic based complexes as pesticides and hundred years later, the foremost usual pesticide – 'Nicotine', was casted-off counter to the pest plum curculio and lice bug (Mrak, 1969). Later, within the time 1828, a second plant derived pesticide, 'Pyrethrum' was made known to fight against different classes of insects. Through the halfway of the nineteenth centenary the primary systematic and scientific investigations started, and soap become potential member of pesticide list and capable to destroy aphids (Ennis and McClellan, 1964; Mrak, 1969). The various types of substances applied and intended for pest control programme, however the all constituents remained of synthetic chemical in origin. An adulterated copper arsenate termed as 'Paris Green' which was applied and investigated to encounter the "Colorado beetle" insects. A specific chemical compound was introduced in the year 1985 termed as "Bordeaux mix", which is mainly composed of copper

sulphate, lime and water, and being practiced by means of one of the utmost significant fungicides (Shepard, 1951; Perkins 1975).

The practice of pesticides and insecticides enhanced during year 1920-1940, and the diversity besides superiority of the constituents exploited elevated by the side of the similar period and spell. During the initial phase of world war II (1940), the common pest control agents were usually restricted to synthetic chemicals like arsenic-based compounds, sulfur-based compounds, sulfur gas, crude oils, Greenland spar, and insect powder DDT while plant derived nicotine, pyrethrum and rotenone were also accessible (Perkins 1975). Arylidene alicyclic ketone insecticide was developed by Shell development company to control the insects and pests during late 1940s (Dorman and Ballard, 1949).

Human has been challenging with insects, rodents, diseases, and weeds for survival throughout its civilization. Various earlier reports of plagues, famine, and pestilence reflects the hazardous effects of these organisms in human health. Since early days, human beings encounter with such organisms and tried to control their growth and restrict them to cause harm. Through the course of evolution and civilization process human developed various techniques and technologies to create tools to combat these harmful pests. To control these pest humans developed a tool i.e. Pesticide and applied it reduce the challenges (Ware and Whitacre, 2004).

A pest is any organisms that:

- troubles human beings, wildlife, crops, buildings, or possessions.
- contends with human beings, livestock and crops for food, feed, or water.
- spreads unwellness to humans, livestock, or crops.

2.2: Chronological Order of Some Significant Incidents Involving Pesticides

(Fishel, 2013; Ware and Whitacre, 2004).

• 12000 BC: initial report of pests associated with human civilization.

• 2000 BC: Practice of sulfur applications as a chemical pesticide through pre-Roman societies.

• 1200 BC: Initial reviews on application of herbicide but nonspecific in target.

• 100 BC: First application of hellebore for management of rodents and pests by Romans.

• 300 AC: initial report of biotic pest management –predatory ants used in citrus crop for limiting and reducing of crop harmful pests by Chinese people.

• 900 AC: Chinese applied arsenic to reduce and limit the number of garden pests.

• 1649 AC: To catch fishes rotenone pesticides applied to paralyze in South America.

• 1690 AC: Tobacco plant leaves derived alkaloid nicotine was used as insecticide.

• 1787 AC: The application of soap to control and reduce the number of pests.

• 1848 AC: In Asian countries rotenone was applied as an insecticide.

• 1850s: To control disease in grape plants the mixture of Lime and copper was introduced in France.

• 1860s: To control the growth of Colorado potato bug Paris green, a synthetic pesticide.

• 1873: DDT insecticide created in the research laboratory.

• 1882: To control fungal infections in plants Bordeaux mixture as antimycotic agent in France.

• 1883: The pressure sprayer was invented for chemical application resulting in economical applications to crops by John Bean.

• 1886: To control citrus plant diseases by hydrogen cyanide.

• 1892: First use to control and reduce the number of gypsy mouth applying lead arsenate in Massachusetts.

• 1894–1900: Various mechanical inventions for pesticide chemical spray tool using vapour pressure and using horse.

• 1907–1911: Manufacture of insect powder lead arsenate at industrial scale started and applied for insect's management.

• 1910: The Federal pesticide Act was established (pioneer to Federal pesticide act).

• 1920s- Structure of pyrethrin I and II elucidated by H. Staudinger and L. Ružička.

• 1921: 1st practice of air craft to use of chemical pesticides in fields.

• 1927: U.S. department of Food and Drug Administration applied arsenic in apple orchard.

• 1932: The first synthetic chemical methyl bromide used for fumigation practices in France country.

• 1932–1939: DDT potential as insecticidal properties investigated and characterized in European country Switzerland.

• 1936: The chemical pentachlorophenol has been proclaimed as a protective effect for wood.

• 1942: throughout world war II, DDT become accessible for U.S. armed practices (citizen usage available and become popular by 1945).

• 1942: Two synthetic chemical phenoxy carboxylic acid and synthetic auxin 2,4-D were applied as herbicide.

• 1944: For rodent control management warfarin chemical was applied.

• 1946: The industrial production of organo-phosphates pesticides started in the Federal Republic of Germany, then accessible to USA.

• 1949- The first synthetic pyrethroid pesticide was developed by Milton S. Schechter *et al.*

• 1950–1960: Industrial revolution and enormous manufacturing investigation for industrial production of multiple categories of synthetic pesticides.

- 1959- The term integrated pest management (IPM) was introduced by Smith, Stern, Bosch and Hagen.
- 1960s- The development of first generation pyrethroids pesticides were done by the Rothamsted institute research team.

• 1961: The bacteria *Bacillus thuringiensis* has potential to control pest growth and listed and registered as biocontrol management.

• 1962: A book named "Silent Spring" written and published by a biologist Dr. Rachel Carson.

• 1965: The synthetic chemical atrazine registered as herbicide but as nonselective weed-killer.

• 1970: For the registration of any synthetic pesticide accountability was fixed with U.S. Environmental Protection Agency.

• 1971: The synthetic chemical glyphosate represented the potential as herbicidal effects.

• 1972: Due to harmful effects like, biomagnification and bioaccumulation caused by DDT pesticides, application of DDT was called off by the independent agency EPA.

• 1973: The foremost photostable and thermostable synthetic pyrethroid pesticide, permethrin was developed.

• 1974: Cypermethrin a pyrethroid pesticide was synthesiszed.

• 1978: EPA released primary list of restricted applications of pesticides.

• 1980s: EPA prohibited several applications of chlorine containing synthetic pesticides.

• 1985: Bifenthrin pesticide was registered with EPA.

• 1987: Cyfluthrin was registered with EPA.

•1996: The commercialization and registration of transgenic crop started; Monsanto acquaint with Roundup Ready® soybeans.

• 1996: The Food Quality Protection Act (FQPA) was passed by US congress on 3rd August.

• 1990s and 2000s: Unions and acquisitions within the chemical industries, Introduction of biopesticide.

2.3: Types of pesticides (Aktar et al 2009)

The pesticides can be grouped in different classes based on target organisms they are mentioned in table 2.1.

Table 2.1: Types of Pesticides and Target Organisms

Types of Pesticides	Target organism
Insecticides	Insects
Herbicides	Plants (basically weeds)
Rodenticides	Rats and Mice
Bactericides	Bacteria
Fungicides	Fungi
Miticides	Mites and Tics
Larvicides	Larvae
Nematicides	Nematodes
Molluscicides	Snails and Slugs

Insecticides: Chemicals which are used to kill insects are called as insecticides and classified in two categories, Broad spectrum insecticides (which kills broad types of insecticides) and Narrow spectrum insecticides (kills specific group of insects). Common insecticides used are, Pyrethroids, Organophosphorus, Carbamates, Organochlorine, Manganese compounds etc.

Herbicides: Chemicals which are targeted to kill unwanted plants. The herbicide chemicals have different in action than the other pesticides. Based on action herbicide is classified in two categories, Selective herbicide and non-selective herbicide. Selective herbicide kills or reduce the growth of selected plants while non- selective herbicide kills or reduces the growth of all types of plants. Commonly herbicides used are, Bipyridyls, Chlorophenoxy, Glyphosate, Acetanilides, and Triazines.

Rodenticides: Chemicals which are targeted to kill the rats, mice and other rodents. Besides rodents these chemicals also kill or control the growth of birds, fishes and other mammals.

Rodenticides are mostly stomach poison, sometimes by contact also it kills to target organisms. Common rodenticides are, Warfarines and Indanodiones.

Bactericides: Chemicals responsible to kill or suppress bacterial population, generally these chemicals are surface sterilizing agents. Mainly Bactericides are disinfectants, antiseptics, or antibiotics. Commonly used disinfectants are, hypochlorites, chloramines, silver nitrate, mercury chloride, copper sulfate. Commonly used antiseptics are, hypochlorites, chloramines, silver nitrate, mercury chloride, copper sulfate iodopovidone, benzoic acid, salicylic acid, triclosan and chlorhexidine. Commonly used antibiotics are, beta-lactam antibiotics, cephalosporins and aminoglycosidic antibiotics.

Fungicides: These are chemicals which potentially target fungi and kill them by direct contact of these chemicals. So, to kill each fungus application of fungicides is done in specific manner either fungi are plant origin or animal origin. Fungicides are widely applied in different types of crop to protect from fungal attack which causes huge loss in crop production. Commonly used fungicides are, Mancozeb, Tricyclazole, Carbendazim, Hexaconazole, Metalaxyl, Difenoconazole etc.

Miticides: These group of pesticides chemical target to kill mites, ticks and bugs especially by direct contact of chemicals. Action of killing mites and ticks is like insecticides. Examples of miticides are organophosphates, carbamates, chlorinated hydrocarbons, nitrophenol derivatives, diphenyl aliphatic derivatives, sulphonates, sulphites and sulphides.

Larvicides: Group of chemicals used to kill or decrease the growth of different forms of larvae of different types of diseases causing organisms like mosquitoes. Organophosphates, Temephos, Methoprene,

Nematicides: Group of chemicals which kills or control the growth of nematodes, a tiny worm resides mostly inside soil and cause damage to crops. These chemicals when come in direct contact with nematodes then only these nematodes are killed. Soil fumigants are also applied to control nematodes in soil. Aluminium and zinc phosphide, Methyl bromide, Ethylene dibromide, DD (dichloropropene plus dichloropropane), and DCP (dichloropropene) are generally used to kill nematodes.

Molluscicides: The group of chemicals used to kill or control snails and slugs. Usually the action chemicals only after ingestion. Baits are often used to attract and kill snails or slugs in specified area. Carbamates, organophosphorus, organochlorine derived pesticides are commonly used molluscicides.

Pesticides have plentiful beneficial properties, like, it helps in crop defense, conservation of food materials and protection from vector-borne diseases. For example, use of pesticides in the stoppage of malaria by targeting limitation and reduction in growth of mosquitoes, for stopping other vector-borne illnesses like, dengue, leishmaniasis and Japanese encephalitis which causes millions of deaths worldwide. Pesticides are BIOCIDES, designed to destroy, diminish or fend off insects, unwanted plant, rodents, fungi or other undesired organisms that can impend community and public health and the economy. The mode of action of these pesticides mostly by affecting on leading enzymes involved in metabolic activities. Pesticides are pervasive in the environment and most of them are synthetic chemicals (National resource council, 1993).

2.4: Global Uses of Pesticides

A database on global consumption of Pesticides total, insecticides, herbicides, pyrethroids, pyrethroids-SeedTr Ins., fungicides and bactericides since year 2011 to 2016 has been obtained from FAOSTAT website, which indicates the total use of these pesticides at 120381757.3 tons worldwide (FAO 2011 and 2012).

2.4.1: Pesticides total consumption: Pesticides consumption by 197 countries, in 2011, Total pesticides consumption recorded as 18311834.71 tons. In 2012, 18666556.84 tons, in 2013, 18583197.24 tons, in 2014, pesticides consumption recorded as 18992535.19 tons, in 2015, 18856484.87 tons and in 2016, 18853571.37 tons. The total amount of pesticides consumption in different forms since 2011-2016 recorded as 112261317.8 tons and consumption of pesticides in India during 2011-2016 has been recorded as 320950 tons which is 3.49 % of total worldwide use. Developing countries uses less amount of pesticides than the developed countries. In fig 2.1-year wise consumption of pesticides in tons is shown during 2011-2016.

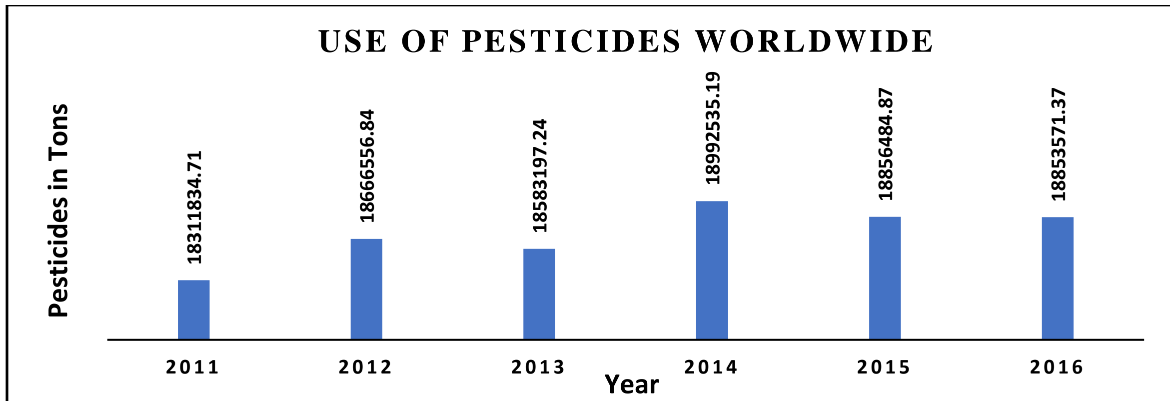


Fig 2.1: Use of pesticides in tons from 2011 to 2016 (Pesticide production is adapted from Food and agriculture organization of the united nations)

Insecticides consumption: The data available on FAOSTAT shows insecticides consumption, in year 2011, 96 countries consumed 232410.86 tons of insecticides, in 2012, 97 countries 295692.32 tons, in 2013 this value reduced to 233717.82 tons consumed by 94 countries, similarly in 2014 consumption of insecticides was reduced to 219374.06 tons by 86 countries, in year 2015, 69 countries consumed 191398.09 tons and in 2016, 59 countries consumed 171123.35 tons. In fig 2.2-year wise consumption of insecticides is represented.

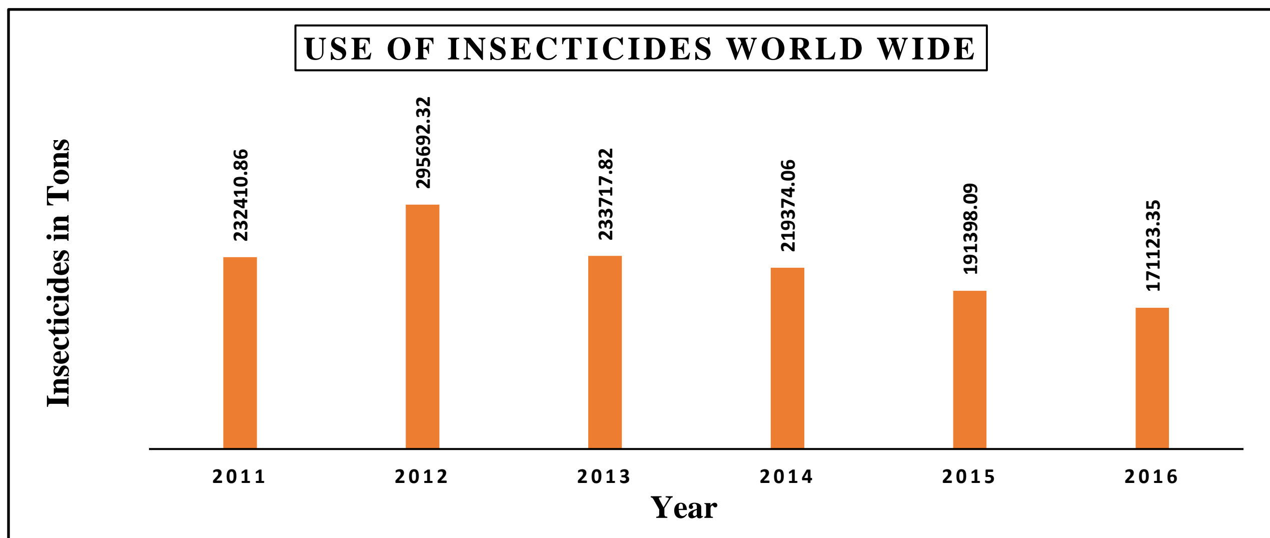


Fig 2.2: Use of insecticides in tons from 2011 to 2016 (Pesticide production is adapted from Food and agriculture organization of the united nations)

Herbicide consumption: Total herbicide consumption during 2011-2016 recorded as 4092099.06 tons by 94 countries. In year 2011, 771165.67 tons herbicide was consumed. In 2012, herbicide consumption was 1020829.7 tons. In 2013, 701883.76 tons of herbicides was consumed, in 2014, 87 countries consumed 863986.84 tons herbicides, in 2015, 68 countries consumed 558495.34 tons and in 2016, 61 countries consumed 480769.82 tons of herbicides. In fig 2.3- the consumption of herbicides is mentioned during year 2011-2016.

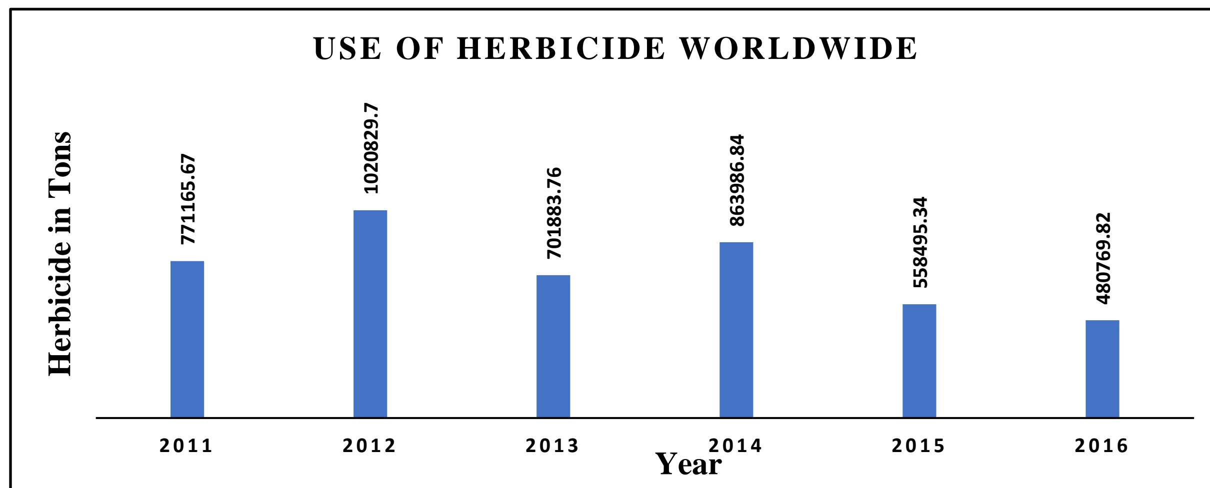


Fig 2.3: Use of herbicides in tons from 2011 to 2016 (Pesticide production is adapted from Food and agriculture organization of the united nations)

Fungicides and bactericides (F and B) consumption: During 2011-2016, consumption of total amount of Fungicides and bactericides worldwide recorded as 2302564.36 tons. In 2011, 401211.88 tons; in 2012, 385797.93 tons; in 2013, 367446.11 tons; in 2014, 399556.33 tons; in 2015, 386441.84 tons and in 2016, 362110.27 tons. In fig 2.4- use of Fungicides and bactericides is shown.

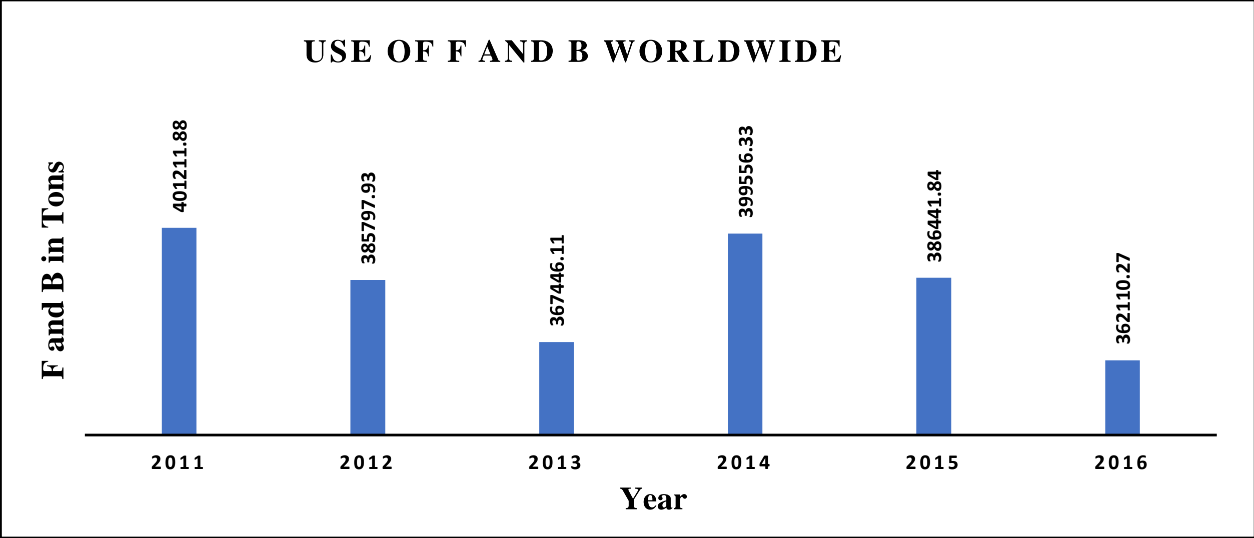


Fig 2.4: Use of Fungicides and herbicides in tons during 2011 to 2016 (Pesticide production is adapted from Food and agriculture organization of the united nations)

Pyrethroids and Pyrethroids-SeedTr Ins consumption: During 2011-2016, pyrethroids consumption was recorded as 46949.1 tons. Pyrethroids-SeedTr Ins consumed during 2011-2016 recorded was 257.46 tons. In fig 2.5- uses of pyrethroids is mentioned.

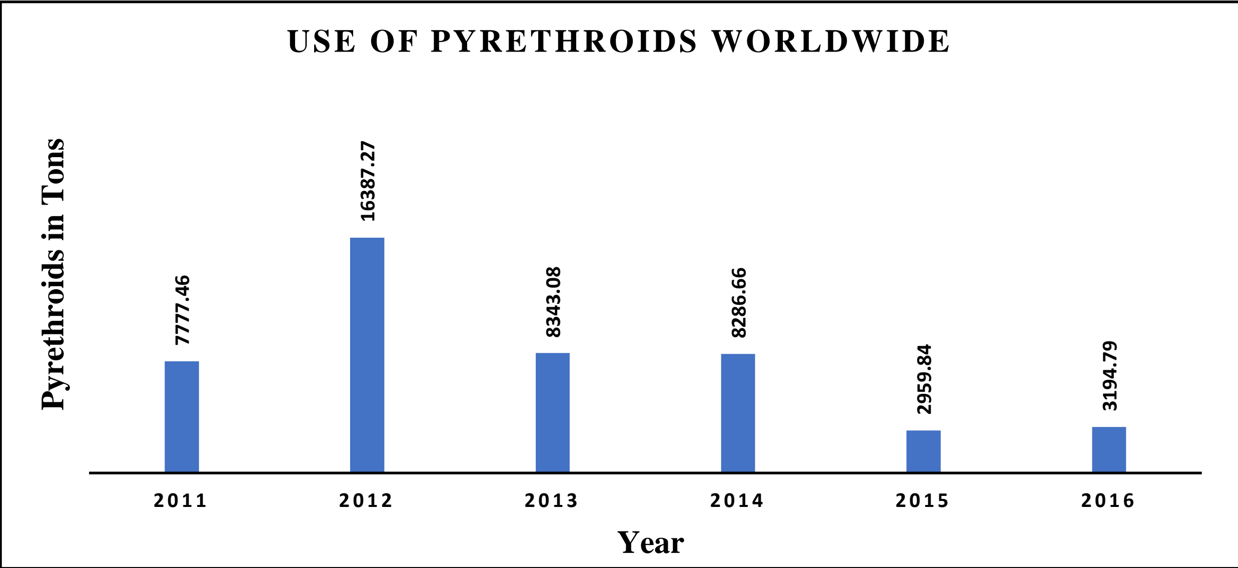


Fig 2.5: Use of Pyrethroids in tons during 2011 to 2016 (Pesticide production is adapted from Food and agriculture organization of the united nations)

2.4.2: Pesticides Usage in India as Per Compound Annual Growth Rate (CAGR) Report 2012-2013 and 2019

State wise consumption of pesticides reflects that major consumptions of pesticides occur in Uttar Pradesh with 9035 tons, Maharashtra with 6617 tons, Andhra Pradesh with 6500 tons, Punjab with 5725 tons, followed by Haryana with 4050 tons. Total consumption of pesticides in India during 2012-2013 recorded as 45619 tons. As per crop land available state wise the average use of pesticides per hectare was found highest in Jammu Kashmir with 2-337 kg/ha trailed through Punjab with 1.377 kg/ha and Haryana with 1.151 kg/ha. These synthetic pesticides mainly used for Kharif and Rabi crop production (Devi *et al.*, 2017). There is huge amount of pesticide production during last five fiscal year in India, in 2013-155, in 2014- 179, in 2015-186, in 2016-188, in 2017-214, 2018-213, 2019-217 thousand metric tons (CAGR report and Statista Research Department). In fig 2.6, showing the production of pesticides in India on the basis of CAGR and statista report 15 Nov 2013-2019.

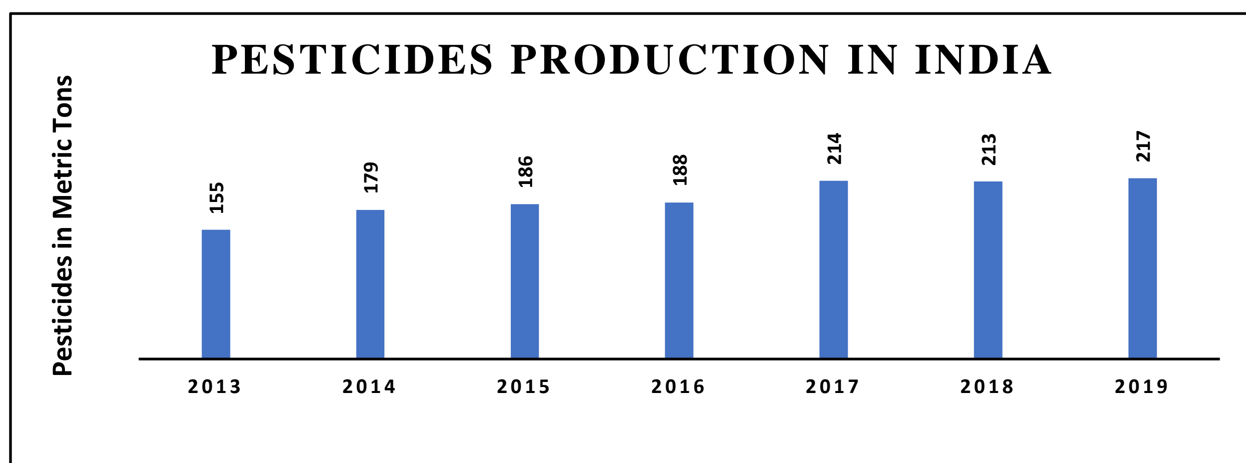


Fig 2.6: Production of Pesticides in India during 2013-2019 (data source CAGR report 2012-2013 and Statista Research Department 2013-2019).

2.5: Synthetic Pyrethroids as an Alternative to Chlorinated and Organophosphate Insecticides

Synthetic pyrethroids are basically derived from pyrethrin, a bioactive compound found in chrysanthemum flowers (Worthing, 1983; Fishel, 2005; Haug and Naumann, 1990). Pyrethroids

marketing started in 1970s for agricultural aspects and home and garden insecticides. Synthetic pyrethroids chemicals are less toxic to mammals and other wildlife animals and biodegrade more efficiently than organochlorine pesticides (Knox, 1984). Organochlorine and other insecticides were more persistent to environment and leading to biomagnification and damaging the neuro system of most of the vertebrates. In earlier days of deltamethrin and fenvalerate applications was significantly less harmful and fewer persistent to the setting, these categories of pesticides don't seem to be touching most non-target organisms than alternative pesticides (Adelsbach and Tjeerdema, 2003). Pyrethroids are one in all the less intensely toxic pesticides to mammals as a result of their apace neutralised by metabolic progressions. In 1980s hottest pyrethroids were- deltamethrin, fenvalerate, and cypermethrin, but due to exposure of those pyrethroid pesticides many poisoning cases are according and recorded in literature. Throughout 1983-1988, 573 case of acute poisoning due to artificial pyrethroid deltamethrin and out of 573 cases, 325 cases were thanks to deltamethrin exposure within which 158 was due to activity and 167 were accidental cases (He *et al.*, 1989). Pyrethrin derived synthetic pesticide pyrethroids are staggeringly fatal toward fish and their tadpoles, they cause influence with dermal receptors and organs controlling the balance (Tomlin 1994). Though, once rats were fed with high dose of 1 mg/kg,000 mg/kg organic phosphate pesticide of body weight exhibited hepatic damages (Hayes 1982). General symptoms of pyrethroids poisoning were burning sensation of skin, irritation in upper respiratory tract and abnormal burning sensation of facial area (Kolmodin-Hedman, *et al.*, 1982; Tucker and Flannigan, 1983; He *et al.*, 1989). The applications of pyrethroid insecticides are extremely lethal to insects, fish and other aquatic organisms and mostly low toxicity to mammals, and other terrestrial vertebrates because of this reason use of pyrethroids have been favoured over other types of insecticides (Casida, 1983). The polychlorinated biphenyls (PCBs) and other pesticides are showing impending effects which are detrimental to the immune system, nervous system, endocrine system etc. and make susceptible to host for various dysfunctions leading to diseased state (Hurley, 1998 and Brouwer *et al* 1999).

Pyrethroids into separated into 2 main categories supported neuroscience, pharmacology, and pharmacologic effects (Gammon and drum sander, 1985).

Types of Synthetic Pyrethroids: Type I or Tourette (T) syndrome and Type II or Choreoathetosis with Salivation (CS) syndrome

- **Type I or T syndrome:** Type I pyrethroids contain mainly allethrin, cismethrin, permethrin and resmethrin; which do not comprise α-cyano group in their structure. The main symptoms observed by their poisoning are tremors due to abnormal behaviour of neurons, hyperexcitations due to altered breathing rate, ataxia, and tremors, in all perilous cases and can lead to paralysis, prostration and death in extreme lethal cases (Verschoyle and Aldridge, 1980). Type I pyrethroids generally affects the central nervous system (CNS) and peripheral nervous system (PNS) and develop symptoms related to Type I disorders (Haschek *et al.*, 2013).

- **Type II or Choreoathetosis with Salivation (CS) syndrome:** Type II pyrethroids include cypermethrin, deltamethrin and fenvalerate which comprise a cyano (-CN) group at the α-carbon of the phenoxybenzylalcohol place. This group of pyrethroids give rise to a more complex syndrome causing excessive salivation, tremors, restlessness, progressing to rolling convulsions (c), piloerection and occasionally both tonic and colonic seizures (Leahey, 1985). In type II pyrethroids exposure CNS is affected and shows impacts and disorders related to CNS damage (Haschek *et al.*, 2013). In fig 2.7 classification of pyrethroids is shown with examples and symptoms.

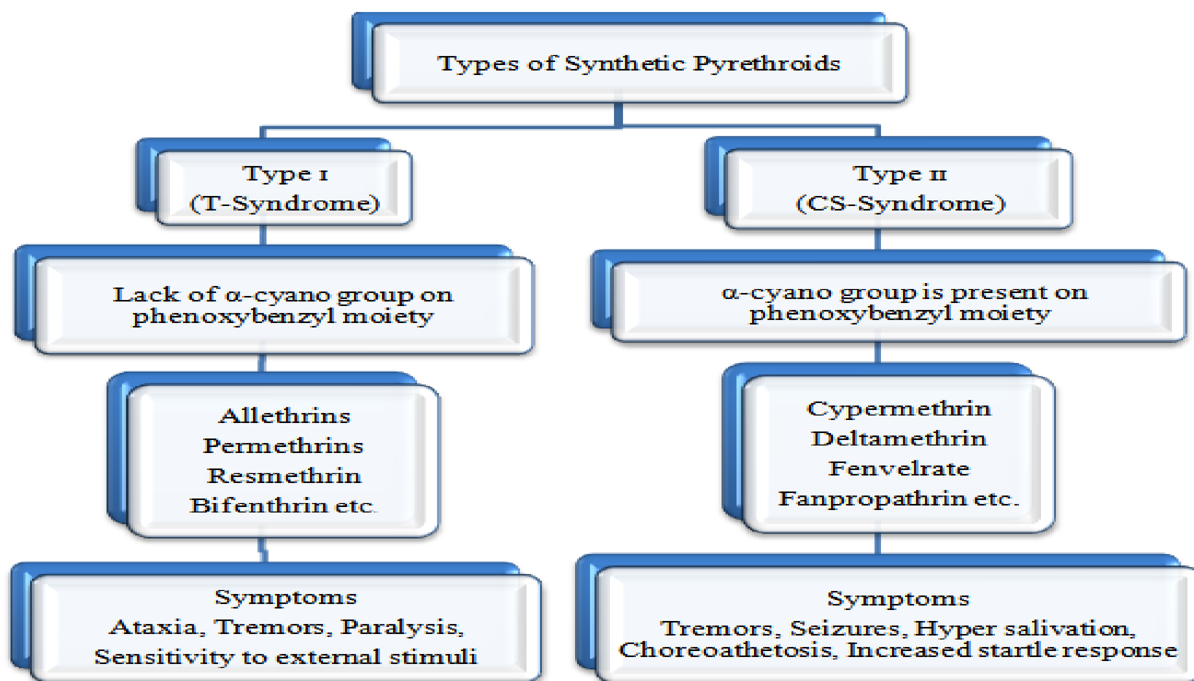


Fig 2.7: Classification of synthetic pyrethroids based on disease symptoms

In table 2.2 common pyrethroid pesticides is mentioned with IUPAC and trade names.

Table 2.2: Common name, trade name and IUPAC name of common pyrethroid pesticides

Pesticide name	Trade names	Chemical name
Allethrin	Many household products	(RS)-3-allyl-2-methyl-4-oxycyclopent-2-enyl (1RS)-cis-trans chrysanthemate
Bifenthrin	Capture®, Talstar®	[1-alpha,3-alpha-(Z)]-(+)-(2 methyl[1,1'-biphenyl]-3yl) methyl 3-(2,chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate
Cyfluthrin	Baythroid®, Tame®	Cyano(4-fluoro-3-phenoxyphenyl)methyl 3-(2,2-dichloro-ethenyl)-2,2-dimethylcyclopropanecarboxylate
Cyhalothrin	Karate®, Warrior®, Demand®, Scimitar®	alpha-cyano-3-phenoxybenzyl 3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate
Cypermethrin	Ammo®, Fury®, Mustang®	(+)-alpha-cyano-3-phenoxybenzyl (+)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethycyclopropanecarboxylate
Deltamethrin	Decis®, DeltaGard®, Demand®	(S)-cyano(3-phenoxybenzyl) (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate
Esfenvalerate	Asana®	(S)-cyano(3-phenoxyphenyl)methyl (S)-4-chloro-alpha-(1-methylethyl)-benzeneacetate
Fenpropathrin	Danitol®, Tame®	RS-alpha-cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate
Fluvalinate	Mavrik®, Zoecon®	Á-RS,2R)-fluvalinate [(RS)-alpha-cyano-3-phenoxybenzyl (R)-2-[2-chloro-4-(trifluoromethyl)anilino]-3-methyl-butanoate]
Permethrin	Ambush®, Pounce®	(3-phenoxyphenyl)methyl (+)-cis,trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate
Resmethrin	Many household products	([5-(phenylmethyl)-3-furanyl]methyl 2,2-dimethyl-3-(2-methyl-1-proenyl) cyclopropanecarboxylate)
Tefluthrin	Force®	2,3,5,6-tetrafluoro-4-methylbenzyl (Z)-(1 RS, 3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-ethyl) -2,2-dimethylcyclopropanecarboxylate
Tetramethrin	Many household products	3,4,5,6-tetrahydrophthalimidomethyl (1RS)-cis,trans-chrysanthemate
Tralomethrin	Scout®	(1R,3S)3[(1',2',2',2',-tetrabromoethyl)]-2,2-dimethylcyclopropanecarboxylic acid (S)-alpha-

In table 2.3 pesticides LD_{50} dose in mg/kg is mentioned for mice, rat and rabbit on the basis of National Pesticide Information Center.

Table 2.3: Various pyrethroid pesticides and their toxicity ranges in mice, rats and rabbits (Data adapted from National Pesticide Information Center, Extension Toxicology network, Toxicology Data Network, and United State Environmental Protection Agency).

Name of Pesticide	Mice Oral LD50 (mg/kg)	Rat Oral LD_{50} (mg/kg)	Rabbit Oral LD_{50} (mg/kg)	Rabbit Dermal LD_{50} (mg/kg)
Allethrin	370	709 (M), 685 (F)	4290	11,332
Bifenthrin	43	375	NA	>2,000
Cyfluthrin	291-609	869–1271	>1,000	>5,000
Cyhalothrin	37-62	144-243	416	632
Cypermethrin	82-779	187 to 326 (M) 150-500 (F)	3000	>2,000
Deltamethrin	21-34	>50(M) 31–139 (F)	NA	>2,000
Esfenvalerate	458	451	NA	2,000
Fenpropathrin	67 (M) 58 (F)	70.6 (M) 66.7 (F)	675 (M) 510 (F)	>2,000
Fluvalinate	2042	282 (M) 261(F)	NA	>20,000
Permethrin	540-2690	430-4000	>4,000	>2,000
Resmethrin	690 (M) 940 (F)	1,244 (M) 1721 (F)	NA	>2,500
Tefluthrin	46 (M) 57 (F)	22 (M) 35 (F)	NA	>2,000
Tetramethrin	>20,000	>2,000	NA	>2,000
Tralomethrin	NA	99	NA	>2,000

In table 2.4 LD_{50} toxicity of selected pyrethroid pesticide in Bird, Fish and Bees is shown.

Table 2.4: LD_{50} Toxicity of Selected Pyrethroid Pesticide in Bird, Fish and Bees (Adapted from National Pesticide Information Center, Extension Toxicology network, Toxicology Data Network, and United State Environmental Protection Agency).

Pesticide name	Bird acute oral LD_{50} (mg/kg)	Fish LC_{50} (ppm)	Bee LD_{50}
Allethrin	Non Toxic	Highly Toxic	Highly Toxic
Bifenthrin	Slightly Toxic	Highly Toxic	Highly Toxic
Cyfluthrin	Non Toxic	Very Highly Toxic	Highly Toxic
Cyhalothrin	Non Toxic	Highly Toxic	Highly Toxic
Cypermethrin	Non Toxic	Very Highly Toxic	Highly Toxic
Deltamethrin	Non Toxic	Highly Toxic	Highly Toxic
Esfenvalerate	Non Toxic	Very Highly Toxic	Highly Toxic
Fenpropathrin	Slightly Toxic	Very Highly Toxic	Highly Toxic
Fluvalinate	Non Toxic	Very Highly Toxic	Moderately Toxic
Permethrin	Non Toxic	Very Highly Toxic	Highly Toxic
Resmethrin	Non Toxic	Very Highly Toxic	Highly Toxic
Tefluthrin	Slightly Toxic	Very Highly Toxic	Highly Toxic
Tetramethrin	Non Toxic	Highly Toxic	—
Tralomethrin	—	Very Highly Toxic	Highly Toxic

Range of LD50 in Bird: Non Toxic = >2,000; Slightly Toxic = 501–2,000; Moderately Toxic = 51–500; Highly Toxic = 10–50; Very Highly Toxic = <10.
Range of LC50 in Fish: Highly Toxic = 0.1 -1; Very Highly toxic = < 0.1
Range of LD50 in Bee: Highly Toxic = Kills upon contact; Moderately Toxic = Kills bee when applied over.

2.6: Physical & chemical properties of deltamethrin

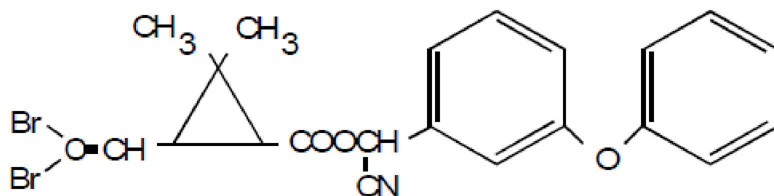
The insecticide deltamethrin was first formed in 1974 and promoted in 1978 for various applications. The IUPAC name of deltamethrin is [(S)-cyano-(3-phenoxyphenyl) methyl] (1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane-1-carboxylate and its molecular formula is $C_{22}H_{19}Br_2No_3$. Deltamethrin is extraordinarily stable on acquaintance to air and

temperature (stability calculated up to 190°C.). It is a lot of stable in acidic as compared to alkalescent media. In table 2.5 physical and chemical properties of deltamethrin.

Table 2.5: Physical and Chemical Properties of Deltamethrin

Physical and Chemical Properties of Deltamethrin	
Chemical formula	$C_{22}H_{19}Br_2NO_3$
Boiling point	300^0C
Molar mass	505.21 g/mole
Density	1.5 g/cm^2
Melting point	98^0C
Vapor pressure	1.5×10^{-8} mm Hg at 25^0C
Octanol-Water Partition coefficient (log Kow)	6.1
Solubility	0.002 to 0.0002 mg/L
Soil Sorption Coefficient (Koc)	7.05×10^5 to 3.14×10^6

Structural Formula:



pH: 5.9 (in a 1% aqueous dispersion) (Grelet, 1990 and Lambert, 1991)

2.7: Deltamethrin exposure to different organisms and effects

There are various studies reported about the toxic effects of deltamethrin, which reflect the nephrotoxicity, hepatotoxicity, splenic toxicity, thymic toxicity, cardiac toxicity, lung toxicity, and immunotoxicity. Some of leading findings are mentioned in brief as below.

In feminine BALB/c mice administered with deltamethrin in two regular oral doses to 2 completely different teams, one cluster with six mg/kg for eighty-four days and another group with fifteen mg/kg for fourteen days. The body substance reaction, the number of plaque-forming cells (PFC), immune cells manufacturing Ig antibodies and interleukin-1 was found decreased considerably. It indicates that, deltamethrin shows the immunological disorder effects in mice (Lukowicz-Ratajczak and Krechniak, 1992). The body covering exposure of deltamethrin in mice with 5 mg/kg for 3 days through injections and one dose of 30 mg/kg injection of Hydrocortone to Balb/c mice causes reversibly upsurge within the development of WBC and phagocyte collections in the marrow however absent in the spleen. There have been no variations were detected in total cell counts and differential cell counts within the marrow and spleen in addition as spleen weights also unchanged. While, action of each the compounds, however, triggered reduction in thymus weights. Hydrocortisone administration caused increase in range of granulocytes however reduced the phagocyte colonies, moreover, deltamethrin was found while not in vitro effects associated with range of granulocytes and phagocyte colonies (Queiroz, 1993). The oral administration of deltamethrin and α-cypermethrin in F344 male rats for 28 days with defined dose of either deltamethrin through 0 mg/kg, 1 mg/kg, 5 mg/kg, or 10 mg/kg body wt./day or α-cypermethrin through 0 mg/kg, 4 mg/kg, 8 mg/kg, or 12 mg/kg body wt./day mixed in soy bean oil give rise to, deltamethrin with five mg/kg or ten mg/kg body wt. resulted into increase in weight of mesenterial humor nodes, decrease in thymus weight in unsusceptible animals and a rise in numbers of SRBC-PFC and natural killer (NK) cells activity of splenic origin though there was no severe effects on the immune system distinguished (Madsen *et al.*, 1996).

2.8: Deltamethrin toxic effects on different organs of animals

There are various research studies explained the toxic effects of deltamethrin and changes in level of signature enzymes and metabolites related to specific organs. Based on effects of deltamethrin

on different organ system present in higher animals and lower animals, toxic effects can be classified as-

2.8.1: NEUROTOXICITY

The deltamethrin a class II pyrethroids leads to opening and activation of sodium channel in brain neurons and cause hyperexcitation symptoms as poisoning effects (Chinn and Narahashi, 1986; Song, *et al.,*1996; Shafer *et al.,* 2004). Deltamethrin exposure in mice with outlined dose of 6 mg/kg for 2 weeks alone or in mix with a sole action of twenty mg/kg of parkinsonian poison, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) will increase neurotransmitters notably dopamine and glutamate (Kirby *et al.,* 1999). The deterioration of nigrostriatal pathway leads to development of Parkinson's disease, when deltamethrin was administered in a mice model for Parkinson's disease development revealed that there was increase in dopamine (Bloomquist *et al.,* 2002). Deltamethrin alters phosphorylation of various proteins concerned in many cellular communication pathway mechanisms, like activation of super molecule protein kinase C in phosphoinositide pathway and caused the increase level of neurochemical and free Ca^{2+}. Calcium-sensitive proteins concerning synaptic communication are the key action goals of type II pyrethroids. (Enan and Matsumura, 1993). Insecticides are targeting specific ion channels extensively by pyrethroids activities on voltage gated sodium channel and causes slow kinetics of both activation and inactivation of sodium channels and consequential extended opening of these channels (Narahashi, 1996). Side effects of neurotoxicity because of introduction of deltamethrin resembles different pyrethroids which is portrayed by hyper excitation, ataxia, coordination misfortune, seizures, inappropriate engine activities and at some point, even loss of motion (Ray and Cremer, 1979; Clark and Brooks, 1989; Dayal *et al.,* 2003). Deltamethrin builds the take-up of dopamine by balancing the dopamine transporter (DAT) and prompting weakened neurotransmission (Kirby *et al.,* 1999 and Elwan *et al.,* 2006). Deltamethrin persuades neuro-disintegration in *in vivo* and *in vitro* and and prompts very much customized cell death process in the brain (Wu and Liu, 2000). The neuronal toxicity brought about by deltamethrin pesticide has been indicated to be related with its metabolic rate in biological system (Anadon *et al.,* 1996). The signs of neurotoxicity due to intoxication of deltamethrin pesticide were realized to be coupled with the ascent in the groupings of deltamethrin pesticide and its intermediate metabolites affecting the brain tissues (Rickard and Brodie, 1985; Anadon *et al.,* 1996). Deltamethrin has portrayed to

have high fascination towards the brain tissues and spinal cord which is part of central nervous system (CNS) as well as affecting sodium channels of neurons were observed to in group of insects as well as vertebrates (Barlow, 2001; Narahashi, 2002; Ray and Fry, 2006; Soderlund, 1989; Soderlund, 2002).

Deltamethrin is likewise portrayed to associate by means of γ-aminobutyric acid (GABA) sensory receptor ionophore complex, which came about into harried synaptic correspondence and neuroexcitation, causes subsequent conduct differences and irregularities (Lawrence and Casida, 1983; Crofton and Reiter, 1987; Aziz *et al.,* 2001). Prior investigations have demonstrated that neurobehavioral variations from the norm due to deltamethrin is dependent on its digestion which is encouraged by synthetic chemical xenobiotic utilizing by cellular respiration involved molecule cytochrome P450s in rodent brain and hepatic tissues (Dayal *et al.,* 2001; Dayal *et al.,* 2003). Deltamethrin pesticide has been accounted for to induce the declaration of cytochrome P450 and cytochrome P450-2B isoenzymes, which are associated with deltamethrin assimilation in the brain and liver of rat (Dayal *et al.,* 1999; Anand *et al.,* 2006). Type II pyrethroids are premise of a decided depolarization and recurrence subordinate transmission piece in sensorial and motor axons just as expanded dull terminating of sensorial closures and muscle filaments (Joy, 1994). Upsurge in Monoamine oxidase action and reduction in Na+ and K+-ATPase movement were set up in a few segments of the CNS in rodents regulated with the cyano-pyrethroid deltamethrin (Husain *et al*., 1996). Concentrates on the destructive impacts of pyrethroid bug sprays in the central and fringe sensory system of warm-blooded animals are normally stressed with the biochemical, pharmacological, and physiological impacts prompting different variations and leading to various abnormalities and disorders (Takahashi and Le Quesne, 1982; Narahashi *et al*., 1996). Morphological changes saw in different investigations are uncommon, and the trial varieties are discrete. In one of the examination discoveries, deltamethrin cause dendritic degeneration of Purkinje neurons of the cerebellar regions of rats and prompting neurotoxicity (Husain *et al*., 1996).

2.8.2: EFFECTS ON ACETYLCHOLINESTRASE

Acetylcholinesterase (AChE), a serine protease compound that separates the synapse acetylcholine at the synaptic parted (the space between two nerve cells) so the following nerve

drive can be transmitted over the synaptic gap (Quinn, 1987). The synergist action of AChE is high, 25000 particles of acetylcholine (ACh) molecules are degraded by every molecule of AChE every second, looming the farthest point allowed by dispersion of the substrate (Radić *et al.*, 1991). AChE originates in various types of conducting tissues: nerve cells and muscles, peripheral and central nervous system tissues, sensory and motor fibers, and cholinergic as well as noncholinergic strands. The rate of activity of AChE is reported higher in motor neurons than sensory neurons (Massoulié *et al.,* 1993; Chacko and Cerf, 1960; Koelle, 1954). Passing of message is governed by neurotransmission, ACh is an envoy atom discharged from pre-synaptic nerve and discharged into the synaptic cleft and ties to ACh receptors which are available on the post-synaptic membrane of neuron, trailed by transferring of the flag and method of correspondence is built up by the one neuron to another neuron. AChE regulates the degradation of Ach, AChE is located on the postsynaptic membrane, and if there is any irregulation in Ach hydrolysis may prompt loss of motion in patient and cause paralysis. After hydrolysis of ACh, choline is discharged and is taken up again by the pre-synaptic neuron and blend of ACh is finished by consolidating of choline atom with acetyl-CoA by the catalysis activity of choline acetyltransferase (Whittaker, 1990; Purves *et al.,* 2008; Pohanka, 2012).

It has been accounted for that deltamethrin and different pyrethroids reasons a significant prolongation of the generally transient upsurge in sodium penetrability of the nerve layer amid excitation, trailed by enduring trains of dreary motivations in the sense organs and a recurrence subordinate dissimilarity of the nerve-driving forces in the neurons (WHO, 1991; WHO, 2010). In the meantime, the procedure of nerve drive advancement, transmission and conduction are for the most part the comparative through the total sensory system, pyrethroids pesticides perhaps act in an indistinguishable method in a few segments of the central and peripheral nervous systems. These deviations in sodium motion in the different tissues may sometime be related with the motivations behind the various components of the sensory system, and the secretory as well as enzymatic assets may also be predisposed. Rao and Rao 1995 reported the variation in action of acetylcholinesterase of rat brain is affected by numerous pyrethroids, like, permethrin and cypermethrin. The up regulation of mRNA of bdnf and c-fos in zebrafish was reported due to exposure of chlorpyrifos, cypermethrin, and deltamethrin (Özdemir, *et al*., 2018).

2.8.3: HEPATOTOXICITY

The liver is the main site of pyrethroid breakdown, absorption and other metabolic activities leading to accumulate a greater concentration of toxic metabolites and generates high amount of reactive oxygen species which damages the liver tissues (Rickard and Brodie, 1985). The cytochrome P450 monooxygenases a xenobiotic degrading enzyme present in hepatic tissues play key role in regulating the metabolism of deltamethrin pesticides (Catinot *et al.,* 1989; De la Riva and Anadon, 1991; Anadon, *et al.,* 1996). Deltamethrin administration in body by any route cause the production of free radical generation in tissues (Manna *et al.,* 2005). The cytochrome P450 monooxygenase enzyme targets ester bonds present in deltamethrin and cleave it into the numerous oxidative metabolic path (Eraslan *et al.,* 2007). There are different reports expressed that deltamethrin is causative specialists behind histological modifications in liver tissues, renal tissues and lungs tissues (Manna *et al.,* 2005; Erdogan *et al.,* 2006; Shona *et al.,* 2010). Deltamethrin induced various histopathological changes in liver and caused raise in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in blood serum and adversely affects its enzymatic activities (Lamfon, 2007; Tewari *et al.*, 2018). There was huge alteration in biomarker enzymes like alkaline phosphatase, lactate dehydrogenase and aminotransferases and histological changes in liver tissues in deltamethrin intoxicated rats (Rjeibi *et al.,* 2016). In deltamethrin administered rats there was significant changes in six liver originated proteins-apolipoproteins IV, apolipoproteins E, haptoglobin, hemopexin, vitamin D binding proteins and fibrinogen gamma chain (Arora *et al.,* 2016). Deltamethrin instigated rodents demonstrated different histological deviations in the liver and these deviations included obstruction of blood vessels and veins, leucocytic penetration, cytoplasmic vacuolization of the hepatocytes and greasy invasion (Lamfon *et al.,* 2007).

2.8.4: NEPHROTOXICITY

Animals treated with deltamethrin leads to numerous histopathological modifications in the different kidney tissues of renal cortex, renal medulla and renal pelvis region (Shona *et al.,* 2010). Significance of leave concentrate of *Oscimum basilicum* was set up on deltamethrin induced nephrotoxicity notwithstanding oxidative stress condition in albino rats, the presentation of deltamethrin in rats caused in an imperative upsurge in the renal substance of malondialdehyde

(MDA) showing improved lipid peroxidation which implicates the renal oxidative nervousness (John *et* al., 1989; Sakr and Al-Amoudi, 2012). Besides, deltamethrin delivered a critical decrease in the exercises of super oxide dismutase (SOD) and catalase (CAT). Antioxidant carrying capacity enzymes available as mobile reinforcement proteins principally SOD, and CAT are the foremost characteristic of marker enzymes to unrestricted radical induced oxidative stress. Enzyme SOD is accountable for synergist disproportionation of particularly receptive and simply detrimental superoxide radicals to hydrogen peroxide, and CAT is accountable for the reactant deterioration of hydrogen peroxide to atomic oxygen and water (Tewari and Gill, 2014). After deltamethrin intoxication in mice and rats a reduce in the grouping of cell reinforcement antioxidant compounds and an upsurge in lipid peroxidation rate had been mentioned (Sayeed *et al.,* 2003, Rehman *et al.,* 2006, Yousef *et al.,* 2006). Exposure to deltamethrin leading the injury of renal tissue discovered and might be brought about to upward push in lipid peroxidation rate and minimize of biomarker cell reinforcement antioxidant proteins in the kidney (Sakr and Al-Amoudi *et al.,* 2012). The above studies also showed that histopathological investigation of kidney of deltamethrin intoxicated rats uncovered many changes such as tubular deterioration, degeneration of glomeruli, leucocytic intrusions and overcrowding of renal blood vessels. The above examinations moreover demonstrated that histological examination of kidney of deltamethrin directed rats printed several adjustments, for example, cylindrical disintegration, degeneration of glomeruli, leucocytic interruptions and congestion of renal veins. Deltamethrin prompted huge increment in serum urea and creatinine which indicates decreased ability of the kidneys to channel these waste gadgets from the blood. Similarly, a few unique reports exhibit an upsurge in the serum urea and creatinine in rats due to deltamethrin hazard and conceivably will be credited to its free radicals invigorated oxidative damage (Mongi *et al.,* 2011). Deltamethrin is not carcinogenic and mutagenic at low dose in swiss mice (Shukla *et al*., 2000; Shukla *et al*., 2001). It has portrayed that the convergence of creatinine and urea in serum relies upon basically on the glomerular infiltration. The change in these two parameters for instance dimension of creatinine and urea collectively with the histological influences on kidney tissues assign a reduce in the glomerular filtration rate due to deltamethrin administration. Defensive influence of lycopene on deltamethrin influenced histological and ultrastructural deviations in kidney tissues of rodents and improved dimension of TNF-α has been observed (El- Gerbed 2012).

2.8.5: REPRODUCTIVE TOXICITY

It has been seen that deltamethrin treatment of grown-up male rats with 1.0 and 2.0 mg/kg for 65 sequential days lessen testis, prostate, and original vesicle weight and abatement sperm tallies and testosterone levels (Abd *et al*., 1994). The weight of the male gonad testis is essentially established on the mass of the detached male gametogenic cells available in the seminiferous tubules and the diminishing in the mass of the male gonad may be because of reduced quantity of germ cells production in seminiferous tubule, avoidance of sperm production and steroid synthesizing enzymes undertaking because of action of bisphenol A (BPA) in F344 rats (Takahashi and Oishi, 2003). It has been reported that deltamethrin lowers sperm amount, sperm movement and alive sperm percentage and provocatively amplified sperm aberrations (Abd *et al.,* 1994). When rats were administered with oxolinic acid at 300, 1000 or 3000 ppm for 4, 26, or 52 weeks then serum luteinizing hormone (LH) concentration were raised up in equally young adult and aged animals administered with 3000 ppm at record studied time period. Despite the fact, the testosterone concentration found to be improved at the initial time point in young adult, however not testified in older, grown-up animals. The decrease in sperm sum total might be due to an antagonistic consequence of deltamethrin on spermatogenesis process, by means of deltamethrin exposure described as the pointers of the chemical toxicity on reproductive system and cause reduced expression and synthesis of testosterone (Yoshida *et al.,* 2002). 4-tert-octylphenol (Organo-Phosphate pesticide) administration with two different dose, first dose of 20 mg and second dose of 80 mg while estradiol valerate dose used with 0.8 μg or 8 μg for three dose per week for duration of 1 month in one group and 2 months in other group resulted into reduced sperm numbers and unfavorably predisposed the size, weight, and histological modifications of the testes, epididymis, prostate glands, seminal vesicles, and coagulating glands (Boockfor and Blake, 1997). Testosterone, primary male sex hormone is irreplaceable to safeguard the assembly and capacity of the male auxiliary organs and sex organ. Also, absence of testosterone intrudes on sperm synthesis movement and results in a decreased number of sperm sum (Boockfor and Blake, 1997). Lipid peroxidase (LPO) is a biomarker showing oxidative hindrance in cells and tissues, which assumes a massive job in identifying the risk of a variety of xenobiotics simply as pesticides. It is shocking that testicles incorporate exceedingly high convergence of glutathione than anything in different organs, which assumes a critical job in the proliferation and variety of spermatogenic cells all the whilst defensive influences on these cells from ROS annihilation (Teaf *et al.,* 1985).

Deltamethrin triggered a vital reduction in the mass of generative organs and instigated damages to testes, epididymis also other accessory sex glands, due to the reduction in serum testosterone concentration (Oda and El-Maddawy, 2012). They likewise portrayed that in deltamethrin exposed rats, there is especially diminished sperm first-rate and had the better grouping of MDA in the testicular tissues uncovering the high technology of LPO and prompts harm of film gathering and capacity (Abd El-Aziz *et al*., 1994, and Andrade *et al*., 2002; Sharma *et al*., 2014). In standard investigations, deltamethrin remedy has been accounted for to lower GSH stage in rodents whilst deltamethrin set off a reduce in GSH sum in mice kidney than the liver (Rehman *et al.,* 2006). FSH and testosterone are most important controllers for spermatogenesis. Organization of 50 µg of oestradiol benzoate utilized for 1, 2, 3, 4, 5 or 10 days in rodents might also reap limit in articulation of gonadotrophin discharge and centralization of testosterone. Minute measure of intratesticular testosterone conceivably lead to breaking down of germ cells from seminiferous epithelium and may want to enlist germ cell apoptosis and succeeding male fruitlessness (Blanco-Rodriguez and Martinez-Garcia, 1998). At the point when mice were uncovered with deltamethrin with 3 and 5.00 µg/kg for 15 and 30 days caused hyalinization, vacuolization, peeled spermatocytes, enlargement and cylindrical disfigurement, cell vacuolated weakening, apical sloughing and multilayered seminiferous epithelia with late spermatids covering the lumen of the seminiferous tubules in the testis (Rashid *et al*., 2012). Deltamethrin presentation to male Swiss mice with one characterized low portion of 3 mg/kg and another high portion of 6 mg/kg set off a generous lessening in body and organ loads, sperm tally, sperm motility percent, sperm reasonability, serum testosterone fixation, sialic acid substance of epididymis and fructose convergence of fundamental vesicle. Deltamethrin directed gatherings of mice likewise showed a significant diminishing in testicular 3β and 17β hydroxysteroid dehydrogenase (HSD) exercises. Histological examinations uncovered noteworthy modifications in the testicles of dosed gatherings (Desai, *et al*., 2016). Thus, deltamethrin and other pyrethroids from various studies suggest that these pesticides induce oxidative damage and histopathological modifications of testis, epididymis and accessory sex glands.

2.8.6: LUNG TOXICITY

All human beings and other animals are is exposed to different types of pesticides present in the environment via several ways. The farmers and family members are most exposed to pesticides

which leads to the harmful effects of pesticides and suffer from various health challenges issues. Most affected organ system due to pesticides exposure is respiratory system, and lung is most affected organ (Damalas and Koutroubas, 2016). When pesticide spray man was exposed with 2.5% deltamethrin shown symptoms of skin burning sensation and breathing problem due to entry of pesticide through skin and inhalation (Shujie *et al.*, 1988). During 1987-1988, a survey was conducted on 3113 pyrethroid spray men (2230 men and 883 women) working in cotton field were interrogated after spraying and health status was observed for 72 hours. Due to pyrethroid exposure 834 of them displayed as irregular facial feelings, faintness, headache, tiredness, nausea, or loss of hunger (Chen *et al*., 1991). 68% of ranchers felt sick after monotonous uses of pesticides in the fields, complete 39 % of ranchers expressed costs flanked by 20,130, and 200 Tanzanian currency per annum on prosperity because of synthetic pesticides toxic quality (Ngowi, *et al.*, 2007).

The rehashed inward breath of pesticides that contains imiprothrin and deltamethrin in rats demonstrated an assortment of immunotoxicity impacts the lung tissue toxicity (Emara and Draz 2007). Organophosphorus pesticides (OPs) are worried in human asthma cases, parathion OP produces hyperactivity in aviation route by restraining M2 muscarinic receptors present on parasympathetic nerves (Proskocil *et. al.*, 2013). Lung cytokines are one of the important endogenous factors gives remarkable incident towards oral pesticide exposure which signifies the damage of lung tissues (Witschi and Last 1991). The 15 mg/kg of deltamethrin organization orally in rats for 30 days prompts different neurotic changes, gentle to direct histological adjustments in lungs, liver, stomach, kidney, testicles and cerebellum which related well with extensive sum in tissue buildups. The rehashed deltamethrin portion prompts poisonous quality and shows decline cytochrome P450 content, antioxidant agent status in rats (Manna *et al*., 2005).

There were morphologic changes seen in the lungs of wistar rats after the inward breath of vaporized of deltamethrin splash utilized for half hour/day used for 45 days in measures of 6.0 mg/m^3 and 12.0 mg/m^3. On light minuscule examination, they watched overwhelming clog, detectable perivascular edema, lympho-plasmocytic invasion through central interstitial pneumonia, macrophage aggregation, emphysema, peri-bronchial lymphoid tissue hyperplasia and drain. On ultrastructural examination they found swollen ciliated cells and with a couple of strange cilia alongside gentle breaking down of alveolar coating cells and edematous alveolar septa (Erdogan *et al*., 2006).

2.8.7: SPLEEN TOXICITY

Exposure of deltamethrin on fingerlings of Nile tilapia (*Oreochromis niloticus L.*) with 5 µg/L dose resulted into behavioural alterations in all fishes and severe morphological modifications in the gills, spleen, kidney, liver and muscles (Yildirim, *et al*., 2006). At the point when Swiss mice were directed with deltamethrin in convergences of 5 mg/kg and 25 mg/kg for 28 days came about into falling apart changes in the liver, spleen and kidneys (Toś-Luty *et al*.,2001). Deltamethrin initiates oxidative stress and prompting caspase subordinate flagging pathway in mitochondria which triggers the apoptosis in murine splenocytes (Kumar *et al*.,2016). The oral administration of acute dose of deltamethrin in tiger salamanders (*Ambystoma tigrinum*) showed damage to organs and there was so many biochemical alterations (Froese *et al*.,2009). A cat fish *Heteropneustes fossilis* when exposed with deltamethrin for 30 days resulted biochemical changes in decrease in haemoglobin and lactate dehydrogenase (LDH), increase in number of erythrocytes. There was also observation of morphological changes kidney, liver and spleen (Kumar *et al*., 1999). Deltamethrin induces apoptotic markers such as oxidative stress, caspase 3 and splenic apoptosis in mice (Kumar and Sharma, 2015).In fig 2.8 various toxicity due to deltamethrin is shown.

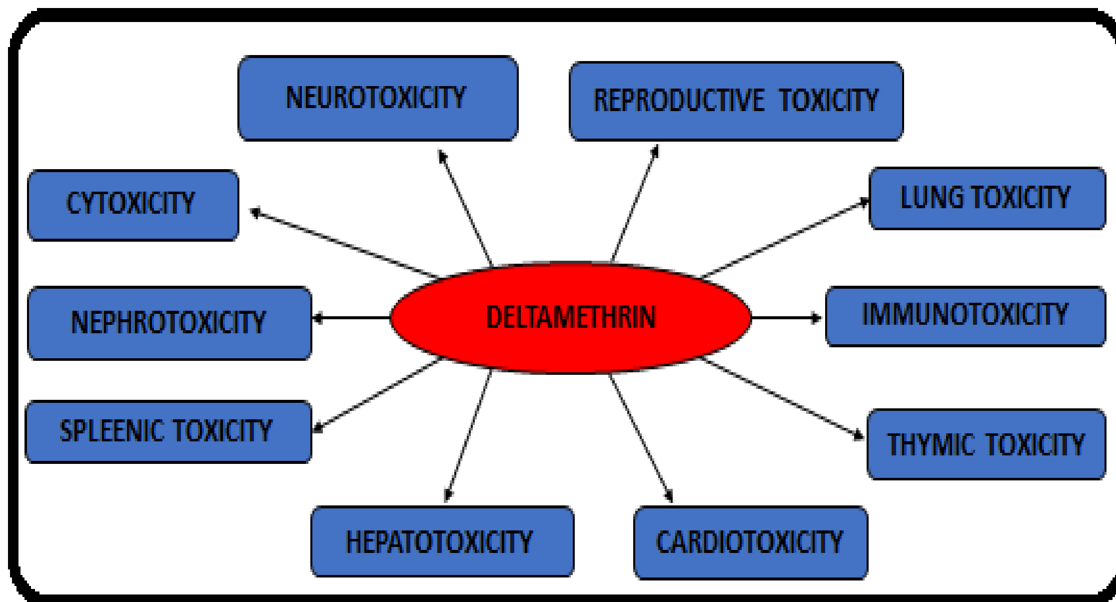


Fig 2.8: Toxic effects of Deltamethrin on various organs and cells

2.8.8: IMMUNOTOXICITY

The invulnerable framework in immune system is both an objective and a target of synthetic initiated damages to cells and tissues. Synthetics and physical stressors, for example, ionizing radiation can harm the insusceptible framework bringing about debilitated insusceptibility and disabled auto-reconnaissance for malignancy and cancer (Wiltrout *et al*., 1978). The insusceptible framework can likewise intercede the harm delivered by exogenous specialists through components, for example, excessive touchiness and autoimmunity. Ecological compound exposures have likewise brought about blends of poisonous and unfavorably susceptible reactions called "dangerous hypersensitive" disorders (Wiltrout *et al*., 1978). The immune gadget is made out of organs, cells and chemical mediators like different classes of cytokines that demonstrate together to distinguish and sequester or murder "outside" substances. Insusceptible organs incorporate the thymus, spleen, and lymphatic framework; invulnerable cells incorporate lymphocytes, other white platelets, and tissue macrophages. Lymphocytes are the essential performing artists in the invulnerable reaction, and are separated into B, T, and common executioner (NK) cells. T-lymphocytes can be additionally subdivided into aide, silencer, and cytotoxic cells. The compound middle people of invulnerable reactions involve antibodies, cytokines and the supplement arrangement of plasma proteins. Insusceptible reactions have been delegated cell-intervened and humoral reactions (Street and Sharma, 1975). Cell-interceded reactions are driven by development and differentiation of T-lymphocytes though Humoral reactions are intervened by B-lymphocytes, which include the generation of antibodies for example IgE, IgA, IgG, IgD and IgM (Kindt *et al*., 2007).

Over the past two decades, toxicological research has indicated that the immune system is a potential target for the adverse effects of insecticides in many animals. International Programmes on Chemical Safety (IPCS) and the Commission of European Communities (CEC) have defined the following basic mechanism for immunotoxicity of such chemicals (WHO, 1992; WHO, 1998; WHO, 1996; WHO, 2016).

- A direct and /or indirect consequence of the synthetic chemical xenobiotics on the immune system

- An immunological grounded host response of an individual to the synthetic compound or it's metabolites

More than the most recent 20 years, investigational signs has assembled that organophosphates can confine with the working of insusceptible framework and utilizes immunotoxicity impacts on research animals like, mice, rats, rabbits, Guinea pigs and so forth through in participation with anticholinergic and non-cholinergic flagging pathways (Wong *et al*., 1992; Barnett and Rodgers, 1994; Brundage and Barnett, 2010; Vial *et al*., 1996). These examinations have included documentation of histopathological changes to invulnerable cells, tissues and organs. Pyrethroids are chief reason behind changed cell pathology, changed development and deviations in lymphocyte sub-populaces and practical modifications to immunocompetent cells (Voccia *et al*., 1999). Now and again, these consequences for insusceptible segments and capacities have been connected to changes in illness and obstruction in uncovered life forms. Pyrethroids were presented as swap for the tireless idea of the organophosphates and chlorinated pesticides. Pyrethroids, especially deltamethrin are viewed as protected however the investigations on immunotoxic impacts of deltamethrin are exceptionally less. Along these lines, there is a need to research on lethal impacts of deltamethrin and different pyrethroids on invulnerable capacity to decide their effect if there should arise an occurrence of human introduction. Fig 2.9 showing biochemical and molecular changes in animals due to deltamethrin pesticide. Biochemical changes are basically on two level

- **Blood parameters**
- **Tissue parametrs**
- **Body weight and organ weight**
- **Molecular changes at gene expression level**

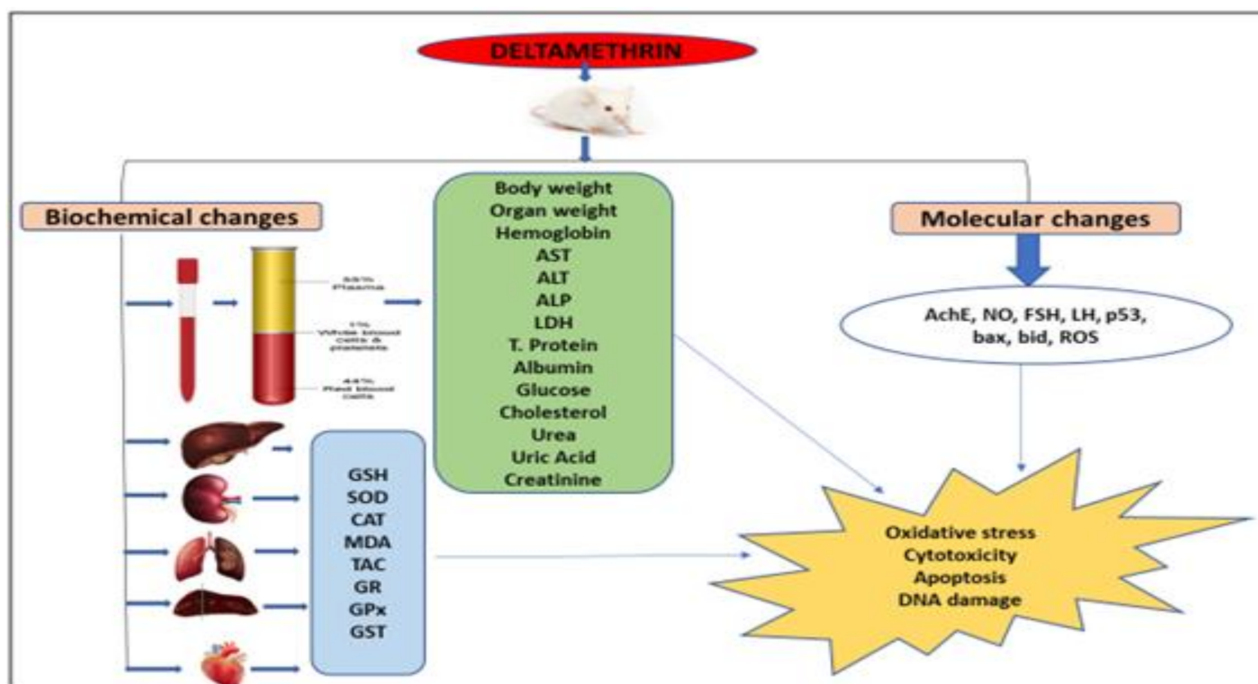


Fig 2.9: Deltamethrin causes biochemical changes and molecular changes leads to oxidative stress, cytotoxicity, apoptosis and DNA damage

2.9: Metabolic Fate of Deltamethrin

The natural and synthetic pyrethroids follow a complex metabolic process and mechanism in living system like microbial cell, animal cell or plant cell. Pyrethrin is a natural compound obtained from *Chrysanthemum* flower and used by Caucasian tribes in early nineteenth century moreover the insecticidal use of these pyrethrin compound is credited to an Armenian named Jumtikoff and his son started marketing of this natural pyrethrin as “PYRETHRUM” in 1828 (Chambers, 1980).

2.9.1: Absorption, distribution, metabolism and excretion

Pyrethroids are easily diffusing through plasma membrane of cell and said to be lipophilic in nature, when pyrethroids are exposed to animals through any routes it would be likely to voluntarily absorbed, either through oral route or dermal route. Due to oral administration of deltamethrin in rats, mice and other experimental animals, it has been quantified that the absorption of deltamethrin is done primarily by the gastrointestinal tract. The different investigations revealed that deltamethrin, an ester subordinate when controlled in exploratory animals have demonstrated

that the deltamethrin is divided quickly by explicit tissue esterase compound present in various organs, as, in the gut wall, liver and kidney via carboxylesterases, cytochrome P450s and microsomal oxidases (Ruzo *et al*., 1978). The chiefly deltamethrin is used in rodents where cleavage of the ester bond to deliver acid and alcohol residues, simultaneously oxidation of different pieces of the deltamethrin molecule previously or after cleavage of the ester bond due to sulfuric acid yield into glycine or glucuronic acid because of oxidation of both acid and alcohol moiety. Approximately 13% to 21% of unaffected deltamethrin is eliminated as waste in rat faecal matter and urine in 2–4 days, while the cyano group of deltamethrin, transformed to thiocyanate and expelled more gradually whereas even after 8 days, approximately 20% of intoxicated deltamethrin taken up in skin and stomach (Rehman *et al*., 2014).

Rats and mice show indistinguishable way of deltamethrin digestion, anyway a reduced measure of end of unaffected deltamethrin, a decreased measure of tissue holding even till 8 days, alike yet not totally approach urinary and fecal metabolites (Ruzo *et al*., 1978). Different investigations expressed the advancement of deltamethrin resilience in rats on continuous administration of deltamethrin which prescribes that the compound acquires its individual digestion by exploratory animals (Anand *et al*., 2006). The methods for digestion of deltamethrin in people have not been very much investigated and thought, however the deficient results accessible which prescribes that they are relied upon to be indistinguishable to those in rodents. For assessment of ingestion, dispersal and end have been determined in three volunteers male human managed with a solitary portion of 3 mg ^{14}C-radiolabeled deltamethrin orally (IPCS, 1990). When the evaluation was done after 1–2 hour of deltamethrin administration, the plasma concentrations found highest with an outward removal. Concentration of deltamethrin when evaluated in blood cells and saliva reported in minute amount. The total amount of 10%–26% deltamethrin administered dose was removed through faecal matter for 5 days and 51%–59% through urine, despite the fact within the first 24 hour, 90% of urinary elimination of deltamethrin. The half-life of deltamethrin in urine was calculated as 10 hour–13.5 hour and found constant and identical with half-life in plasma. Change of deltamethrin in the metabolite 3-(2, 2-dibromovinyl)-2, 2-dimethylcyclopropane carboxylic acid gives the breakdown of esters by hydrolysis in individuals (He *et al*., 1991). There was low rate of retention of deltamethrin by dermal way in rodents, and just 3.6% of the amount connected to skin is consumed and removed outside the body in initial 24 hour (IPCS, 1990). The lipophilicity of deltamethrin and the low measure of

assimilation is opposing. There are no distributed proof and data on dermal assimilation of deltamethrin in people. (IPCS, 1990). The lipophilicity of deltamethrin and the low amount of absorption is contradictory. There are no published evidence and information on dermal absorption of deltamethrin in humans. In fig 2.10 metabolic fate of deltamethrin pesticide is shown and table 2.6 the various applications of deltamethrin pesticides are shown.

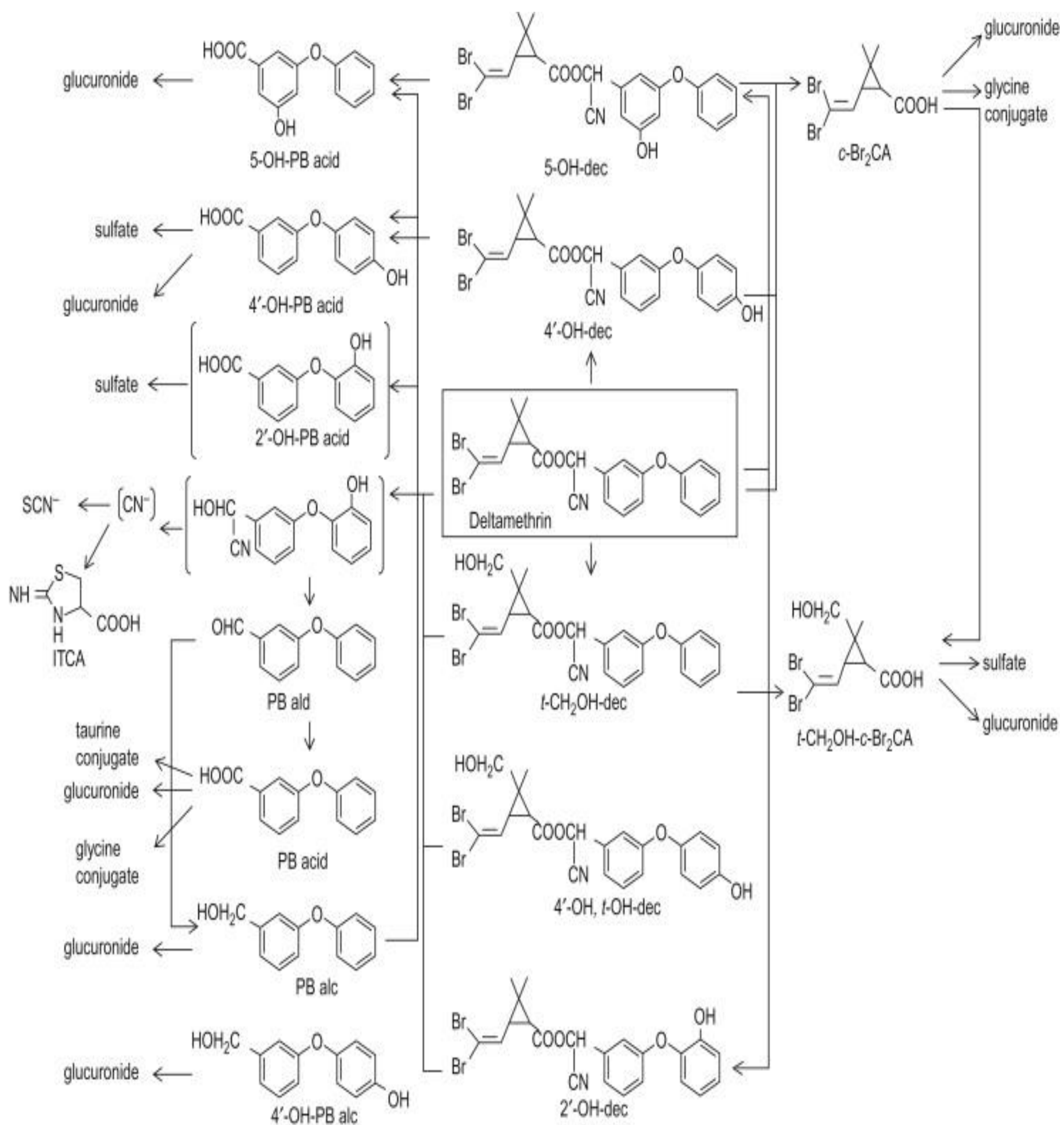


Fig 2.10: Deltamethrin metabolism pathways in different animals (Kaneko, 2010)

Table 2.6: Various applications of Deltamethrin pesticide

Applications of Deltamethrin Pyrethroids	
Insects	Aphids, Beetles, Bollworm, Bud-Worm, Caterpillars, Coding moths, Totrix Moths, Weevils, Whitefly, Winter Moths, Mosquito
Crops	Alfalfa, Beet, Cerels, Coffee, Cotton, Figs, Fruits, Hops, Maize, Oilseed, Rapeseed, Olives, Oil Palms, Potatoes, Rice, Soybeans, Sunflower, Tea, Tobacco, Vegetables
Other locations & applications	Households, forests, animal houses, stored products.

2.10: Amelioration of harmful effect of deltamethrin pesticide

The pyrethroids class II pesticide, deltamethrin causes damage to vital organs of the human beings and other animals. It creates various challenges in animal system. So, to encounter or diminish or reduce the harmful effects of deltamethrin, worldwide several research studies have been conducted and various research studies are under progress. Findings of various research studies showed positive effects and reduced the effects of deltamethrin and improved the status of organs damage, tissue damage and restoration of expression level of biochemical enzymes.

The hepato-protective effects of methanolic seed extracts derived from *Amaranthus spinosus* reported in deltamethrin induced rats. The phenolic and flavonoid constituents of *A. spinosus* seed separates can possibly diminish the liver damage in deltamethrin incited rats and rebuilding of hepatic dimensions of biomarker compounds like, catalase (CAT), superoxide dismutase (SOD), lipid peroxidation (MDA), decreased glutathione synthase (GSH) also glutathione peroxidase (GPx) to ordinary dimensions (Rjeibi *et. al.*, 2016). Olive fruit extract (OFE) and oleuropein (OLE) introduction in rats, enhanced the serum and hepato-renal biomarkers concentration of lipid peroxidation (MDA) in deltamethrin administered rats. OFE and OLE

treated rats also showed the improvement in oxidative status, reducing the inflammation and apoptosis (Maalej *et. al.*, 2017). Turmeric diet has shown protective effects against deltamethrin induced rats, the antioxidant potential of turmeric has reduced the oxidative stress challenges, restoration in expression of antioxidant enzymatic activity and recovered with neural antioxidant damages (Shivanoor *et. al.*, 2016). The leaves extract of *Globularia alypum* have shown protective nephrotoxicity in deltamethrin induced rats. The bioactive compounds present in leaves extract of *G. alypum* improved the renal damages and restored the increased concentration of plasma creatinine, urea and uric acid while reduced the level of elevated MDA (Feriani *et. al.*, 2017).

The citrus natural products inferred flavonoid naringin, improved the harmed memory, uneasiness, locomotor, and emotionality exercises in rodents inebriated to deltamethrin. Deltamethrin introduction prompts decline in the dimension and grouping of acetylcholinesterase, Na+/K+, Ca2+, and Mg2+ ATPase, enzymatic and non-enzymatic cancer prevention agents exercises in cortex and hippocampus locale and upsurge the exercises of TBARS. Flavonoid naringin tweaks the irregularities in oxidative biomarkers, acetylcholine esterase, ATPase, also social and behavioural execution of rats (Mani and Sadiq, 2014). The aqueous concentrate of green tea leaves regulated the neurotoxicity and oxidative harms because of 0.6 mg/kg of deltamethrin treated rats. The raised dimension of MDA, nitric oxide, and DNA discontinuity and articulation dimension of apoptotic qualities was decreased by green tea leaves removes with 25 mg/kg/day dose (Ogaly *et. al.*, 2015). The histopathological and ultrastructural changes in the thyroid brought about by deltamethrin was diminished because of lycopene (Abdul-Hamid and Salah, 2013). The impact of ginger (*Zingiber officinale Roscoe*) extricate on deltamethrin treated testicular harm in albino rats. Rats were directed with deltamethrin at a characterized portion of 1/10th of LD50, 3 dosages week by week for about a month and a half coming about into diminishing in body weight and testicles weights. There was critical improvement noted in sperm cell counts and sperm motility because of ginger concentrate in deltamethrin exposed animal system. Histopathological examines uncovered that declined spermatogenic cells and demolition of Leydig cells were likewise reestablished by diminishing Bax expression in Leydig cells and p53 expression diminished in spermatocytes of testicles. As indicated by these discoveries, it very well may be expressed that, deltamethrin expanded oxidative pressure and activated apoptosis in testicles of pale skinned person rodents while concentrates of ginger decreased oxidative pressure and reestablished the histological changes (Sakr and Al-Amoudi, 2012). The developmental toxicity

of deltamethrin in combination with phenoxybenzoic acid in the larval stage of zebrafish (Kuder and Gundala, 2018). Immunomodulatory specialists mainly antioxidants can support or anticipate the immunological affectability of an individual creature by meddlesome with its administrative components. These might be because of antigens, antigen free and may straight through creation of go between and effector molecules by the particularly immunocompetent cells. In this way, antigen free resistance is along these lines not the same as the one achieved by moderate vaccination process or by uncomplicated inoculation utilizing antibodies (Devasagayam and Sainis, 2002). The essential focus of the immunomodulatory compounds is accepted to be the macrophages, which assume a key job in the age of insusceptible reaction. It is realized that the initiated macrophages show not just expanded phagocytosis and intracellular slaughtering of pathogens by creating effector particles like free radicals and nitric oxide, yet in addition produce cytokines like interleukin (IL)-1, IL-6, IL-12 and tumor necrosis factor (TNF)-α and so on. These cytokines may, thusly, trigger T cells or NK cells for immunomodulation impacts. The immunomodulatory specialists may specifically invigorate either cell interceded invulnerability or humoral resistance by enrolling either TH1 or TH2 kind of cell reaction, correspondingly. It is presently understood that upgrade of TH1 type of T cell reaction might be of helpful centrality for an assortment of intercellular pathogens, similar to protozoan parasites or mycobacteria; while TH2 kind of reaction might be valuable against extracellular pathogens (Knight, 2000; Hajian, 2015; Aslani, and Ghobadi 2016).

2.10.1: Immunomodulation by herbal products

The variety of insusceptible reaction by actualizing remedial plant determined items as a conceivable gainful measure has happened in the antiquated Vedic sacred writing, the Ayurvdea, and has been knowledgeable about Indian customary drug for many centuries. (Agarwal and Singh, 1999). Restorative plants are advanced with constituents that are entranced to empower explicit and non-explicit invulnerable capacities. Particularly the regulation of working of granulocytes, macrophages, normal executioner cells and supplement framework are the key end-purposes of immunomodulation. Agarwal and Singh (1999) have looked into Indian therapeutic plants that have immunomodulatory properties. A few plants are accounted for to have antioxidant fixings which can possibly decrease the immunotoxicity. Therefore, it would be ideal to select a plant with dual activities of immunomodulation and antioxidant response for evaluation of its efficacy to

protect the host from neurotoxicity, nephrotoxicity, lung toxicity, thyroid toxicity and immunotoxicity effects of synthetic pesticides.

Since prehistoric period the plants are the companion of humans and gives proof of various profitable medications for the treatment of an immense scope of diseases. Therefore, the discovery and invention of new effective therapeutic agents is immediately desirable, especially to overcome deltamethrin induced malfunctions in the humans and to be disease free.

There are wide variety of herbal plants listed in various medicinal journal and papers which are known to exert their immuno-modulatory effect in response to various oxidative stress and immune toxicological conditions. *Nigella sativa* L. seeds are known for their immuno-modulatory activity against fungal pathogens and have antioxidant activity as well (Rogozhin, 2010). Similarly, *Syzygium cumini*, commonly recognized as Jamun, is a tropical plant that produces purple coloured ovoid fleshy fruit and has strong antioxidant activity (Benherlal, 2007).

Review of literature reveals that several plants possess immunomodulatory as well as antioxidant properties. A portion of the noticeable precedents are *Acacia catechu, Achillea wilhelmsii, Aconitum heterophyllum, Actinidia eriantha Benth, Actinidia macrosperma, Aesculus indica, Allium sativum, Allium cepa, Aloe vera, Andographis paniculata, Andrographis paniculata, Angelica sinensis, Apocynum cannabinum L., Arnica montena , Asparagus racemosus, Azadirachta indica, Bacopa monerri, Balanite roxburghi, Baliospermum montanum, Berberis aristata, Boerhaavia diffusa, Boswellia carteri, Boswellia serreta, Caesalpinia bonducella, Camellia sinensis L., Centella asiatica, Chelidonium majus L., Citrus aurantium L., Coptis chinensis, Commiphora wightii, Corchorus olitorius, Corydalis turtschaninovii, Curcuma longa, Cynodon dactylon, Dioscorea japonica, Emblica officinalis, Gelsemium elegans, Glycine max, Glycyrrhiza glabra, Gmeline arborea, Helichrysum italicum, Heracleum persicum, Hemidesmus indicus, Hibiscusrosa sinensis, Holarrhena antidysenterica, Humulus lupulus, Jatropha curcas L., Juglans regia, Leonurus japonicas, Lycoris radiate, Mangifera indica,Morus alba Linn., Myrtus communis L., Nigella sativa, Nyctanthes arbortristis, Oldenlandia corymbosa,Panax ginseng, Picrorhiza scrophulariiflora, Picrorhiza kurroa, Piper betel, Piper longum,Plantago asiatica L., Pteridium aquilinum, Randia dumatorum, Schisandra arisanensis , Sphaeranthus indicus, Syzygium cumini, Terminalia arjuna, Tinospora cordifolia, Trigonella*

foenum-graecum, Terminalia chebula, Tinospora cordifolia, Toxicodendron pubescens, Tripterygium wilfordii, Tylophora indica, Viscum collection, Withania somnifera and Zingiber aromaticum and a lot more plants still to be explored. (Kumar *et al*., 2017; Zaid *et al*., 2015; Bhardwaj *et al*., 2013 and 2014; Ghosh *et al*., 2006; Bin-Hafeez *et al*., 2001; Premkumar *et al*., 2006; Agarwal and Singh, 1999; Agarwal and Singh, 1999). Along these lines, investigating a tremendous rundown of immunomodulatory properties of plants two plants have been chosen for variation study action, first one is *N. sativa* and second is *Syzygium cumini*. The seed part obtained from these plants were selected to investigate the immunomodulatory action in *in-vitro* and *in-vivo* state. In-silico study related to bioactive compounds derived from these seed extracts were evaluated for immunomodulation.

2.10.2: *Nigella sativa*

A large amount of plants in the world and their ingredients have the potential to prevent the disease. *Nigella sativa*, commonly identified as black cumin or kalonji is a dicotyledonous plant found in mainly dry atmosphere in the southern Europe, North Africa, and Asia Minor, South and Southwest Asia (Goreja, 2003; BSBI List, 2007). *Nigella sativa* belongs to the botanical family of Ranunculaceae is an annual flowering plant. Linnaeus observed and reported about *Nigella sativa* for the first time reported in the year of 1753 (Jansen *et al.*,1981). Kalonji is an oval shaped seed composed of 3-7 combined follicles has length ranging from 2.5mm to 3.5 mm and width of 1.5mm to 2mm and used for culinary purposes (Amin and Hosseinzadeh, 2016; Al-Sheddi *et al*., 2014). It is documented in Ayurveda that seeds of *N. sativa* (black seed) belongs to Ranunculaceae family, considered as a traditional protective and curative remedy for the treatment of various diseases. *N. sativa* seeds were utilized for seasoning sustenance and different purposes in Egypt (Zohary, and Hopf, 2000). Since, ancient time use of *N. sativa* to cure various diseases in Persia, Middle east, Pakistan, Saudi Arabia and India (Hassanien, *et al*., 2014).

N. sativa seeds used by people for cooking purpose, to cure digestive disorders, recuperate joint aches, obesity, cold, headache, asthma, rheumatic diseases etc in India, Pakistan, Bangladesh, China and Middle East countries (Naz, 2011). *N. sativa* containing bioactive compounds like; nigellone, dithymoquinone, thymoquinone, thymol and thymohydroquinone and have potent as antimicrobial agents (Forouzanfar *et al.,* 2014). These bioactive compounds are

considered as responsible for antioxidant activities, antimicrobial activities and responsible for antitumor as well as anti-cancerous activity (Morsi, 2000; Arora and Kaur, 1999; Halawani, 2009; Salomi *et al.,* 1992; Thabrew *et al.,* 2005; Khan *et al.*, 2016; Manju *et al.*, 2016). Bioactive compounds present in seed extracts of *N. sativa L.* have been reported for antiproliferative and anticancerous effects on various cell lines (Badary and Gamal, 2001; Farah and Begum, 2003; Salim and Fukushima 2003; Gali-Muhtasib *et al.*, 2004; El-Mahdy, *et al.*, 2005; Al-Haj 2010; Al-Sheddi *et al.*, 2014).

In the middle east countries, *N. sativa* is considered as one of the blessed plants by prophet Mohammad. This plant is accepted in society to cure all diseases except death and this concept of plant was popularized by very popular Islamic scholar Al-Bukhari and plant *N. sativa* was adulated for "*Habbat Albarakah*", with the term "*Albarakah*" meaning blessings and society accepted it as "Prophetic medicine" (Salem, 2005; Randhawa, 2008). Ibn Sena (Avicenna) a popular Persian physician and philosopher mentioned *N. sativa* in his medicinal discourse "Canon of Medicine" as energy booster, anti-tiredness, therapy for fever, common colds, pain, toothache, skin diseases, wounds, fungal infections, parasitic infection and in treating bites and stings by venomous animals (Luetjohann, 1998). The seed content of *N. sativa* has been stated to have many healing belongings such as, immunomodulator, bronchodilator, antitumorigenic, antihistaminic, memory enhancer, antidiabetic, antihypertensive, anti-inflammatory, antibacterial, antifungal, anti-neurotoxic, hepatoprotective, gastroprotective and antiapoptotic. These healing properties of *N. sativa* seeds are credited to its quinone, nigellone, thymoquinone, thymohydroquinone, thymol, carvacrol, p-cymene and other chemical constituents (Ghosheh *et al.*, 1999; Anwar, 2005; Gali-Muhtasib, *et al.*, 2006; Jukic *et al.*, 2007; Kanter, 2008; Kanter, 2008; Azzubaidi, 2011; Norsharina, *et al.*, 2011; Alhebshi *et al.*, 2013).

In India, traditionally plant *Nigella sativa* is utilized as culinary purposes, antioxidant, antibacterial, antifungal and anti-proliferative activity. Kalonji enhances an attractive smell to the dishes, and an indication of flavour to the food that you can't resist to eat. In India, dry roasted kalonji is used for flavouring curries, bread, sauces, dal, stir-fried vegetables, salads and even savouries such as samosa, papdis and kachori among others. It is broadly outlined that *N. sativa* seeds conveying abundant measure of dietary advantages like proteins ranges 20% to 27%, sugar might also trade from 23.5% to 33.2%, water content extending from 5.52% to 7.43% and fiery

remains (ash) ranges 3.77 to 4.92%. The seeds have been uncovered to comprise a fixed oil range between 32% to 53%. It has been accounted for that minerals (Calcium, phosphorus, potassium, sodium and iron) content material went from 1.79% to 3.74% and rough fiber content 5.5% around (Al-Jassir, 1992; Al-Ghamdi, 2001; Atta, 2003; Nergiz and Otles, 1993). In table 2.7 taxonomic position of *Nigella sativa* L.and in fig 2.11 different plant parts of *N. sativa* is mentioned.

Table 2.7: Taxonomic position of *Nigella sativa* L. (Adapted from United State Department of Agriculture: Natural Resource Conservation Service)

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Magnoliidae
Order	Ranunculales
Family	Ranunculaceae
Genus	Nigella L.
Species	*Nigella sativa L.*

Fig 2.11: Various parts of *Nigella sativa* plant, Flower, Drying funnel and Seeds

2.10.3: Bioactive compounds present in seeds of *Nigella sativa*

A great number of uses of *Nigella sativa* hope to scientists to analyze the components of *N. sativa* by HPLC, NMR and GCMS analysis (Bourgou *et al*., 2012). Thymoquinone is a constituent of *N. sativa* extract, has potential to prevent the microbial, oxidant as well as proliferative activity. In in vivo condition α-hederin is another ingredient of *N. sativa* prevent the proliferative properties of cancerous cells (Huat and Swamy, 2003). In numerous, in vitro studies reported that many experiments conducted on cell lines of extract of *Nigella sativa* shows prevention on oxidant as well as on the cancerous activity (Randhawa and Alghamadi,2002).

Table 2.8: List of bioactive compounds reported in seed extract of *N. sativa* using GC-MS and other techniques (Joulain and Konig, 1998; Adams, 2007; Venkatachallam *et al*., 2010).

Tricyclene	Borneol	α-Copaene
Camphene	Pinocarvone	α-Longifolene
β-Pinene	Dihydrocarvone	Aromadendrene
Sabinene	Ocimenone (E)	Thymohydroquinone
β-Myrcene	Thymoquinone	Davanone
1,8-Cineole	Thymol	Palmitic acid
α-Terpinene	Carvacrol	Octadecanoic acid
Limonene	2-Undecanone	Quinones
γ-Terpinene	α-Longipinene	Diterpenes
cis-Sabinene hydrate	Citronellyl acetate	Alkane
Linalool	Cyclosativene	Alkenes
Terpinolene	α-Longicyclene	Fatty acids
Citronellyl acetate	Pimaradiene	Fatty acid esters
α-Thujene	α-Pinene	t-Anethole
Menthatriene	8-Heptadecene	Sesquiterpene

2.10.4: Structure of some bioactive compounds present in seeds of *N. sativa*

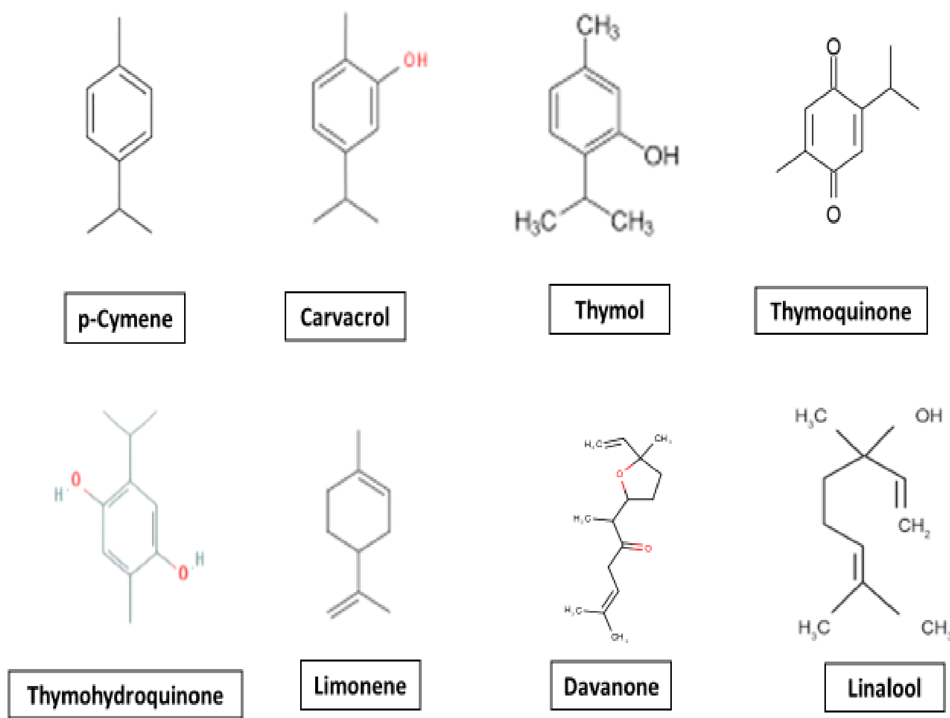


Fig 2.12: Structure of selected compounds present in *Nigella sativa* extracts

Previous literature has shown the antimicrobial nature of *N. sativa* seeds on diverse pathogenic and non-pathogenic microbes. The essential oil obtained from the seeds of *N. sativa* has been also reported anti-bacterial effects on a group of gram-positive bacteria like, *S. aureus* and other group of gram-negative bacteria like, *E. coli* (Ahmad *et al.,* 2013). *Nigella sativa* contains crude extracts or active phytochemicals known as quinones, thymoquinone and dithymoquinone and cause hepatoprotectivity (Daba and Abd El-Rehman, 1998; Nagi *et al*., 1999). The phytoconstituents got from *N. sativa* have shown the upliftment of invulnerability in vertebrates and furthermore demonstrates against harmful properties (Haq *et al*., 1999; El-Dakhakhny *et al*., 2002; El- Aziz *et al*., 2005). Black seeds contain in excess of a hundred extremely treasured parts and roughly 30% w/w of a fixed oil and 0.4-0.45% w/w of an unstable oil according to previous research studies (El- Tahir *et al*., 1993). The fixed oil essentially incorporates of unsaturated fats in its chemical constituents (Houghton *et al*., 1995).

Nigella sativa is a pool of revelation of antimicrobial exercise (El-Fatatry *et al*., 1975), antibacterial towards gram positive as properly as gram negative (El-Kamali *et al*., 1998), antioxidant as cell reinforcement (Burits and Bucar, 2000; Al-Enazi, 2007), vindicating anti-inflammatory activity (Houghton *et al*., 1995), antitumoral and anti-cancerous (Worthen *et al*., 1998), antihypertensive achievement (El-Tahir *et al*., 1993) and hypoglycemic activity (Al-Hader *et al*., 1993). Many researches throughout the world show that *N. sativa* threatening the microbial organism up to a great limit. It shows that the different extract of *N. sativa* prohibited the bacterial like gram+ and gram –nature, fungi as well as pathogenic yeast.

There are so many reports that reveal the presences of pharmacologically active components in the N. sativa are mainly nigellone, dithymoquinone, thymoquinone, thymol and thymohydroquinone. *Nigella sativa* seed composition includes protein 20-23%, carbohydrate 23.5 to 32.3%, moisture content 5.52 to 7.43% and ash 3.77 to 4.92 (Forouzanfar *et al*., 2014). Other important compounds obtained when essential oil derived from *Nigella sativa* seeds were analysed by GC/MS includes *p*-cymene, carvacrol 4-terpineol, tanethol, α-pinene and longifolene have been also reported. The seeds of N. sativa are enriched with unsaturated fatty acids that consist of linolenic acid as most important fatty acid, oleic acid, dihomolinoleic acid and eicodadienoic acid, whilst the fundamental parts of saturated fatty acids are palmitic and stearic acid. The essential sterol existing in the seeds is α-sitosterol which ranges between 44% and 54% of the whole sterols (Ramadan and Morsel, 2002; AlHaj *et al*., 2010; Khader and Eckl 2014).

The bioactive ingredients of seeds of *Nigella sativa* have been described to beneficial against various kinds of cancer around the world, like extracts of *Nigella sativa* shown inhibitory effects on chemical carcinogenesis skin cancer (Salomi *et al*., 1989; Salomi *et al*., 1991; Salomi *et al*., 1992). One of the bioactive compound α-hederin present in seeds of *Nigella sativa* has been reported showing cytotoxic and immunopotenciating effects, antitumor activity in Lewis lung carcinoma in BDF1 mice (Swamy and Tan, 2000; Huat and Swamy, 2003), effects of volatile oil derived from *N. sativa* shown anticancerous effects on colon cancer in rat (Salim and Fukushima, 2003), thymoquinone has potential to induce the apoptosis in myeloblastic leukemia a type of blood cancer (El-Mahdy *et al*., 2005), in human hepatoma HepG2 cells *N. sativa* shown cytotoxic effects (Thabrew *et al*., 2005). The seed extracts and bioactive compounds of *N. sativa* protects

the neurodegeneration in toluene exposed rats especially in frontal cortex and brain stem injury (Kanter, 2008; Kanter 2008).

2.10.5: *Syzygium cumini* L.

Syzygium cumini, generally known as Jamun, has a place with family Myrtaceae is a tropical perineal tree that yields purple ovoid meaty fruit. Its seed has customarily been utilized in India for the treatment of diabetes. Considering the accessible ethno-pharmacological information, further examinations were stretched out to comprehend the synthetic organization and antioxidant for cell reinforcement exercises of three anatomically unmistakable parts of fruit: the mash, kernel, seed and seed coat (Bitencourt, *et al*., 2015). *S. cumini* has been accounted for to have acetyl oleanolic acid, triterpenoids, ellagic acid, isoquercetin, quercetin, kaempferol, suzyginin A, suzyginin B and myricetin in more than a few focuses relying upon the season and atmosphere. The majority of these mixed compounds have been accounted for having antioxidant for cell reinforcement and free radical hunting exercises (Jagetia and Baliga, 2002; Tanaka, 1996; Korina and Afana'ev,1997).

Table 2.9: Taxonomic position of *Syzygium cumini* L. (Adapted from United State Department of Agriculture: Natural Resource Conservation Service)

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Myrtales
Family	Myrtaceae
Genus	Syzygium L.
Species	*Syzygium cumini L.*

Fig 2.13: Various parts of *Syzygium cumini* plant, Flower, Fruits and Seeds

The notable work of *Syzgium cumini* as customary helpful medication reflects its pharmacological importance. The eatable fleshy mash of plant frames 3/4th of the entire fruit. Different mineral and nutrients are discovered, for example, Ca, Cl, Cu, Fe, K, Mg, Na, P, S, vitamin A, vitamin C, riboflavin, nicotinic acid, choline, and folic acid. Principally glucose and fructose are the main sugars present in fruit i.e. ready natural product with low measure of sucrose. Naturally, jamun fruit contains significantly maleic acid (0.59% of the fruit) and little amount of oxalic acid (Ivan, 2006). Tannins predominantly include gallic acid demonstrates the astringent idea of the jamun fruit (Evan and Trease, 2007). Tannins are likewise capable for gastro-defensive and anti-ulcerogenic action (Kokate *et al*., 2008). The purple shade of the fruit is because of quality of a couple cyanidin diglycosides accessibility.

It has been accounted for that ethanolic concentrate of *Syzygium cumini* bark mitigating action against histamine, serotonin and prostaglandin (Pandey and Khan, 2002). Watery concentrate of seed indicates stamped increment in complete hemoglobin, support up body weight and drop down free radical development in tissue (Prince *et al*., 1998). Basic oil constituents in the leaves of *Syzygium cumini* demonstrated better antibacterial action (Shafi *et al*., 2002). Leaf extricate indicated action against *Escherichia coli* and *Staphylococcus aureus*.

It has been additionally recorded that ethanolic concentrate of *Syzygium cumini* seed part let down the oxidative pressure prompted pathogenesis and movement of diabetic tissue dysfunctions. This movement was affirmed with an expanded dimensions of plasma glucose, nutrient E content, ceruloplasmin, lipid peroxidation rates while diminished dimensions of nutrient C and glutathione in diabetic rodents, however *S. cumini* seed part remove recuperated parameters back to the normal dimensions (Ravi *et al*.,2004).

The utilization of plant and regular items might be beneficial derived from *Syzygium cumini* L. seeds is a, medium to long tree, and it has been attributed to have a few therapeutic properties incited in the old literature about traditional medicinal practices (Warrier, 1995). The products derived from various parts of *S. cumini* are utilized to cure a polygenic disorder diabetes, throat infections, splenopathy, urethrorrhea, eczema and ringworm infection. The leaves of *S. cumini* are having antibiotic properties and utilized to reinforce the teeth and gums. The leaves extract of *S. cumini* have been broadly used to cure diabetes, stoppage constipation, removes blockage in blood vessels (Bhandary, 1995) leucorrhoea, stomachache, hyperthermia, gastropathy, strangury, dermopathy and to avoid blood discharge in the fecal matter (Rastogi *et al*., 1990). A hypoglycemic impact of *S. cumini* leaves and antipyretic, antioxidant as cancer prevention agent exercises for jamun seed have additionally been accounted for (Teixeira, et.al., 1992; Arun, 2011; Chakraborty *et al*., 1985; Ravi, *et al*., 2004; Banerjee, *et al*., 2005).

2.10.6: Bioactive compounds present in seeds of *Syzygium cumini*

The seed extracts of *S. cumini L.* contains so many bioactive compounds like; quercetin, isoquercitin, myricetin, ellagic acid, gallic acid, ferulic acid, kaempferol etc. These bioactive compounds are mainly responsible for antioxidant, antimicrobial, antidiabetic, anti-inflammatory and anti-cancerous activities (Tanaka *et al*., 1996). Due to presence of tannin, steroids, flavanoids, alkaloids and saponin, so it can be used to treat many infectious diseases and many other uses. GCMS analysis revealed presence of many organic compounds in seed extracts of *S. cumini* some of them are mentioned below in table 2.10.

Table 2.10: Bioactive compounds reported in seed extract of *S. cumini* using GC-MS and other techniqes (Ayyanar and Subhash babu, 2012; Neha and Vibha, 2013; AH *et al*., 2019)

Tetradecanoic acid	Cadinene	Oleic acid
α-Caryophyllene	Oxirane	n-hexadecanoic acid
Decandiol	Nondecanoic acid	Cyclooctasiloxane
Bicyclo	Caryophyllene oxide	Octadecanal
Octadienol	Isogeranio	Hexadecamethyl
Pinene	Camphene	Myrcene
Limonene	cis-Ocimene	Copaene
Terpineol	Cadinene	Humulene
Geranyl butyrate	Dihydrocarvyl acetate	Caryophyllene
Lauric acid	Myristic acid	Palmitic acid
Stearic acid	Oleic acid	Linoleic acid
Malvalic acid	Sterculic acid	Vernolic acid

Neha and Vibha reported 34 compounds in methanolic extract and 25 chemically active compounds in ethanolic extract derived from seed of *S. cumini* during their GC-MS analysis. Presence of compounds in extracts also differ due to different solvents used for extraction method, like wise methanolic extract shows presence of different types of compounds than ethanolic extract or aqueous extract or hexane or petroleum ether derived extracts.

2.10.7: Structure of important bioactive compounds present in seeds of *S. cumini*

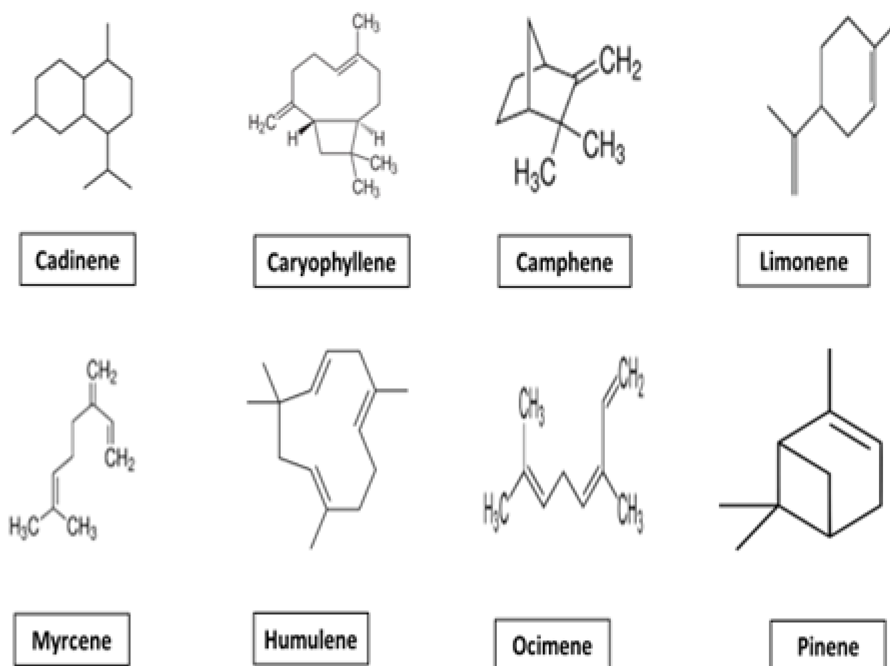


Fig 2.14: Structure of selected compounds present in *Nigella sativa* extracts

2.11: ROS generation mechanism in biological systems

Reactive species encompass elements that do not inevitably have unpaired electrons, but these elements are in fact sensitive because of unsteadiness and highly unstable in nature (Halliwell and Gutteridge, 1999). The atoms, molecules, or ions which bear with unpaired electrons are highly reactive to their targets are considered as free radicals (Lushchak, 2011). The free radicals are mainly classified in two categories, first oxygen derived free radicals and second is nitrogen derived free radicals, these two categories of free radicals target largely the several physiological paths and cause generation of unwanted metabolites which cause damage to cell and ultimately leads to death of cell (Halliwell and Gutteridge, 1999). In all living organisms, there are many types of oxygen derivatives like peroxides and transition metals. About 5% of ROS produced by electron transport chain process in aerobic organisms, the molecular oxygen (O_2) is reduced to water (H_2O) throughout oxidative phosphorylation and cellular respiration, during this highly reactive species, partly reduced metabolites like, radical of superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), and the hydroxyl radical (HO^*) (Pereira, *et al*., 2012; Kelly *et al*., 1998). All these

oxygen derivatives cause degeneration effect towards living cell and the consequence of such oxidizing metabolites resulting into illness of healthy individual (El-Bahr, 2013). The penetrability of molecular oxygen into electron transport chain in mitochondria represents energy generation into cells. The electron transport chain along with oxidative phosphorylation resulted to high energy phosphate bond adenosine triphosphate or ATP. As a result of metabolism of oxygen compounds superoxide radical anion, hydrogen peroxide which are free radicals produced (Devasagayam *et. al*. 2004). Nowadays, Reactive Oxygen Species is most interesting field of research in life sciences and especially due to pesticides exposure. ROS play very critical role in damaging the pulmonary tissues due to pesticide exposure and developing symptoms of hypoxemia, hemorrhage, edema and penetration of inflammatory cells. ROS establishes oxidative stress leads to generate toxicological impacts in cells, tissues and organs which may be lethal for organisms (Bagchi and Bagchi, 1995). Several investigators have provided details regarding the unthinking characters of oxidative hindrance and cell reactions in different natural frameworks because of introduction of manufactured synthetics particularly pesticides. Oxidative stress happens after the degree of reactive species assembly surpasses the degree of reactive species disintegration by antioxidant systems present in living organisms, once the level of reactive species goes beyond the limit of oxidation started damaging and destroying the various cellular compartments and triggering the death of cell (Almeida *et al*., 2005). Pesticides mainly cause the production of ROS in aerobic organisms. There are numerous biologically active enzymes available in biological systems which contribute their role in generation of ROS in various metabolic pathways, thus establishing an extra basis of ROS production in cells. Endogenous antioxidant systems available in cells recruited to encounter and nullify the effects of ROS but when the level of ROS turn out to be higher than the rate of available antioxidant defense mechanism then oxidative stress is generated and leading the injury of cells, tissues, swelling and inflammation, lethal diseases development, tumorigenic and aging impacts in organisms (Wallace, 1997; Valavanidis, *et al*., 2006). Endogenous defense antioxidant enzymes mainly include, superoxide dismutase (SOD), catalase and peroxidases which play major role to encounter and nullify against various ROS. SOD encounter with super oxide ions $O^{\bullet -}_2$ while catalase and peroxidases encounter with H_2O_2. SOD is a metalloenzyme present in prokaryotic cell as well as in eukaryotic cell and play role as first line of antioxidant defense system. Roots of free radical generation can be categorized in two factors, first is endogenous factor which includes mainly high

concentration of O_2, mitochondrial damage and leakage, swelling and inflammation of cells and tissues, higher rate of respiration while the second factor is exogenous which include mainly, pollution, pesticides, radiation, deprived nutrition, lifestyle, psychological stress conditions etc.(Poljsak and Milisav., 2012).

HYPOTHESIS AND OBJETIVES

3.1: HYPOTHESIS

The inclusive goal of this present research study was to evaluate the hypothesis that deltamethrin causes the toxicological impacts in *in-vitro* and *in-vivo* model system. In *in-vitro* model system cytotoxicity effects of deltamethrin was evaluated alone and with phytochemical extracts derived from seeds of *Nigella sativa* and *Syzygium cumini*. Phytochemical constituents present in the extracts derived from seeds of *Nigella sativa* and *Syzygium cumini* was tested alone as well as in combination. The extracts were also evaluated for primary biochemical analysis for constituents available in it, antioxidant analysis, antibacterial and antifungal analysis. In *in-vivo* model system, female swiss mice were taken to test the effects of deltamethrin to analyze the nephrotoxicity, hepatotoxicity, lung toxicity, splenic toxicity, cardiac toxicity and immunotoxicity. For the evaluation of protective and immunomodulatory effects of seed extracts of *Nigella sativa* and *Syzygium cumini* were used in deltamethrin induced mice model.

So, considering all above evidences in the cognizance, following hypotheses had been outlined to assessment are

1. The phytoconstituents present in seeds extracts of *Nigella sativa* and *Syzygium cumini* encompasses flavonoids, alkaloids, saponins, tannins and other bioactive compounds which play vital role in antioxidant activity, cytotoxicity, antibacterial activity, antifungal activity, and immunomodulatory activity.
2. Deltamethrin, a class II synthetic pyrethroid pesticide causes production of reactive oxygen species and leads to oxidative stress condition in *in-vitro* and *in-vivo* state which leads to impairment of cells and tissues of vital organs, respectively.
3. Deltamethrin exposure with defined dose and period in animals causes nephrotoxicity, hepatotoxicity, lung toxicity, splenic toxicity, cardiac toxicity and immunotoxicity.

4. Bioactive compounds present in seed extracts of *Nigella sativa* and *Syzyium cumini* have protective effects and immunomodulatory effects in deltamethrin induced cell lines and mice model system.

3.2: OBJECTIVES

To test the above stated hypotheses following objectives were finalized to find out relevancy of the hypotheses in current research study

1. To investigate the biochemical composition, antioxidant properties, antibacterial, and antifungal activities of seed extracts of *Nigella sativa* and *Syzygium cumini.*
2. To investigate the cytotoxic effects of deltamethrin and seed extracts of *Nigella sativa* and *Syzygium cumini* in *in-vitro* state.
3. To investigate the biochemical properties, immunotoxicity and immunomodulatory effects of deltamethrin and seed extracts of *Nigella sativa* and *Syzygium cumini* in Swiss albino mice.
4. To study the histopathological deterioration due to exposure of deltamethrin and protective effects of seed extracts of *Nigella sativa* and *Syzygium cumini* in Swiss albino mice.
5. To study the immunomodulatory effects of deltamethrin and bioactive compounds of *Nigella sativa* and *Syzygium cumini* with MHC I and MHCII molecule of human and mice origin using bioinformatics tools.

MATERIALS AND METHODS

4.1: Procurement of *Nigella sativa* and *Syzygium cumini* seeds

Seeds were obtained from ayurvedic store in a local market of Phagwara, Kapurthala, Punjab, India and identified by taxonomist available in Department of Botany, School of Bioengineering and Biosciences, Lovely Professional University, Punjab, India. Once it was confirmed and assured the seeds obtained belongs to *Nigella sativa* and *Syzygium cumini* then only it was used for further processing and analysis.

4.2: Phytochemical extracts from seeds of *Nigella sativa* and *Syzygium cumini*

30gm of *Nigella sativa* seeds and *Syzygium cumini* were air dried to avoid excess moisture content in seeds then seeds were coarsely crushed by using grinder and packed into thimble and extraction was done in 500ml of double distilled water, acetone, ethanol, and methanol, respectively. Their extraction was carried out in two ways the first method was used extraction by using Soxhlet apparatus using 15 cycle for 2 hours approximately and in second method seeds were crushed in mortar-pestle and powder was soaked in different solvent for 18-24 hours. The extracts were concentrated under vacuum drier and stored in the refrigerator and used for phytochemical analysis, antioxidant analysis, antibacterial activity, antifungal activity and cytotoxicity effects on cell lines.

4.3: Experimental design for Phytochemical studies

Phytochemical extracts derived from seed extracts of *Syzygium cumini* and *Nigella sativa* were isolated by polar and nonpolar solvent extraction pattern, the solvents used for extraction were decided by looking to earlier research studies. The solvents (Ethyl Alcohol, Methyl Alcohol, Acetone, and Double Distilled water) were used for extraction, all used solvents were of technical grade to avoid impurities in separated extracts. Then followed by antioxidant properties,

antibacterial, antifungal effects, and qualitative biochemical analysis for presence and absence of bioactive compounds of seed extracts of *Syzygium cumini* and *Nigella sativa* were performed.

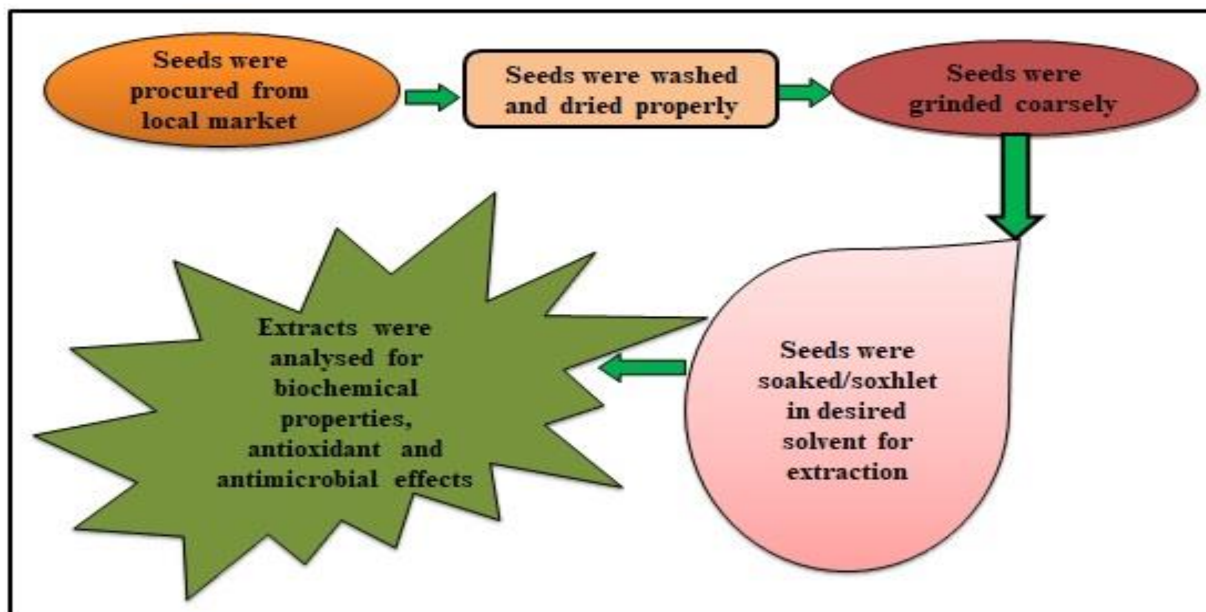


Fig 4.1: Experimental design flow for phytochemical evaluation of extracts derived from seeds of *S. cumini* and *N. sativa*

4.4: BIOCHEMICAL ANALYSIS OF SEED EXTRACTS

The phytochemical tests were performed using various reagents of phytochemical tests which determines the presence or absence of alkaloids, flavonoids, saponins, sugars, steroids, glycosides, and tannins.

4.4.1: Test for Flavonoids:

10% aqueous sodium hydroxide is added in 2 ml of the extract. It is heated to get warm which produces a yellow colouration followed by addition of 3-4 drops of dilute hydrochloric acid (HCl). The colour change from yellow to a colourless denotes flavonoid is present.

4.4.2: Test for Steroids:

a. Liberman Burchard's test:

Plant extracts were inoculated with acetic anhydride (2-3 ml) and a small amount of glacial acetic acid followed by addition of concentrated. After addition of few drops of sulphuric acid (H_2SO_4) and noticed appearance of bluish-green colour that implied the occurrence of steroids.

b. Salwoski's test:

When two to three drops of concentrated sulfuric acid (H_2SO_4) was supplemented to the test tube containing extracts, then formation of a layer at the bottom of tube appeared. At the inter phase, reddish-brown colour develops and it shows the attendance of steroidal ring.

4.4.3: Assessment intended for Alkaloids:

In 10ml of the solvent extract was mixed with five (5) ml of 1.5% v/v hydrochloric acid (HCl). Then it was filtered. This remainder can be further used for testing the occurrence of alkaloids.

Dragendorff's reagent assessment for alkaloids:

Few drops of Dragendorff's reagent were supplemented in 1ml of filtrate. It leads to form an orange-brown colour precipitate that indicates occurrence of alkaloids.

Mayer's reagent assessment for alkaloids:

Addition of few drops of Mayer's reagent to a 1ml of filtered extract in a test tube gives formation of a creamy colour precipitate which determines the occurrence of alkaloids.

4.4.4: Assessment for Tannin:

To 5 ml of filtrate extract, 1ml of 5% ferric chloride ($FeCl_2$) solution is added. It was allowed to react for few seconds. Then a dark green colour was obtained which indicates occurrence of tannin.

4.4.5: Assessment for Saponins:

a) **Assessment of foam**: 1ml solution of the extract was diluted using distilled water 20 ml, the sample was vigorously shaken in the test tube for 15 minutes, the development of stable foam was observed, this concludes the existence of saponins in extracts.

b) **Haemolysis test for saponins**: To the 2 ml of extract sample, addition of 2 ml of aqueous NaCl solution was carried out in a test tube. Then inoculation of three drops of blood to the tube via syringe and smoothly mixed without shaking by inverting the tube and it was then placed aside undisturbed for 15 min. The accumulation of the red blood corpuscles in the bottom of tube indicates the existence of saponins.

4.4.6: Test for Phenolic Compounds

The phenolic compounds were estimated by using following standard biochemical protocols.

a) Using $FeCl_3$:

Addition of seed extracts in solution of ferric chloride (neutral) and observed violet colour formation, indicated the phenolic compounds are present.

b) Using 10% NaCl:

The seed extracts were added into 10% sodium chloride solution and found cream colour which indicated that presence of phenolic compound.

4.4.7: Test for carbohydrates

a) Molisch's test: To 2ml of extract test sample, 4-7 drops of Molisch's reagent is added. Then test tube is kept in slant position and a small amount of concentrated H_2SO_4 acid is added slowly at downwards without shaking. A purple colour ring appeared between sulphuric acid and test layer which a positive result.

b) Barfoed's test: Two ml of seed extract in a test tube taken then 3 ml of Barfoed's reagent (a solution containing mixture of cupric acetate and acetic acid) is supplemented in it. After this solution of seed extract and Barfoed's reagent is boiled; the brick red precipitate is observed, this indicates the presence of monosaccharides or disaccharides.

4.4.8: Reducing sugar

Benedict's test:

After addition of 5ml of Benedict's reagent to 10ml of extract, the mixture is boiled for 2 minutes and formation of a brick red coloured precipitate indicates the presence of carbohydrates.

Fehling's test:

1 ml mixture of equal parts of Fehling's solution first and Fehling's solution second is supplemented to 10 ml of seed extract. Then it is boiled for few minutes, the brick red colour precipitate is found to form indicating the existence of reducing carbohydrates in seed extracts.

4.4.9: Test for Glycosides

Molisch's test:

In 2ml of seed extract and mixed with 2-3 drops of freshly prepared Molisch's reagent. Followed by addition of 2ml of concentrated sulfuric acid carefully through the inner side wall of the test tube. A reddish-violet circle presence designates the occurrence of glycosides, but it is not formed in the test sample, so, the result is negative.

4.5: Free radical scavenging assay

4.5.1: By Using DPPH:

The inhibitor action of the plant seed extracts was scrutinized which support the scavenging impact on the firm DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical activity. A methanolic solution of DPPH (0.1 mM) 10 ml was ready. Five check samples with fluctuating extract concentration of 25 mg, 50 mg, 75 mg, 100 mg, and 150 mg were added and analysed for antioxidant potential. All the samples were made up to 2000µl by including 1000µl of 0.1mM DPPH resolution and distilled water wherever required. The reaction combination was jolted smartly and incubated at room temperature for 30 min. The absorbance of samples was evaluated spectrophotometrically at specific wavelength of 517nm. A blank solution containing the indistinguishable quantity of methyl alcohol (CH_3OH) and DPPH was conjointly prepared (Fontana *et al*., 2001; Thaipong *et*

al., 2006; Hazra *et al*., 2008). The novel scavenging actions of the tested samples containing seed extracts articulated as a proportion of inhibition due to absorbance of light were estimated in line with the subsequent equivalence as below-

Percent (%) inhibition of DPPH activity = $[(A_B - A_A) / A_B] \times 100$

Where A_A and A_B are the absorbance standards of the trial samples and of the blank sample, correspondingly.

A percentage inhibition contrasted with volume curve was designed to evaluate the efficiency of plant seed extracts.

Different dilutions of the essential oil and its active remain components were mixed with 1 mL of 0.1mM of DPPH solution and concluding volume finished up to 2 ml.

Mixed solution was vigorously shaken and incubated at 37^0C for 30 minutes. Absorbance of the seed extracts samples was recited at specific wavelength of 517 nm with UV spectrophotometer.

4.5.2: Superoxide Anion Scavenging Evaluation:

The non-enzymatic phenazine methosulfate-nicotinamide purine dinucleotide (PMS/NADH) system generates superoxide radicals, that cause the reduction of nitro blue tetrazolium (NBT) to purple formazan. The one mil volume of final reaction mixture contained phosphate buffer (0.1 mM, pH 7.8), NADH (3 mM), NBT (1mM), PMS (0.3 mM) and various concentrations of extracts (25, 50, 75, 100, and150 mg) of sample determination. Once incubation for five min at temperature is over, then the absorbance at 560 nm monochromatic wavelength exploitation UV-Vis photometer was measured against the associate blank solution answer to figure out the number of formazans generated. All tests were performed thrice (Fontana *et al*., 2001; Hazra *et al*., 2008).

- Superoxide anions were generated in test samples that contained 100 µl each of, 1.0mM NBT, 3.0mM NADH and 0.3mM PMS then the concluding volume was maintained to 1ml by 0.1 M of phosphate buffer solution (pH 7.8) at room temperature.
- The reaction mixture containing NBT and NADH with or without extract was incubated at room temperature 25^0 C intended for 2 minutes.

- The chemical reaction was initiated by addition of PMS. Then absorbance was recorded at 560 nm wavelength by spectrophotometer of test samples and compared with blank sample after 10 min of incubation time.

The absorbance reflected in percent inhibition and evaluated by means of the subsequent calculation.

Percent (%) superoxide scavenging activity =100 ($A_{Control}$-A_{Sample}/$A_{Control}$)

4.5.3: Ferrous Ion Chelating Activity:

The iron (ferrous) particle chelating program was evaluated by a standard technique, the response was done in HEPES buffer solution (20 mM, pH 7.4). Quickly, different fixations (25 mg, 50 mg, 75 mg, 100 mg and 150 mg) of seed extracts were supplemented to 12.5 µM ferrous sulfate solution and the response stood in progress by the addition of 100 µl of ferrozine solution (5 mM). The blend was shaken vivaciously then incubated for duration of 20 min at 25^0C room temperature, at that point the absorbance has been estimated at 562 nm wavelength by spectrophotometer. All trial samples were repeated for absorbance estimation thrice. EDTA solution remained as a positive control.

- The chemical reaction mixture in seed extracts was initiated by addition of 100 µl FeCl2 solution (0.6mM in distilled water) then maintained with 900µl of methanol.
- The controls have all the reaction substances excluding the extracts or EDTA. The mixtures were shaken cautiously then incubated at ambient temperature for 5 min.
- 100 µl of freshly prepared ferrozine (5mM in methanol) has been supplemented to the mixture and was shaken again, then kept it for the additional reaction to be carried out at room temperature used for 10 min to generate complex of Fe^{2+} -ferrozine.
- The absorbance of the Fe^{2+}–ferrozine complex was estimated at 562 nm and compared with a methanol blank solution. The effect of iron chelation was evaluated as a percentage of absorbance in chelating activity, by means of the calculation as below-

Percent (%) chelating activity = 100($A_{Control}$ – A_{Sample}) / $A_{Control}$

4.6: ANTIMICROBIAL ASSAY

4.6.1: Antibacterial assay (Ncube *et al*., 2008)

The antibacterial screening was performed by disc (Whatman filter paper) diffusion method. About 20 ml of Mueller Hinton agar is poured into a petri dish. After it is solidified, the bacterial strain was swabbed upon the agar plate. The sterile discs were impregnated with silver nanoparticle solution and a positive control standard antibiotic and were placed inverted on the swabbed plate. The antimicrobial activity is determined after 24 hours of incubation at 37°C. The diameters of zone of inhibition produced by the discs are then compared with the standard antibiotics (Chloramphenicol and Gentamycin).

- Medium was properly autoclaved and sterilized then poured in to Petriplate
- After solidifying media bacterial population was inoculated and kept for incubation at 37^0C for 24 hours
- The diameters of zone of inhibition produced by the discs are then compared with the standard antibiotics.

Test organisms: *Bacillus subtillus* (MTCC 121), *Salmonella typhimurium* (MTCC 3231), *Bacillus subtillus* (MTCC 441), *Salmonella enteric* (MTCC 1164), *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 1305)*, Staphylococcus aureus* (MTCC 7443), Escherichia coli (MTCC 40), *Bacillus cereus* (MTCC430), *Bacillus cereus* (MTCC2086). These microorganisms were procured from Institute of Microbial Technology (IMTECH) Chandigarh, India.

4.6.2: Antifungal assay

Phytochemical extracts were analyzed for antifungal assay against four different strains of fungi obtained from microbial repository of Institute of Microbial Technology (IMTECH), Chandigarh, India. *Saccharomyces cerevisiae* (MTCC 464), *Candida albicans* (MTCC 183), *Aspergillus niger* (MTCC 281) and *Aspergillus parasiticus* (MTCC 696) were tested against phytochemical extracts. Disc diffusion method was used, and potato dextrose agar (PDA) media was used for fungal growth. 15 µl/disc at the concentration of 10mg/ml, 20mg/ml and 30 mg/ml were dissolved in 1 ml of DMSO. Petri plates were incubated at 25-28^0 C for 24 hours then width of zone of inhibition was quantified in milli meter (mm).

- PDA media was autoclaved and sterilized properly
- Media was poured in Petriplate, after solidification of media fungal cells were inoculated
- Phytochemical extracts and antibiotics were kept in Petriplate for diffusion
- After incubation for 24 hours at 25-28^{0}C, zone of inhibition was observed and measured

4.7: In in-vitro cytotoxicity and anti-cancerous analysis

The cytotoxicity analysis of deltamethrin (DM), ethanolic and methanolic seed extracts of *Syzygium cumini* and *Nigella sativa* on SW480, A549 and HeLa cell lines-experimental design. Anti-cancerous and anti-toxic effects of extracts were also evaluated. The design of in-vitro experiments is mentioned in table 4.1.

Table 4.1: Experimental design of cytotoxicity and protective effects of extracts

S. No.	Group (48 hr)	Concentration
1	Control (Vinblastin)	1μM
2	DM	100 μg/ml
3	DM	200 μg/ml
4	NSM	200 μg/ml
5	NSE	200 μg/ml
6	SCM	200 μg/ml
7	SCE	200 μg/ml
8	DM + NSM	100 μg/ml + 200 μg/ml
9	DM + NSE	100 μg/ml + 200 μg/ml
10	DM + SCM	100 μg/ml + 200 μg/ml
11	DM + SCE	100 μg/ml + 200 μg/ml
12	DM + NSE + SCE	100 μg/ml + 50 μg/ml + 50 μg/ml
13	DM + SCM + NSM	100 μg/ml + 50 μg/ml + 50 μg/ml

4.8: Procurement of mice for *in-vivo* studies

The experimental procedures were permitted and approved by Institutional Animal Ethics Committee, Lovely Professional University, Phagwara, Punjab, India. Thirty-six Swiss albino female mice (8-10-week age) were purchased from and transported from Central Animal Facility, National Institute of Pharmaceutical Education and Research (NIPER), S.A.S. NAGAR to Lovely Professional University. These mice were kept at Animal House Facility, Lovely Institute of Technology (Pharmacy), Lovely Professional University, Phagwara, Punjab for four weeks for acclimatization to prevent transportation stress, promote environmental adaptation and friendly handling before initiation of dosing and other experimental approaches. Mice were housed in polycarbonate laboratory animal cages with room temperature of around 22-24°C, relative humidity maintained in the range of 50±10 % and 12:12 hour light and dark cycle was followed throughout the experimental procedure. Light - dark cycle (photoperiod) was controlled between 8 A.M. to 8 P.M. with hygienic and sanitary conditions adequately maintained as per guidelines. The mice were provided feed in pellet form supplied by Godrej Agrovet Limited, Khanna, Punjab, India and drinking water *ad libitum*. After completion of 10 days or 20 days period of dosing, the mice were then sacrificed with overdose (40mg/kg) of anesthesia sodium pentabarbiturate, subsequently blood sample was collected via cardiac puncture, blood was kept in EDTA treated vials and then carried out centrifugation at 2500 rpm for 10 min and serum was separated out for heamatological parameters analysis. Immediately the liver, kidney, spleen, lungs and heart were dissected, collected and then washed with ice-cold saline. Organs were kept in 10% saline and stored at 4°C, further tissue homogenate (10%) in 0.15 M KCl was prepared at 4°C by using tissue homogenizer for biochemical analysis.

Table 4.2: In *in-vivo* Mice experimental design approved by IAEC, LPU, Phagwara, Punjab

(IAEC Approval No: - LPU/IAEC/2017/Protocol No. 17)

S. No.	Group (N=6)	Dose	Route
1	Deltamethrin (DM)	18mg/kg in corn oil for 10 days	Gavage
2	DM + Nigella Sativa (NS)	18 mg/kg in corn oil for 10 days	Gavage
		100 mg/kg corn oil for 10 days	
3	DM + Syzygium cumini (SC)	18 mg/kg in corn oil	Gavage
		100 mg/kg in corn oil	
4	DM + (NS + SC)	18 mg/kg in corn oil for 10 days	Gavage
		100 mg/kg (50mg NS+ 50 mg SC) mg/kg in corn oil for 10 days	
5	Control	Corn oil 2ml/kg for 10 days	Gavage

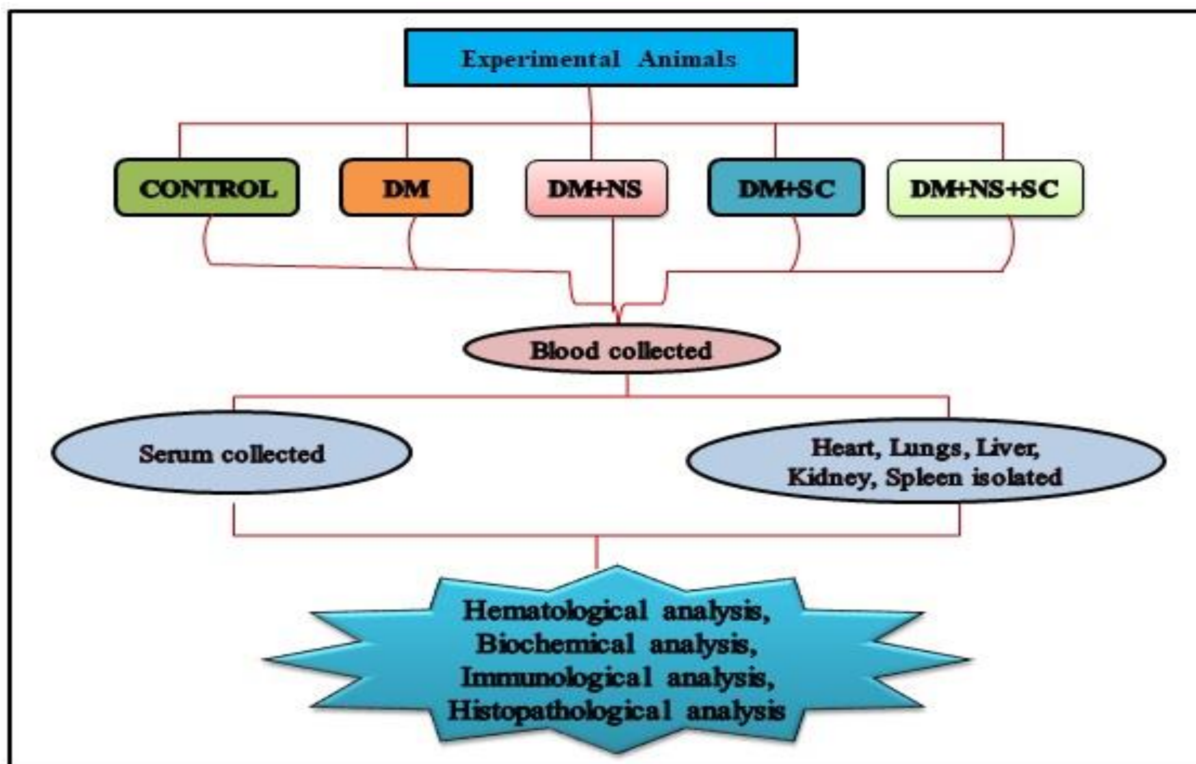


Fig4.2: Flow design of Mice experiment

Immune compromise model of mice was developed by two days limited supply of food and water then followed by two days stopping supply of food, before giving dose of deltamethrin pesticide.

4.9: Evaluation of haematological, biochemical and immunological parameters:

Hematological analysis

The hematological constraints were investigated for red blood corpuscles (RBC), white blood corpuscles (WBC), packed cell volume (PCV) and hemoglobin (Hb) concentration. Mentioned above hematological parameters were evaluated by employing standard protocol (Brown, 1976; Cole, 1974, Schalm *et al*., 1976; Dacie and Lewis, 2001). The total leucokyte count (TLC), also known as the total white blood count (WBC) was detected via hemocytometer (Schalm *et al*., 1976; Baker, *et al*., 1998).

In-vivo studies

a) Size, and weight of Liver, lungs, heart, kidney and spleen was measured, hematological effects, erythrocyte (RBC) sum total, total leukocyte number (TLC), and differential leukocyte count (DLC) were analysed as per standard methods.

b) Humoral immunity function test (plaque forming cell; PFC assay) (Raisuddin *et al*, 1991): The sheep red blood cells (SRBC) obtained from Bajaj Blood supplier, New Delhi and prepared at a cell density of 5×10^8 cells / ml in phosphate buffer saline (PBS). Cunningham chambers was prepared using 'doubled-sided' tape (Scotch Brand, St Paul, USA). The slides were kept in a plastic box with a wet cotton swab and incubated at 37 ^{0}C for 1 hour in incubator. The plaques formed were counted under a light microscope and expressed as PFC per 10^6 spleen cells

c) Haemagglutinin titre assay: Haemagglutinin titre (HT) assay was performed as per the procedure given by Bin-Hafeez *et al.* (2001). On the fifth day after immunization, blood was collected from the tail of each mice for serum preparation. Serial two-fold dilution of serum was made in PBS (pH 7.2) in 96-well microtiter plate and mixed with 50 µL of 1% SRBC suspension in PBS. After mixing, the plates were kept at room temperature for 2 h. The value of antibody titer was assigned to the highest serum dilution showing visible hemagglutination.

d) Cellular immunity function test (delayed Type of hypersensitivity, DTH response): To determine cellular immunity protocol by Raisuddin *et al*. (1991) was followed. On the day of termination of the treatment with plant extract, animals were immunized with $1x10^8$ SRBC, subcutaneously. On the fifth day of immunization, all the animals were again challenged with $1x10^9$ cells in the left hind footpad. The right footpad was injected with the same volume of normal saline, which served as the trauma control for nonspecific swelling. Increase in footpad thickness was measured 24 h after the challenge by using a dial caliper.

e) Macrophage phagocytosis (oxidative burst assay) was performed as per Fujuki and Yano, 1997 method.

f) Lymphocyte proliferation assay was performed as per Benencia *et al*, 1994 and 1995 method.

Biochemical parameters of in *in-vivo* studies

a) Estimation of lipid peroxidation (Utley *et al*, 1967; Aitken *et al*, 1993), Glutathione S-transferase assay (Habig *et al*,1974), Glutathione Peroxidase assay (Mohandas *et al*,1984), Estimation of reduced glutathione (Jollow *et al*, 1974)

b) SDH (Beatty *et al*, 1966), Catalase assay (Claiborne *et al*, 1985), ALPase (Bessey *et al*, 1946), ACPase (Bessey *et al*, 1946), ATPase (Quinn and White, 1968), Cholesterol (Zlatkis *et al*, 1953), NO

c) Estimation of aspartate aminotransferase (AST), (Reitman and Frankel,1957) and alanine aminotransferase (ALT), (Reitman and Frankel,1957).

d) Protein estimation by the method of Lowry *et al*, 1951.

4.10: Dissection and separation of selected organs for the histopathological studies

The selected organs were liver, kidney, lungs, heart, and spleen isolated after dissection and fixed in a solution of 4% paraformaldehyde and kept at 4°C in refrigerator for 12-16 hours for histopathological analysis.

Preparation of 4% Paraformaldehyde

1. 90 ml of distilled H_2O was taken in a Borosil glass beaker then warmed up to 60°C with a hot plate with magnetic stirrer for uniformity.

2. During stirring, 4 g of paraformaldehyde powder supplemented to the warmed water and sustained the temperature at 60°C.

3. Then followed by addition of single drop of 2N NaOH solution (1 drop per 100 ml) in warmed solution containing paraformaldehyde. The solution turns out to be clear in 2-4 minutes (Precaution: Avoided heating mixed solution more than 70°C).

4. When solution turned out to be clear in appearance beaker was taken away from hot plate then supplemented with 10 ml of 10X PBS.

5. The pH of mixed solution was maintained to 7.2 and volume was completed to 100ml by adding distilled water.

6. Then above obtained mixed solution remained filtered as well enclosed through foil to guard from the sunlight or any other source of light.

7. The above solution can be used instantly, or aliquots can be kept at -20°C in deep freezer for further applications as required.

4.11: Chemicals and Kits

The pesticide Deltamethrin (technical grade) by 98.50 % purity was obtained from Heranba Industries Limited, Vapi, Valsad, Gujarat India. The deltamethrin pesticide solution was prepared by dissolving fixed quantity of deltamethrin pesticide in technical grade corn oil. The solution was kept in refrigerator and was brought to room temperature before oral administration. The anesthetic solution of xylazine-ketamine has been prepared by mixing of xylazine (Xylazin®) 0.5 ml (10 mg) and Ketamine (Aneket®) 2 ml (100 mg) in 7.5 ml of normal saline. Media designed for bacterial and fungal growth purchased from HiMedia®, all additional chemicals exploited during experiment were of the uppermost technical grade accessible commercially.

4.12: Blood collection

After giving anesthesia, paraffin plate was used to fix mice on it by using pins then blood was collected from tail vein or by doing cardiac puncture of mice in the vial containing EDTA using 1 ml tuberculin syringe. The obtained blood was used to evaluate meant for total leukocyte count (TLC) in addition differential leukocyte count (DLC). The remaining blood trials were centrifuged at 3000 rpm and sera of the centrifuged blood samples were moved into Eppendorf tubes and analyzed various biochemical parameters investigation.

Preparation of Xylazine-Ketamine solution

0.5 ml of Xylazine (20mg/ml), 2.0 ml of Ketamine (50mg/ml), and 7.5 ml of pyrogen free saline mixed all together and final Volume maintained upto 10.0 ml.

Phosphate Buffer Saline (PBS)

$NaCl_2$ 8.0 g, KCl 200 mg, KH_2PO_4 120 mg, Na_2HPO_4 522 mg, Distilled water- 500 ml

4.13: Total Leukocyte Counts and Differential Leukocyte Count

4.13.1: Total leukocyte count (TLC)

For evaluation of total leukocyte or white blood cells count following standard procedure was adapted –

1. In a glass vial, 380 µl of the WBC diluting fluid were taken and mixed with blood sample.

2. In vial containing 380 µl WBC diluting fluid 20 µl of mice blood was supplemented in their ampoules and mixed appropriately.

3. The mixed blood was loaded to haemocytometer then observed under light microscope with specific magnification (10X).

4. The blood cells present in in four quadrangles of haemocytometer chamber then summed up after counting.

5. Total leukocytes count (TLC) was estimated subsequently putting the standards in equation as mentioned under:

$$\text{Cells}/\ \mu L = \frac{\text{Total number of cells in 4 squares} \times \text{Dilution factor}}{\text{Volume factor (0.4)}}$$

Where,

Volume factor = 4 × (width × length × height) = 0.4

Dilution factor for blood= reciprocal of dilution (*i.e.* 20)

4.13.2: Differential leukocyte count (DLC)

For evaluation of differential leukocyte count following procedure was adapted:

1. A clean slide loaded with a drop of mice blood then generated a clear smear by using another slide with plane edge with a slant of 45°.

2. Leishman stain was flooded over air dried smear of blood and kept for 30-45 seconds.

3. Buffer was supplemented to the stained slides, then mixed properly. For 10-12 minutes slide was lay by untouched for staining.

4. Tap water was used gently over slides and washed properly then slides were kept for air drying.

5. Colony counter was implemented to count a total of 100 Cells under light microscope at 100× magnification.

4.14: Lipid profile

After completion of treatment, homogenized hepatic and cardiac tissue and blood was exploited for biochemical examination of various parameters named as follows:

4.14.1: Total cholesterol (TC)

Blood sample volume 0.1 ml taken and was finally volume maintained up to 10 ml by supplementing ferric acetate–uranyl acetate reagent. A total lipid extract of volume 0.1 ml was collected in an aliquot and formerly dehydrated through evaporation. The dried extract of sample with standard were made up to final volume of 3 ml through the solution o ferric chloride–uranyl acetate reagent. Followed by addition of 2 ml of sulfuric acid-ferrous sulfate reagent solution into all the vials and then ingredients were mixed properly. After 20 minutes of incubation the colour developed was read at 540 nm by using UV-Vis spectrophotometer or colorimeter. The total cholesterol (TC) level was represented as mg/dl.

4.14.2: Triglyceride levels (TG)

4 ml of isopropanol was mixed with 0.1 ml of blood sample followed by the addition of 0.4 g alumina, this mixture was shaken about 15 minutes and then for 10 minutes this mixture was centrifuged at 2000 rpm. After that, supernatant fluid (2.0 ml) was collected and then added to the well labeled tubes. After 0.6 ml of the saponification regent was supplemented, the tube was placed in hot water bath tub for saponification reaction at specific 65°C till 15 minutes. After cooling, then addition of sodium meta periodate (1.0 ml) was supplemented trailed by addition of 0.5 ml acetyl acetone reagent. Then contents were mixed, and the tubes were placed in water bath at 65°C for 30 minutes. The contents of the tube were allowed to cool and then absorbance i.e. logarithm of the reciprocal of the transmittance was recorded at specific wavelength 430 nm and compared with a blank by UV-vis spectrophotometer or colorimeter. Triglyceride presence was measured in mg/ dl plasma.

- VLDL-C = Total Serum Triglycerides/5
- LDL- C = Total serum cholesterol- total serum triglycerides- HDL-C/ 5

4.14.3: Lipoproteins levels

Lipoproteins levels like HDL-cholesterol (HDL-C) (Burstein *et al*., 1970), triglycerides (TG) level (McGowan *et al*., 1983), LDL-cholesterol (LDL-C) level, VLDL-cholesterol (VLDL-C) level were estimated by means of standard protocols (Friedewald *et al*., 1972).

4.14.4: Insulin Level

To investigate the level of insulin in experimental groups blood was withdrawn from tail vein in vials containing 10 mg/ml EDTA. The collected blood sample in vial was centrifuged for 5 min at 300 rpm and then blood plasma was collected and aliquoted subsequently stored at -20^{0}C in deep freezer and further used to evaluate insulin presence. The insulin level in plasma was represented as µIU/ml.

4.14.5: Glucose Level

After overnight fasting of mice, blood samples were collected immediately into vials containing a little amount of an anticoagulant agent EDTA or mixture of potassium oxalate and sodium fluoride. Blood was collected by tail vein after anesthesia treatment. The level of blood glucose in plasma were examined by using glucose oxidase-peroxidase (GOD-POD) process. Then colorimetric method was exploited for estimating the enzymatic responses of enzyme invertase and glucose oxidase-peroxidase (GOD-POD) (Park *et al*., 2007).

4.14.6: Protein estimations (Lowry *et al*., 1951).

The tissue homogenate (0.1 ml) containing normal saline (0.9 ml) and 10% TCA (1.5ml) was held at 40ºC for 4 hours. The solution was further centrifuged to separate protein precipitate. One ml of 0.1 Normality NaOH solution was exploited to dissolve proteins. In the diluted sample addition of 0.5ml alkaline copper sulfate was prepared using 1 ml of 1.0% w/v of copper sulphate + 1 ml of 2% w/v of sodium potassium tartarate + 48 ml of 2.0% w/v of sodium carbonate in 0.1N NaOH solution, and followed by incubation at 37ºC, for 30 min, then 0.5 ml folin-calteau reagent added. Exactly after 30 min, optical density of the blue colour was measured at 625 nm.

Along with the blank, BSA, standard protein solution (20-100 µg) was also run. Standard curve of BSA was plotted on the basis of which concentration of protein in the sample were calculated in terms of mg protein /100g tissue weight.

4.14.7: Oxidative stress markers

4.14.7.1: Lipid peroxide levels (Ohkawa et. al., 1979)

0.2 ml of each subcellular fraction having 3 to10 mg protein was used and inoculated with 1 ml of 20 % acetic acid and then followed by addition of 0.2 ml of 8 % aqueous SDS. The prepared solution was accustomed to 4 pH by the adding of concentrated NaOH solution if needed. The reaction mixture, was prepared at final volume of 4ml, contained 1.5 ml of 0.8% TBA solution and adequate volume of distilled water after adjusting pH. Boiling water bath was used to incubate the reaction mixture tubes for one hour. Maintained the temperature at room temperature, then three ml of n-butanol was supplemented into the above obtained solution then followed by centrifugation at 10,000 × g till 15-minutes. After centrifugation, there was development of a clear fraction of butanol then applied for noting the absorbance using UV-Vis

spectrophotometer at specific wavelength of 532 nm. Simultaneously, 2.5 normality malondialdehyde (MDA) chemical solution applied and compared to track as standard.

4.14.7.2: Catalase assay (Aebi, 1974)

Addition of tissue supernatant as cytosolic portion by means of catalytic enzyme cause and inoculated H_2O_2 phosphate buffer (3.0 ml) into the cuvette, and reaction mixture prepared. The absorbance was declined at 240 nm wavelength was recorded after each 30 seconds till 3 minutes i.e 6 records were noted, each time absorbance was reduced.

4.14.7.3: Superoxide dismutase assay (McCord & Fridovich, 1969)

Tissue homogenate was poured in centrifuge tube. Then centrifugation carried out at 10,000x g for 15min in a refrigerated centrifuge. Ammonium sulphate (313mg/ml) was inoculated in the supernatant separated from each sample and the final concentration was made up to 50%. Then vials were carefully shaken afterward incubated till 4 hours at 4^0C. Then, again centrifugation carried out at 14,000x g at 4^0C for 30 min. Dialysis of obtained supernatant of sample was showed deviation from cooled triple distilled water (TDW). The dialysis bags were filled and afterward exposed to an enzyme. Subsequently, another setup of two-reaction combination run parallelly. The vials in the first investigational setup was treated with 0.3 ml of 1.5mM nitro blue tetrazolium (NBT), 1 ml of 20.4 mM of pyrophosphate buffer at pH 9.2; 0.2 ml of, 0.93 mM phenazine methosulphate then 1ml of triple distilled water added and followed by 0.2 ml of enzyme source. The reference vials of second setup have all the same above reagents contents except the enzyme source. Simultaneously, both the chemical reactions were carried through the supplementation of 0.1 ml solution of 2.34 mM NADH. Later on, into each test tube, addition of 1 ml of glacial acetic acid was done; after an interval of 90 seconds, for inspection of the chemical reaction subsequently 0.2 ml of enzyme source was supplemented in the sample tubes. By using UV-Vis spectrophotometer the absorbance of the experimental tubes was esitmated at 560 nm and compared with a blank test tube containing mixed solution of Buffer + PMS + NBT + TDW.

Calculation: Enzyme activity unit was outlined because the amount of biocatalyst i.e. enzyme, essential to retard the reduction in optical density of Nitroblue tetrazolium up to 50%, in one min at specific wavelength of 560 nm underneath the evaluation. Outcomes of enzymatic activity of SOD were stated as unit/mg protein.

4.14.7.4: Glutathione reductase (Hazelton & Lang, 1985)

The mixture used in reaction consisting of 0.1mM NADPH (0.1 ml), 3.0mM GSSG (0.2 ml), 1.0 mM EDTA (0.1 ml), 0.1m Tris buffer (2.5 ml) and 0.1 ml volume of diluted tissue cytosolic supernatant (enzyme source) was made of 3.0 ml total volume. The reaction was initiated with the addition of tissue supernatant and other reagents. Oxidation of NADPH was followed at 340 nm. Reference reaction was also run simultaneously.

Calculation: Enzymatic action was evaluated by means of the molar extinction coefficient of oxidising agent NADPH ($6.22 \times 10^3 M^{-1}cm^{-1}$) then the outcomes of enzymatic responses were denoted as n mole of NADPH oxidized each minute each mg protein.

4.14.8: Assessment of inflammatory markers

Nitric oxide synthase assay:

In cardiac tissues, nitric oxide synthase (NOS) activity was investigated by the commercially available kit (Bioxytech Nitric oxide; Catalog No. 22113; OXIS International, Inc. U.S.A).

Reagent

Assay buffer, preparation of Co-Factor, Nitrate reductase, Standard Nitrate, Griess Reagent R1 and R2 and NADPH, Lactate Dehydrogenase.

Standard: Nitrate standard dilution were prepared at 1/10 in assay buffer. At the time of testing, serial dilutions were made from 0-35 nmoles/ml

Procedure:

The tissue sample was homogenized in PBS and pH was maintained with 7.4, then sample was centrifuged at 10,000 x g till 20 minutes. In two wells of microtiter plate blank was prepared by adding 200 µl water or assay buffer added. In remaining wells 60 µl of homogenized sample was poured. Then subsequently inoculation of 10µl of 1mM NADPH solution in each well was done, followed by incubation at room temperature after addition of 10µl of nitrate reductase in each well of reaction mixture. Then addition of 10 µl of cofactor and 10 µl of lactate dehydrogenase solution to each well. Then incubation for

20 minutes at 25^0C, then in each well of microtiter addition of 50 µl of Griess reagent R1 and Griess reagent R2. Then microtiter plate was kept at room temperature till 10 minutes for the development of the colour due to heat inactivation. Then at specific wavelength of 540 nm was used to evaluate the absorbance. The calculation of NOs activity was evaluated by the following equation:

NOS Activity = {(A 540 - *b*) / *m*} (200 / VS) / TRXN = nmoles / ml /min

Where: A540 - sample absorbance at 540 nm.

b - y-intercept (linear regression of the standard curve)

m - slope (linear regression of the standard curve)

VS - volume of sample poured to the well (mL)

TRXN - NOS reaction incubation time in minutes.

Final volume makes up to 200 mL for the reaction in the experiment.

Cytokines Assay

After 10 days in control group of mice and deltamethrin induced group of mice while after 20 days in deltamethrin + seed extracts treated mice blood was collected through tail vein in vials comprising anticoagulating agent,10 mg/ml EDTA. Then blood was centrifuged at 300 rpm till 5 min due to it, the plasma gets separated and aliquoted in another vial. This plasma was stored at 4^0C for insulin valuation and determination of levels of serum cytokines specially, IL-6 and TNF-α. The Invitrogen kit was used to determine cytokines assay by means of the quantitative sandwich enzyme immunoassay method. A micro plate was pre-coated with monoclonal antibody precise designed for mice IL-6 and TNF- α. Once controls standards, and samples were loaded in well through micropipette then binding of these sample molecules was achieved with immobilized antibody present in well. Unbound molecules were washed out by washing solution provided in kit, then addition of an enzyme-linked polyclonal antibody into each well was done precise for mice IL-6, and TNF- α. Again, washing was completed through washing solution to eliminate any boundless antibody-enzyme reagent in well, then followed by addition of substrate solution in the

wells. A yellow colour was produced from blue colour due to enzymatic activity while stop solution was added to well as mentioned in the kit. The color intensity produced in wells were measured in quantity of bounded molecules with pre-coated antibody in wells of mice IL-6 and TNF- α. The sample values are then read off and compared with the standard curve.

4.14.9: Study of Liver function test

To evaluate liver function test namely aspartate aminotransferase (AST), alanine amino transferase (ALT) and gamma glutamyl transferase (GGT) by using standard procedures.

4.14.9.1: Aspartate aminotransferase (AST) or serum glutamic-oxaloacetic transaminase (SGOT)

The method of Reitman and Frankel (1975) was employed for the evaluation of aspartate transaminase.

Reagent: 0.1 M of Phosphate buffer with Ph 7.4 prepared, 0.4 N solution Sodium hydroxide (NaOH) then in 20.5 ml of 1N NaOH,2.66 g of DL-aspartate and 38 mg of α-oxaloglutarate were dissolved by mild heating system. The final volume was maintained to 100 ml by adding buffer. 1mM 2,4-dinitrophenyl hydrazine was dissolved in 2N HCl. 10 mg of standard sodium pyruvate was dissolved in 100 ml of phosphate buffer 0.1 M and maintained the pH 7.4.

Procedure: One ml of substrate (buffered) was supplemented to 0.1 ml of serum or enzyme source (0.1 ml) then kept at 37°C till 60 minutes. Followed by addition of one ml of DNPH reagent to detention of the chemical response. One ml of distilled water was added in the blank tubes, serum was not introduced in vials to make a blank reference. Test tubes were kept at room temperature till 15 minutes for incubation. Then in all test tubes 10 ml of 0.4 N NaOH solution was supplemented and absorbance was recorded at precise wavelength of 520 nm in a UV-Vis spectrophotometer. The activity of biocatalyst enzyme was stated as IU/1 for serum and µM of pyruvate liberated /hr /mg protein present in tissue.

4.14.9.2Alanine amino transferase (ALT) or Serum glutamic pyruvic transaminase (SGPT)

The Reitman and Frankel method (1975) was implemented for the evaluation of aspartate transaminase.

Reagent: In 20.5 ml of 1N NaOH solution, 0.1M of Phosphate buffer solution with pH 7.4 + 0.4 N NaOH solution + 2.66 g of DL-aspartate substrate and 38 mg of α-oxaloglutarate were dissolved by mild heating. The final volume was maintained up to 100 ml by adding 1Mm 2,4-dinitrophenyl hydrazine (DNPH) dissolved in 2N HCl buffer while 10 mg of standard sodium pyruvate was dissolved in 100 ml of 0.1 M phosphate buffer with pH 7.4.

Procedure: 1.78 g of DL-alanine i.e. racemic mixture of alanine amino acid and 38 mg of α-oxaloglutarate were dissolved in standard buffer solution followed by addition of 0.5 ml of NaOH and the final volume maintained up to 100 ml by adding buffer solution in it. The activity of biocatalyst enzyme was stated as IU/l for serum and μM of pyruvate liberated /hr /mg protein present in tissue.

Statistical Analysis: All the data are expressed as Mean±SEM. Statistical analysis were achieved with the GraphPad Prism 5 software package. Comparison between groups was made by one-way analysis of variance (ANOVA) taking significance at $P<0.01$ followed by Newman-Keuls Test.

Preparation of blocks and sectioning of tissues by using microtome

The histopathological studies were performed using Scheuer and Chalk, 1986.

The different isolated organs which were stored in paraformaldehyde or formalin solution were handled for tissue block grounding as mentioned below:

Processing:

Different isolated organs which were immersed in formalin were isolated and kept firstly in 70% of ethyl alcohol till 60 minutes, followed by 80% ethyl alcohol treatment for next 60 minutes, then for next 60 minutes organs were kept in 90% ethyl alcohol and finally with 100% ethyl alcohol till 60 minutes. After alcohol treatment of tissues were kept in acetone I for 15 minutes and followed by acetone II for next 15 minutes. Then the tissues were kept in benzene I and benzene II for 15 minutes each.

After these, tissues of various organs remained reserved overnight in the liquefied pure wax at maximum temperature of 60°C. Then tissue blocks were organized subsequent day through dropping the tissues in paraffin wax then it was cooled at room temperature and followed by

cooling at 4°C.Then by irregular trimming of extra wax covering tissues in blocks were completed. Then these blocks were kept for additional histopathological examinations using microscope after thin slice of tissue development followed by staining process.

4.14.15: Paraffin sectioning

The 4-5 µm thick tissue sections were obtained with the help of rotary microtome. Sections were taken on plain glass slides very carefully for Hematoxylin and Eosin staining which are commonly used for routine histopathological studies and on clean and clear lead crystal slides for immunohistochemistry.

Staining of tissues by Mayer's method

Once thin layers of tissue were developed then it was kept on slides then these slides were treated for staining with hematoxylin and eosin stains and for decolorizing some other chemicals were used as following method:

Processing

Slides were firstly treated with xylene twice for 10 min each then followed by treatment with Alcohol 100%, 90%, 80%, and 70% for 2 min each, then slides were treated with double distilled water for 2 minutes then slides were applied with hematoxylin till 20 minutes. Then slides were washed with running tap water gently for 18 minutes, followed by eosin stain for 2 min then slides were dipped into graded alcohol 70%, 80%, 90%, and100% followed by addition of xylene and slides were kept for 5 minutes then slides were mounted properly for microscopic observation.

Preparation of hematoxylin solution:

1 gm of hematoxylin crystals were dissolved in 1000 ml of distilled water then addition of 0.2 gm of sodium iodate, 50 gm of potassium alum followed by addition of 1 gm citric acid crystals and finally 50 gm of choral hydrate were mixed properly to prepare hematoxylin stain. The final volume was remained 1000 ml.

Preparation of stock solution eosin:

For preparation of eosin stock solution, 1 gm of eosin Y (Water soluble) was dissolved in 20 ml of double distilled water then followed by addition of 80 ml of 95% alcohol in above solution. The final volume of eosin stock solution was 100 ml. Working solution of eosin was prepared in the ratio of 1:3 i.e. 25 ml of stock solution mixed with 75 ml of 80 % alcohol.

4.16: Computational Analysis of Deltamethrin and Bioactive Molecules Derived from Seed Extracts of *N. sativa* and *S. cumini*

4.16.1: Molecular docking with PatchDock

Deltamethrin and reported six important bioactive molecules present in seed extracts of *N. sativa L.* and *S. cumini L.* were selected as ligand molecules for interaction with MHC I and MHC II molecules with human and mice origin. Immunomodulatory effects were evaluated with the binding energy and score. IUPAC name and general formula of L1-L7 and physicochemical properties of L1-L7 molecules were predicted while molecular properties. Prediction of bioactivity score of the title compounds L1-L7 with GPCR ligand; Ion channel modulator, Kinase inhibitor; Nuclear receptor ligand; Protease inhibitor, Enzyme inhibitor. Prediction of Drug- likeness model score of the ligands L1- L7. Prediction of solubility and toxicity of the title compounds L1-L7 evaluation of pharmacokinetics profile of the title compounds L1-L7 were analyzed.The structure of total seven ligand molecules were extracted from database; L1- Deltamethrin, L2- Thymoquinone, L3- Dithymoquinone, L4-Gallic acid, L5-Antimellin, L6-Quercetin, L7-ferulic acid. Then the structure of MHC I and MHC II of human and mice origin was extracted and further analyzed for binding energy with seven ligand molecules. Interaction of L1 (Deltamethrin) and MHC molecules. Prediction of ADMET profile of the title compounds L1-L7 and prediction of Binding energy and Inhibition constant of ligands L1-L7 calculated.

4.16.2: Docking of “MHC-I and MHC-II” with “deltamethrin” with the help of Autodock

Molecular docking studies were also performed for analysis of interaction ability of deltamethrin molecule with MHC I and MHC II which may affect the immunomodulation effects.

The tool used in this study was used Auto dock 4 was developed by Morris *et al.* in 2009 after its first release in 1990. This tool enables to perform the docking of macromolecule with the ligand

which it performs efficiently by foretelling binding conformations and binding energies between macromolecule and ligand under consideration. This program performs by initially looking for hefty conformational space for the ligand in the region of protein which is carried out by Lamarckian genetic algorithm explained by Morris *et al*. (1998). The software Autodock uses grid-based method for calculation of binding energies of the conformations. In this approach target macromolecule is entrenched in a grid after which the investigative atom is placed chronologically in each grid point and their respective binding energy is noted in each grid. This binding energy is used lately during the docking imitation. This tool also allows the user to carry out simulated annealing search and traditional genetic algorithm search (Morris *et al*., 2009).

4.16.3: Installation of the tools

The latest available version of the AutoDock was downloaded from http://autodock.scripps.edu/downloads/autodock-registration/autodock-4-2-download-page/ for the windows. The program was installed in the default directory C:\Program Files (x86)\.

4.16.4: Retrieval of the Macromolecule and ligand

The macromolecule used in the study was MHC-1. This macromolecule was docked with the ligand deltamethrin with the help of autodock4. As the tools read pdb file format of the macromolecule and ligand thus 3D structure of MHC-1 was retrieved from the https://www.rcsb.org/ in pdb format. Similarly, deltamethrin structure was retrieved from https://pubchem.ncbi.nlm.nih.gov in the sdf format which was then converted to pdbqt format by using Open Babel (O'Boyle, *et al.*, 2011). In this tool select input format as sdf and output format as pdbqt. After this browse the sdf file of deltamethrin in the browsing tab present below input format option and set the output file destination C:\workspace. Finally, both the pdb and pdbqt files of the macromolecule and ligand were kept under C:\workspace (folder name).

4.16.5: Setting up the default directory

In Autodock set the default directory by navigating File> Prefrences>Set> under Startup directory enter C:\workspace>click Set> Click Make default. In this way default directory is made in which all the docking files will be stored by default.

4.16.6: Setting up the Macromolecule

AutoDock tool was opened by clicking on its desktop shortcut. Under the main menu click File> Read Molecule> browse the workspace Folder > select the macromolecule file named as MHC_1.pdb > click open.

Remove water by Edit> click on Delete Water.

Hydrogen were added to the macromolecule by Edit> Hydrogens> add> All Hydrogens> ok.

Merge Non-Polar hydrogen by Edit>Hydrogen>Merge Non-Polar.

Click Edit>Charges>Compute Gasteiger>Total Gasteiger charge added = -27.9913>Click ok.

File>Save>Write pdb>Ok. This will write the newly modified pdb file to the workspace folder.

Now click on Grid> Macromolecule> choose> select macromolecule> ok> save as "protein .pdbqt" in the same folder containing the pdb files (Srinivasan and Rose, 1999).

4.16.7: Setting up the ligand

In AutoDock click on Ligand> input> open> select ligand file "deltamethrin.pdbqt"> open. AutoDock automatically modifies the ligand in terms addition of charges and also detects the frequency of rotatable bonds in ligand. In this case 15 aromatic carbons were found and seven rotatable bonds were detected. TORSDOF was set to 7 automatically with respect to detected rotatable bonds.

Click Ligand> Torsion Tree> Detect root. Root will be indicated by green circle in the Autodock visualisation window.

4.16.8: Setting the Grid Space

At this point we will set the boundaries of the grid box for the macromolecule under which the ligand will look for the potential binding.

Click on Grid>Macrolecule>Choose>Select the file MHC_1.pdb>save the modified Macromolecule in pdbqt extension in the same workspace destination.

Click on Grid>Set map types>choose ligand>select deltamethrin>click ligand.

Click on Grid>Grid Box. Entered the following values to set the grid box (Figure 1).

X center = 1.11

Y center = 10.412

Z center = 18.775

Number of points in x dimension = 66

Number of points in y dimension = 102

Number of points in z dimension = 98

Spacing = 0.931 angstrom

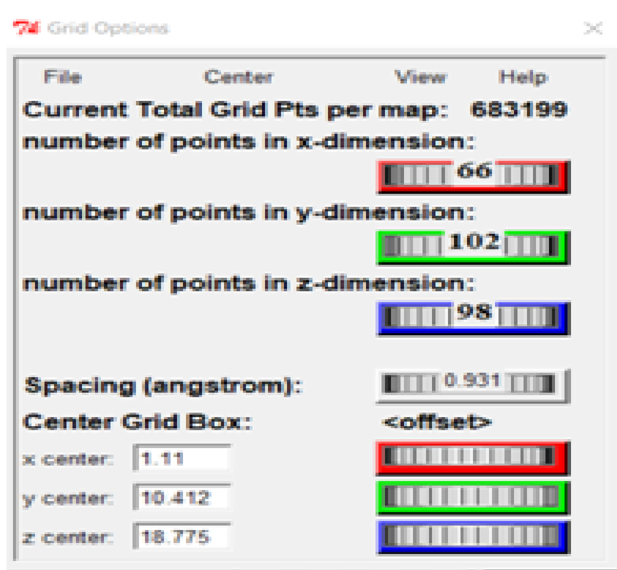


Fig 4.3: Autodock Grid Box Window

Click on File>Close saving current.

Click on Grid>output>save gpf>give file name "grid.gpf">save to the workspace folder.

4.16.9: Performing Autogrid

Click on Run>Run Autogrid> Autogrid window will appear. Browse and select the following path under the provided options in Autogrid window.

Program Pathname = C:/Program Files (x86)/The Scripps Research Institute/Autodock/4.2.6/autogrid4.exe

Parameter Filename = C:/workspace/grid.gpf

Log file name = C:/workspace/grid.glg (automatically writes while the parameter filename path was added)

Click Launch, Autogrid will be launched and AutoDock process manager window will appear. On the successful completion the process manager window will disappear.

4.16.10: Performing Autodock

Click on Docking>Macromolecule>Set rigid file name>select MHC_1.pdbqt.

Again click on Docking>Ligand>Choose>Select deltamethrin.pdbqt>click select ligand> click on accept by setting the default parameters.

Again Docking>Search parameter>Genetic algorithm>click Accept.

Again Docking>Docking parameters>Accept (use defaults).

Again Docking>Other options>AutoDock 4.2 parameters>Accept.

Again Docking>Output>Lamarckian GA 4.2>enter file name dock.dpf>save in the workspace folder.

Click on Run>Run Autodock> Autodock window will appear. Browse and select the following path under the provided options in Auto dock window.

Program Pathname = C:/Program Files(x86)/TheScrippsResearch Institute/Autodock/4.2.6/autodock4.exe

Parameter Filename = C:/workspace/dock.dpf

Log file name = C:/workspace/dock.dlg (automatically writed while the parameter filename path was added)

Click Launch, AutoDock will be launched and AutoDock process manager window will appear. On the successful completion the process manager window will disappear.

4.16.11: AutoDock Analysis

Click on Analyze>Dockings>open>open dock.dlg file from the workspace (created after the completion of Autodock).

Again, click on Analyze>Macromolecule>choose>select MHC_1.pdb

Again, click on Analyze>conformations>Play,ranked by energy.

After this all the conformations can be viewed and analyzed one by one.

RESULTS AND DISCUSSION

OBJECTIVE1: To investigate the biochemical composition, antioxidant properties, antibacterial, and antifungal activities of seed extracts of *Nigella sativa* and *Syzygium cumini.*

5.1: BIOCHEMICAL COSTITUENTS OF SEED EXTRACTS

Table 5.1: Qualitative tests of phytoconstituents in different extracts of *N. sativa* and *S. cumini* is denoted as + (plus sign) and absence is denoted as – (minus sign)

Phytoconstituents	Test	Phytochemical extracts							
		NSE	NSM	NSW	NSA	SCE	SCM	SCW	SCA
Alkaloids	Mayer's test	+	+	+	+	+	+	+	+
	Dragendorff's test	+	+	+ +	+ +	+	+	+ +	+
Steroids	Salkowski's test	+	+	+	+	+	+	+	+
Flavonoids	Flavonoids test	+	+	+	+	+	+	+	+
Tannins	Ferric chloride	+	+	+	+	+	+	+	+
Saponin	Saponin test	+	+	+	+	+	+	+	+
Glycosides	Molish's test	-	-	-	-	-	-	-	-
Proteins & amino acids	Biuret test	+	+	+	+	+	+	+	+
Reducing sugar	Benedict's test	-	-	-	-	-	-	-	-
	Fehling's test	-	-	-	-	-	-	-	-

In the above table 5.1 qualitative analysis of phytoconstituents present in seed extracts were performed by standard methods. Four different solvents like ethanol, methanol, water and acetone were used for extraction purpose for both type of seeds derived from *Nigella sativa* and *Syzygium cumini.* In both type of seed extracts glycosides and reducing sugar were not reported while alkaloids, steroids, flavonoids, tannins, saponins, proteins and amino acids were present in

extracts. Presence of these biomolecules in seed extracts may play vital role in antioxidant activities, antimicrobial activities, cytotoxicity activities and immunomodulation activities. These biomolecules can play role in reducing the toxic effects as well, can improve the functioning of vital organs of vertebrates in defined dose and can boost the immune system to achieve better health state of an individual. Ahmad *et al*. reported in 2013 about therapeutic potential of *Nigella sativa* due to phytoconstituents present in extracts. Jassir in 1992 explained the chemical composition of *N. sativa* is similar with our findings in terms of qualitative analysis.

5.2: ANTIOXIDANT ACTIVITIES

5.2.1: DPPH SCAVENGING ACTIVITY

A. ***Nigella sativa* extracts**

Table 5.2: DPPH scavenging activity of *N. sativa* extracts, values are represented as Mean of triplicate of each extract and Standard Deviation. (NSE-*N. sativa* ethanolic extract, NSM- *N. sativa* methanolic extract, NSW- *N. sativa* water extract and NSA- *N. sativa* acetonic extract)

Conc (µg/ml)	NSE	NSM	NSW	NSA
25	18.46±0.06	20.33±0.29	15.37±0.15	17.40±0.17
50	26.40 ±0.17	28.30±0.17	18.30±0.10	23.13±0.12
75	32.46±0.06	34.17±0.29	24.37±0.15	27.17±0.29
100	37.13±0.12	40.40±0.17	30.30±0.17	33.40±0.17
150	42.30±0.17	45.53±0.06	34.30±0.17	39.30±0.17

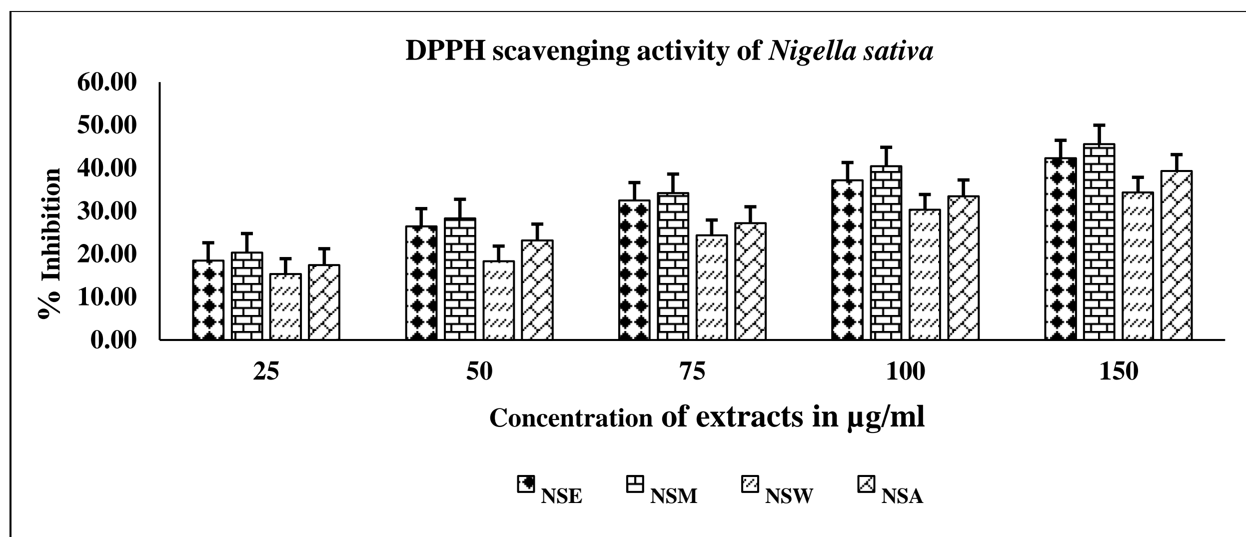


Fig 5.1: DPPH radical scavenging activity analysis shown with Standard Error Bar for *N. sativa* extracts with different concentrations. (NSE-*N. sativa* ethanolic extract, NSM- *N. sativa* methanolic extract, NSW- *N. sativa* water extract and NSA- *N. sativa* acetonic extract)

As concentration of extracts increasing the scavenging of DPPH radical increased in all types of extracts which confers the antioxidant potential of seed extracts of *N. sativa*. Maximum antioxidant activity was observed in NSM i.e. *N. sativa* methanolic extract while minimum activity was recorded in aqueous extract (NSW). At exceeding the extract concentration to 200 µg/ml there was no significant difference in % inhibition was observed than 150 µg/ml extract concentration.

***B. Syzygium cumini* extracts**

Table 5.3: DPPH activity of *S. cumini* extracts, values are Mean of Triplicate of Each Extract with Standard Deviation. (SCE- *S. cumini* ethanolic extract, SCM- *S. cumini* methanolic extract, SCW- *S. cumini* water extract and SCA- *S. cumini* acetonic extract)

Conc (µg/ml)	SCE	SCM	SCW	SCA
25	12.3±0.17	18.33±0.29	12.67±0.58	14.33±0.58
50	16.73 ±0.40	24.07±0.12	16.33±0.29	17.33±0.58
75	20.40±0.17	30.33±0.29	22.67±0.58	23.67±0.58
100	24.17±0.29	36.17±0.29	27.33±0.58	28.33±0.58
150	32.17±0.29	40.67±0.58	31.33±0.58	32.33±0.58

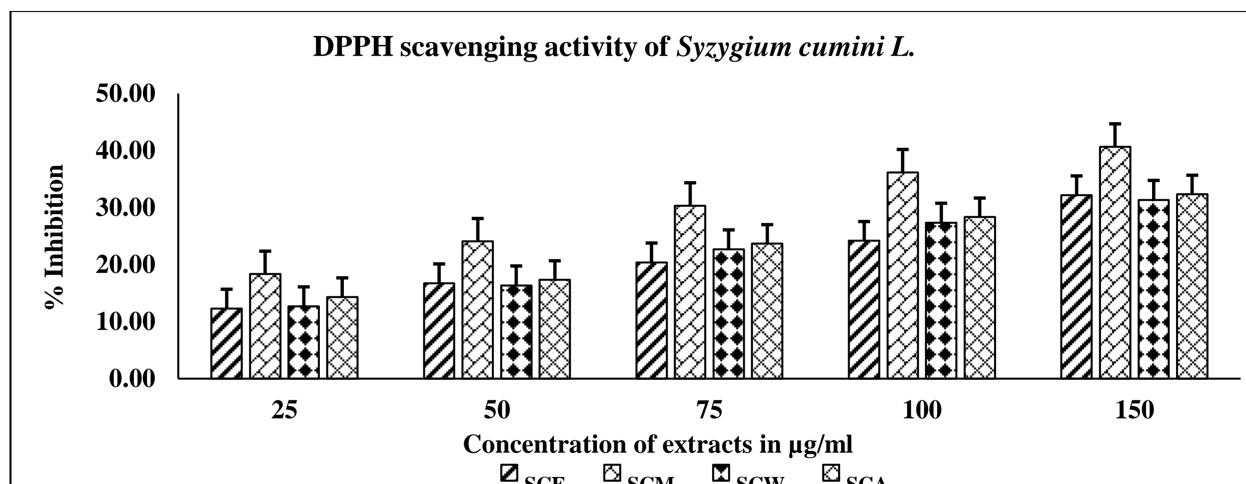


Fig 5.2: DPPH radical scavenging activity of *S. cumini* extracts with Different Concentrations and shown with standard error bar. (SCE- *S. cumini* ethanolic extract, SCM- *S. cumini* methanolic extract, SCW- *S. cumini* water extract and SCA- *S. cumini* acetonic extract)

As concentration of extracts increased the scavenging activity of DPPH radicals increased. Maximum was recorded in methanolic extract of *S. cumini* seeds while minimum was recorded in aqueous extract. Seed extracts have been found capable of doing the antioxidant activity.

5.2.2: SUPEROXIDE SCAVENGING ACTIVITY

A. *Nigella sativa* extracts

Table 5.4: Super oxide scavenging activity of different extracts of *N. sativa*. Values are Mean of Triplicate of Each Extract with Standard Deviation. . (NSE-*N. sativa* ethanolic extract, NSM- *N. sativa* methanolic extract, NSW- *N. sativa* water extract and NSA- *N. sativa* acetonic extract)

Mean ±SD of extracts				
Conc (µg/ml)	NSE	NSM	NSW	NSA
25	17.69±0.70	20.27±1.60	14.37±0.15	23.40±0.92
50	26.47 ±0.70	25.20±0.87	21.50±0.89	31.93±0.99
75	34.07±1.21	35.07±1.17	29.07±0.99	43.27±0.99
100	41.53±1.22	41.40±1.56	36.87±0.31	51.07±0.81
150	53.50±0.95	47.80±0.69	42.50±1.05	57.67±1.10

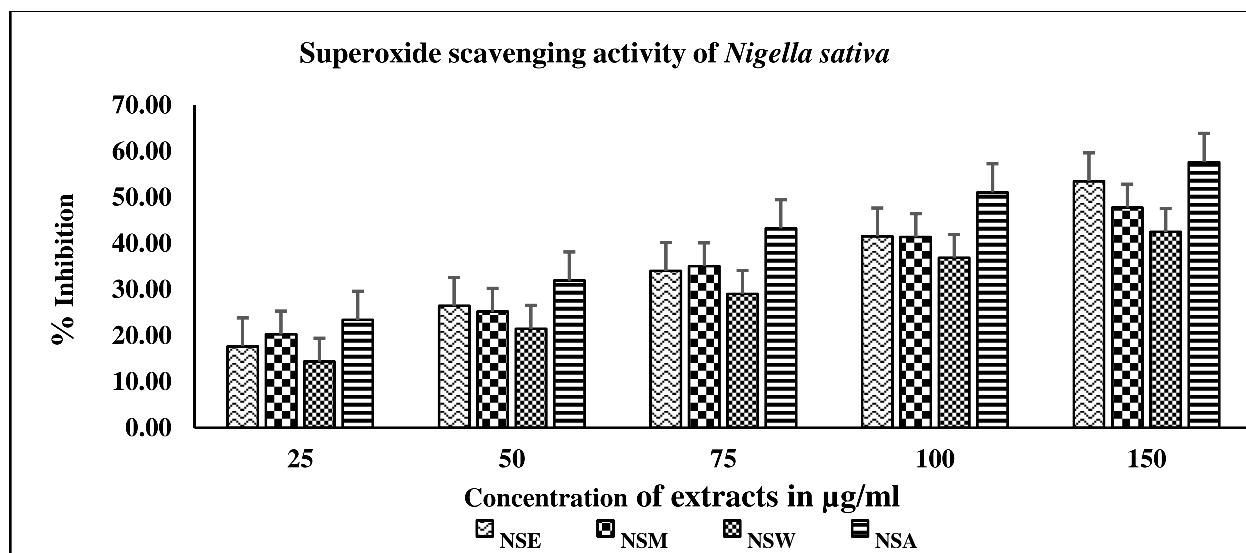


Fig 5.3: Superoxide ions scavenging activity by seed extracts of *N. sativa* with different concentrations. (NSE-*N. sativa* ethanolic extract, NSM- *N. sativa* methanolic extract, NSW- *N. sativa* water extract and NSA- *N. sativa* acetonic extract)

As concentration of extracts were increased the scavenging activity of superoxide ions increased. Maximum scavenging activity was observed and recorded for acetonic extracts of seeds derived from *N. sativa* followed by methanolic extract then ethanolic and least was recorded for aqueous extract. Superoxide scavenging activity of these extracts establish the antioxidant potential of seed extracts. All types of extracts shown the superoxide scavenging activity.

B. *Syzygium cumini* extracts

Table 5.5: Superoxide scavenging activity of *S. cumini* extracts, values are mentioned as Mean of triplicate of each extract with Standard Deviation. (SCE- *S. cumini* ethanolic extract, SCM- *S. cumini* methanolic extract, SCW- *S. cumini* water extract and SCA- *S. cumini* acetonic extract)

Conc (µg/ml)	SCE	SCM	SCW	SCA
25	12.37±0.15	14.33±0.23	17.23±0.30	14.64±0.31
50	18.73 ±0.42	21.83±0.49	30.19±0.60	23.59±0.70
75	25.73±0.61	31.27±0.99	44.00±0.55	33.67±0.64
100	35.73±1.79	39.07±0.99	58.27±0.40	44.36±0.74
150	34.73±0.76	47.23±0.74	79.66±0.40	56.21±0.31

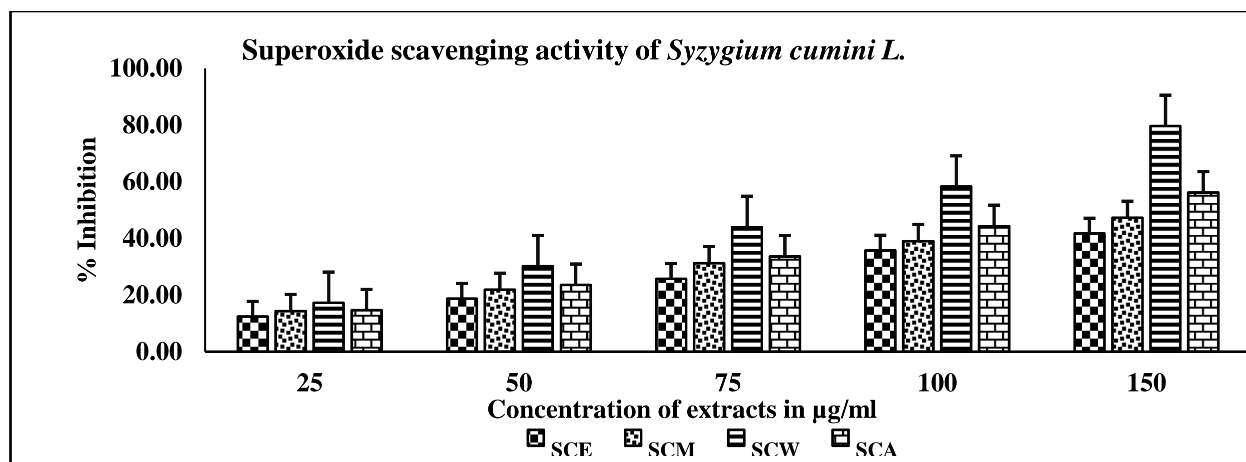


Fig 5.4: Superoxide ions scavenging activity by seed extracts of *S. cumini* with different concentrations. (SCE- *S. cumini* ethanolic extract, SCM- *S. cumini* methanolic extract, SCW- *S. cumini* water extract and SCA- *S. cumini* acetonic extract)

As concentration of extracts increased likewise scavenging activity for superoxide ions were increased. Maximum scavenging activity was recorded for aqueous extract of *S, cumini* while minimum was recorded for ethanolic extracts. Scavenging activity of extracts derived from seeds of *S. cumini* establish the antioxidant potential of extract. When the concentration of extracts was taken 200 µg/ml the %inhibition was found almost near to 150 µg/ml extract concentration, which is not shown in data.

5.2.3: IRON CHELATION ACTIVITY

A. *Nigella sativa* extracts

Table 5.6: Iron chelation activity of *N. sativa* extracts. Values are represented as Mean of Triplicate of Each Extract with Standard Deviation at different concentration. (NSE-*N. sativa* ethanolic extract, NSM- *N. sativa* methanolic extract, NSW- *N. sativa* water extract and NSA- *N. sativa* acetonic extract)

Conc (µg/ml)	NSE	NSM	NSW	NSA
25	21.07±0.61	22.60±0.53	16.93±0.61	20.97±0.57
50	33.27 ±0.99	32.40±0.20	24.93±0.81	28.33±0.12
75	40.80±0.60	41.40±0.20	31.33±0.64	36.50±0.30
100	50.13±1.27	48.60±0.20	37.47±1.03	43.90±0.46
150	59.73±0.99	56.60±0.53	46.90±0.56	53.73±0.12

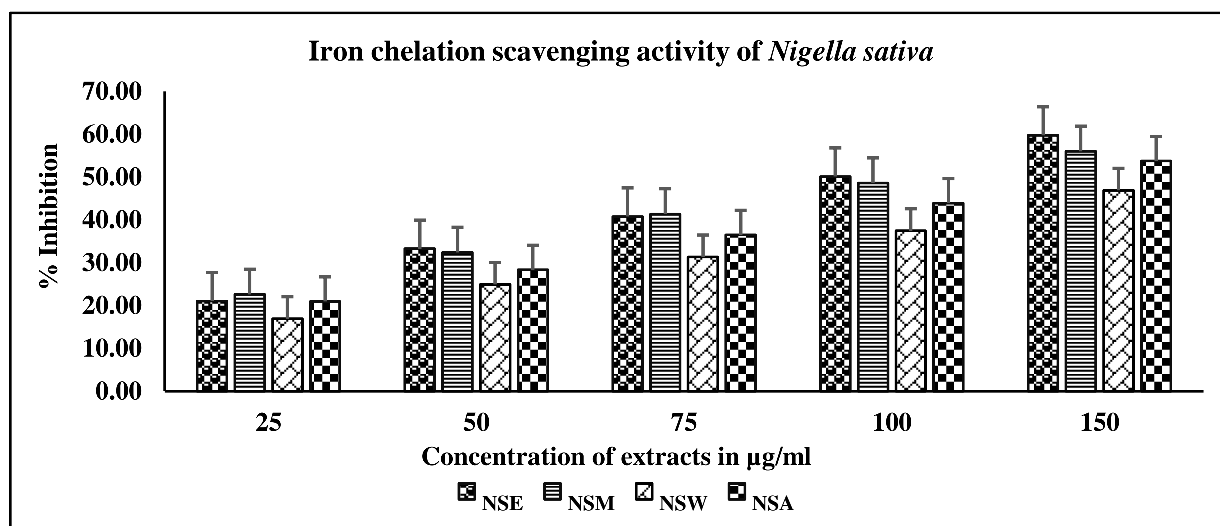


Fig 5.5: Iron chelation activity by seed extracts of *N. sativa* with different concentrations (NSE-*N. sativa* ethanolic extract, NSM- *N. sativa* methanolic extract, NSW- *N. sativa* water extract and NSA- *N. sativa* acetonic extract)

As concentration of extracts were increased the chelation activity of ferrous ions were also increased. Maximum activity was recorded in ethanolic extracts of *N. sativa*. The chelation activity defines the antioxidant potential of seed extracts derived from *N. sativa.*

C. *Syzygium cumini* extracts

Table 5.7: Iron chelation activity of *S. cumini* extracts, values are Mean of triplicate of each extract with Standard Deviation. (SCE- *S. cumini* ethanolic extract, SCM- *S. cumini* methanolic extract, SCW- *S. cumini* water extract and SCA- *S. cumini* acetonic extract)

Conc (µg/mL)	SCE	SCM	SCW	SCA
25	16.37±0.15	20.37±0.15	15.37±0.15	23.37±0.51
50	22.43 ±0.15	28.07±0.42	21.50±0.10	30.53±0.31
75	28.73±0.42	35.40±0.20	28.33±0.64	38.67±0.12
100	33.87±0.55	40.93±0.81	32.67±0.12	45.43±0.21
150	42.63±0.15	48.20±0.61	40.57±0.12	54.87±0.31

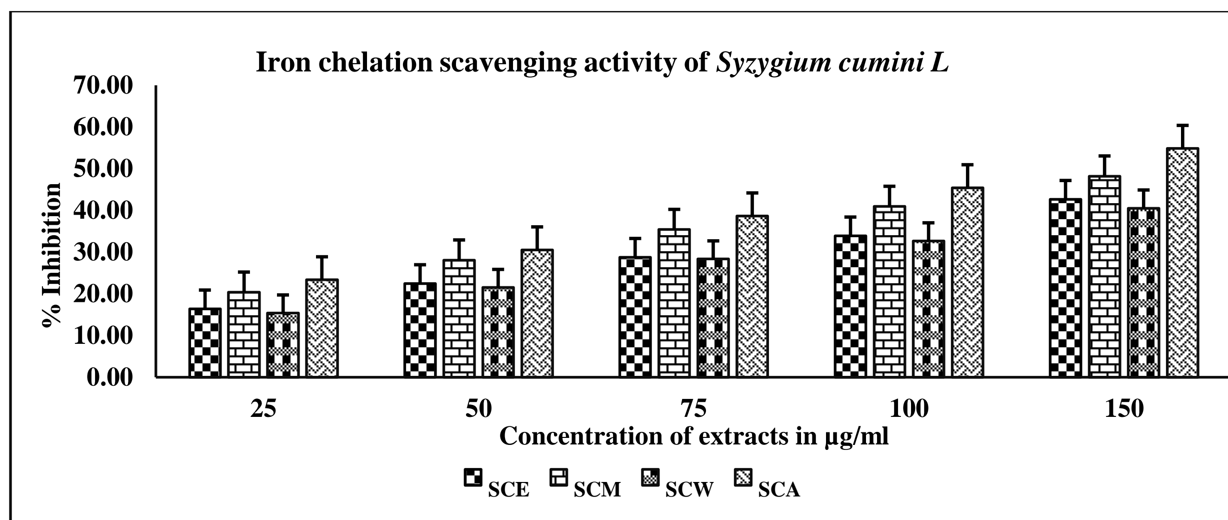


Fig 5.6: Iron chelation activity by seed extracts of *S. cumini* with different concentrations (SCE- *S. cumini* ethanolic extract, SCM- *S. cumini* methanolic extract, SCW- *S. cumini* water extract and SCA- *S. cumini* acetonic extract)

As concentration of extracts were increased the chelation activity of ferrous ions were also increased. Maximum activity was recorded in acetonic extracts of *S. cumini*. The chelation activity defines the antioxidant potential of seed extracts derived from *S. cumini.*

5.3: FTIR SPECTRUMS OF DIFFERENT EXTRACTS

5.3.1: FTIR spectrum of *Nigella sativa* extracts (Nurrulhidayah *et al*., 2011)

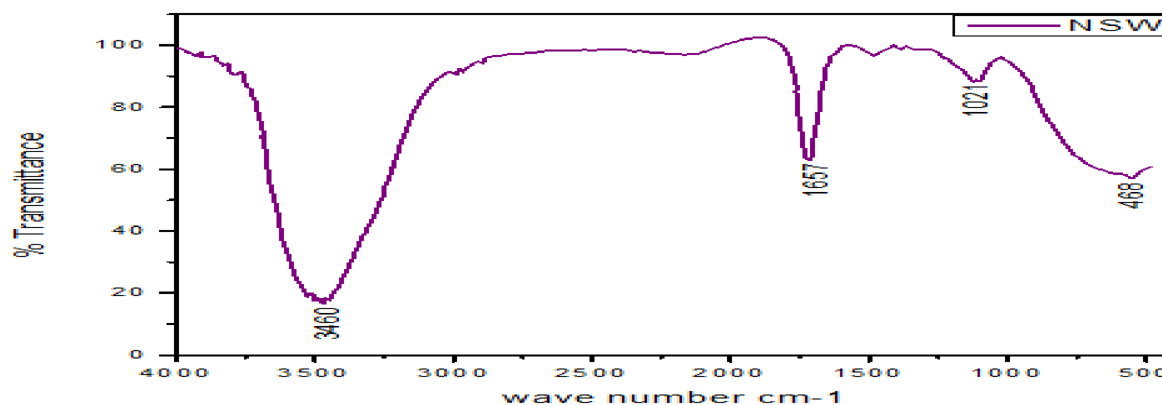


Fig 5.7: FTIR spectrum of aqueous extract *Nigella sativa*

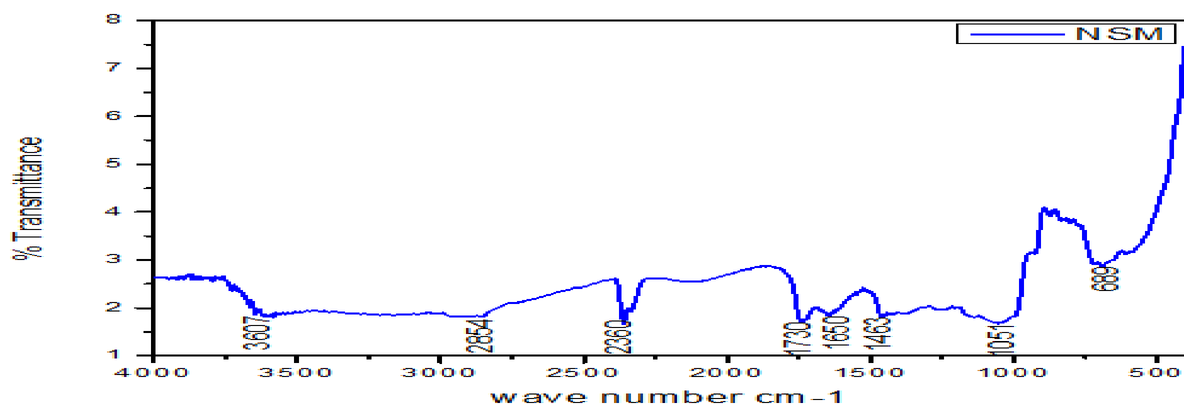


Fig 5.8: FTIR spectrum of methanolic extract of *Nigella sativa*

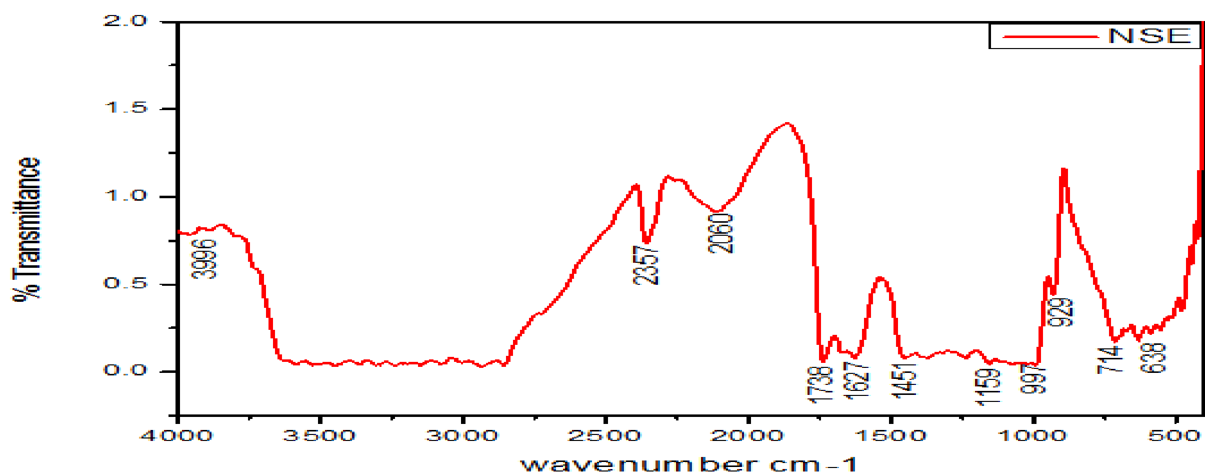


Fig 5.9: FTIR spectrum of ethanolic extract of *Nigella sativa*

Different peak value obtained in all the above three extracts in fig 5.7, fig 5.8, and fig 5.9 confirms the availability of different functional group containing molecule. Likewise, the peak value 3996, 3607, and 3440 reflects the high energy stretch and show availability of N-H bond which determines the presence of Amine group in extracts. Similarly, the obtained peak 2854, 2360 and 2357 is corelated with stretch of C-H bond and simultaneously alkane group containing biomolecules can be established. The peak value obtained at 1738, 1730, 1650, and 1637 reflect the presence of stretch of C=O bond which is present in different organic acids. The peak value 1461 and 1453 reflect the presence of bending of C-H bond against alkane group containing biomolecules. The peak value 1159 (ester), 1051, and 1021 depicts the presence of ester group and alkyl group containing molecules. The peak value 714, 689, 638, 477, and 468 confers the availability of alkene group containing biomolecules.

The FT-IR spectrum mentioned in fig 5.7, fig 5.8 and fig 5.9 reveal the presence of various bioactive molecules containing molecules in seed extracts of *N. sativa* which may play vital role as an antioxidant agent as well as antimicrobial activity. These biomolecules may play important role in reducing the various toxicity effects in vertebrates and can improve the functioning of vital organs and these molecules can also affect the immunomodulation effects in the organisms by triggering various pathways of immune system either humoral or cell mediated immune system pathway.

5.3.2: FTIR spectrum of *Syzygium cumini* extracts

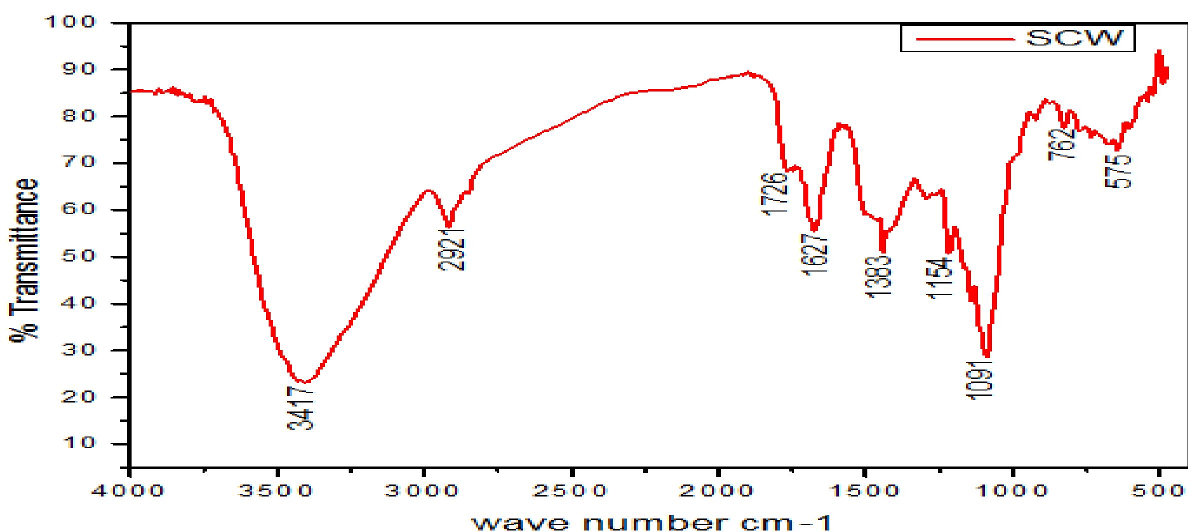


Fig 5.10: FTIR spectrum of aqueous extract of *Syzygium cumini*

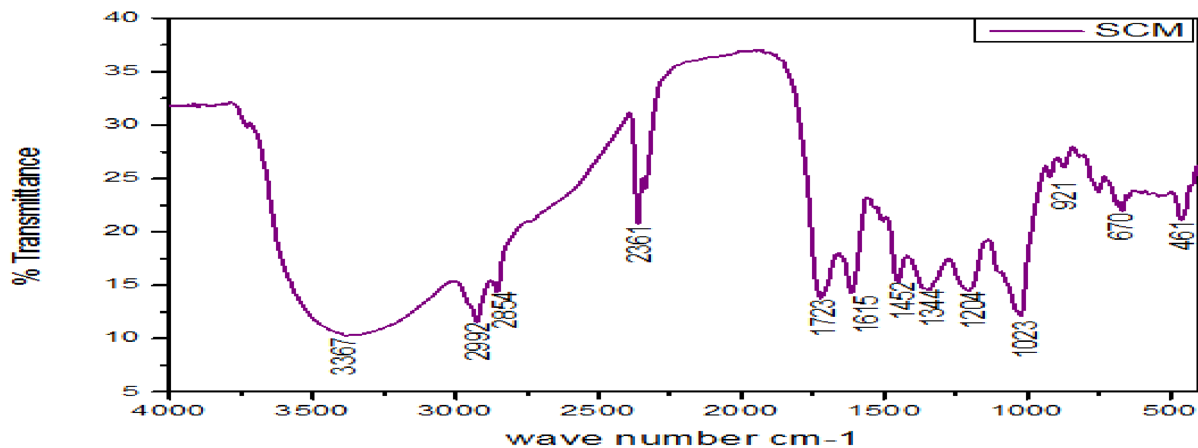


Fig 5.11: FTIR spectrum of methanolic extract of *Syzygium cumini*

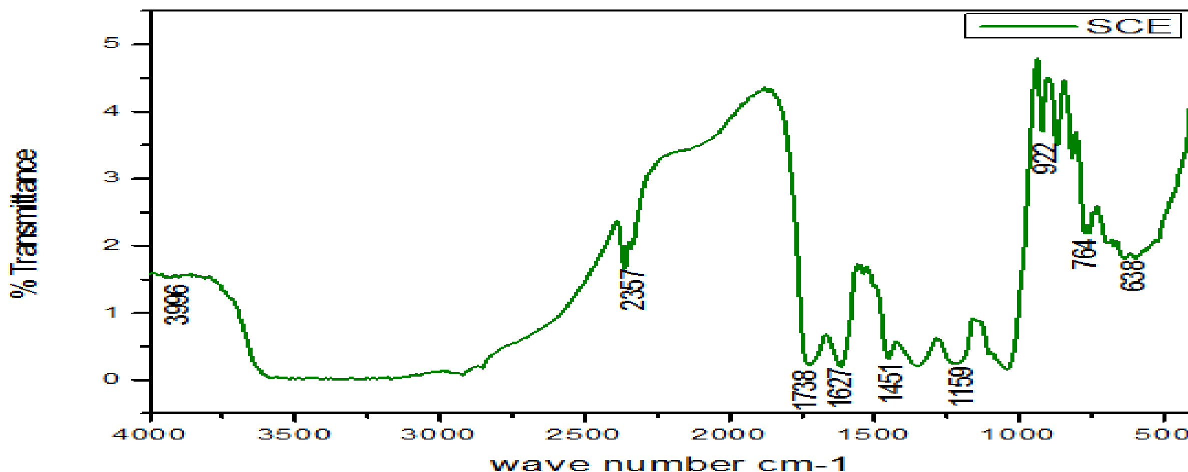


Fig 5.12: FTIR spectrum of ethanolic extract of *Syzygium cumini*

The presence of various functional group in seed extracts of *Syzygium cumini* can be confirmed with various peak obtained in above FTIR spectrum in fig 5.10, fig 5.11 and 5.12. The peak values are ranging from wavelength 4000-500 cm^{-1}. The peak values 3417, 3370 and 3361 reveals the presence of alcohol and carboxylic acids containing biomolecules and confers the presence of O-H and C-O stretch. The peak values 2925, 2923, 2854, 2361, and 2360 reveals the presence of N-H stretch. The peak value at wavelength 1721, 1726, 1627, 1616, and 1615 confers the availability of amine group (N-H stretch), aromatic ring (C=C stretch), and nitro compounds (NO_2). The peak at wavelength 1452, 1451, 1383, 1362, 1344, 1204, and 1154 reflects the presence of biomolecules containing functional group like, alkenes (C=C stretch), and C-N stretch mainly. The peak value at wavelength 1077, 1040, 1023, 1019 depicts the presence of mainly C=S stretch and C-N stretch containing biomolecules. The peak value obtained at wavelength below 1000 are 921, 871, 870, 817, 774, 762, 751, 670, 637, 598, and 575 confers the presence of mainly alkenes and alkynes (C-H bend) containing biomolecules.

These biomolecules are mainly present into alkaloids, flavonoids, tannins, saponins, amino acids, proteins and other bioactive ingredients of phytoconstituents which are major factor of antioxidant activity, antimicrobial activity, cytotoxicity and immunomodulating agents. The bioactive compounds are also able to reduce the toxic effects due to exposure of pesticides and other toxic agents in *in vitro* as well as *in vivo* conditions in vertebrates and invertebrates.

5.4: HPLC SPECTRUM AND CALCULATIONS

5.4.1: HPLC of *N. sativa* extract

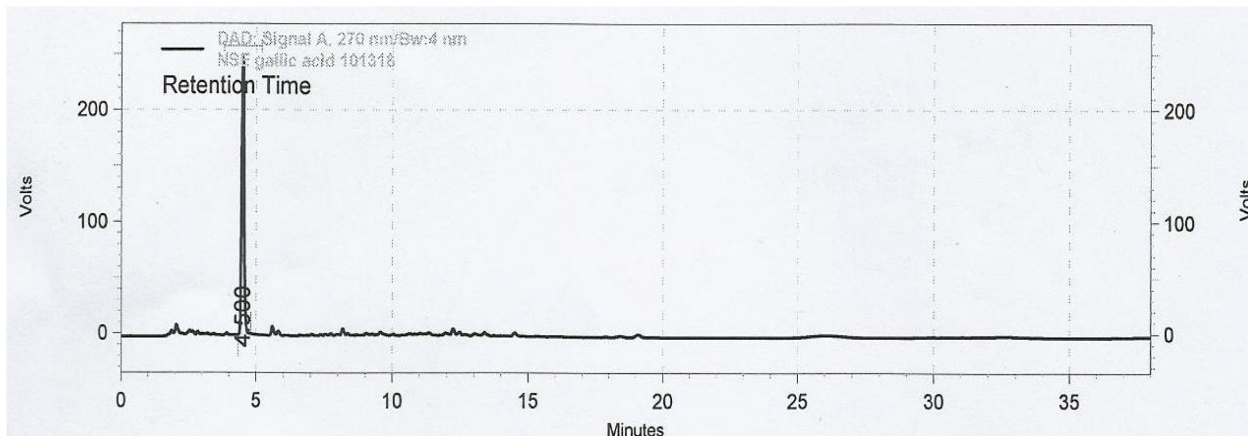


Fig 5.13: HPLC chromatogram of *Nigella sativa* ethanolic extracts with standard Gallic acid

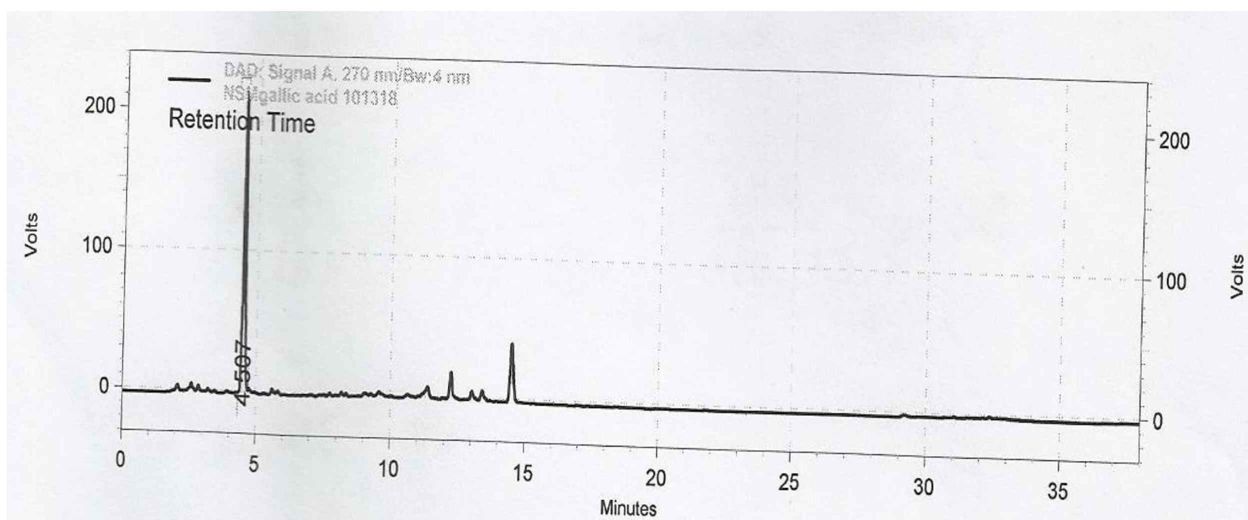


Fig 5.14: HPLC chromatogram of *Nigella sativa* methanolic extracts with standard Gallic acid

Calculation:

Nigella sativa ethanolic extract analysis: based on peak values mentioned in fig 5.13 and 5.14 the following observation are

Retention time: 4.500 minute

Wavelength: 270nm.

Injection volume: 5µl

Assay: Area of test= 3155738

Area of Standard= 5816657

$$\frac{3155738}{5816657} \times \frac{11.35}{50} \times \frac{100}{218.01} \times 99\% = 6.74\%$$

Test solution =180.77 mg in 100ml of water

Nigella sativa methanolic extract analysis:

Retention time: 4.507 minute

Wavelength: 270nm.

Injection volume: 5µl

Assay: Area of test= 3155738

Area of Standard= 5816657

$$\frac{2738007}{5816657} \times \frac{11.35}{50} \times \frac{100}{218.01} \times 99\% = 2.07\%$$

Test solution= 510.73 mg in 100 ml of water

5.4.2: HPLC spectrum of seed extracts of *Syzygium cumini*

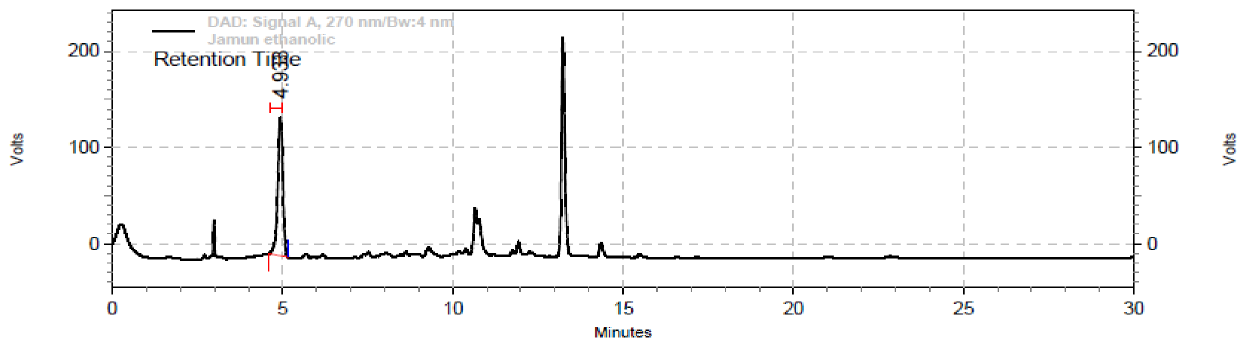


Fig 5.15: HPLC chromatogram of *Syzygium cumini L.* ethanolic extracts with standard Gallic acid

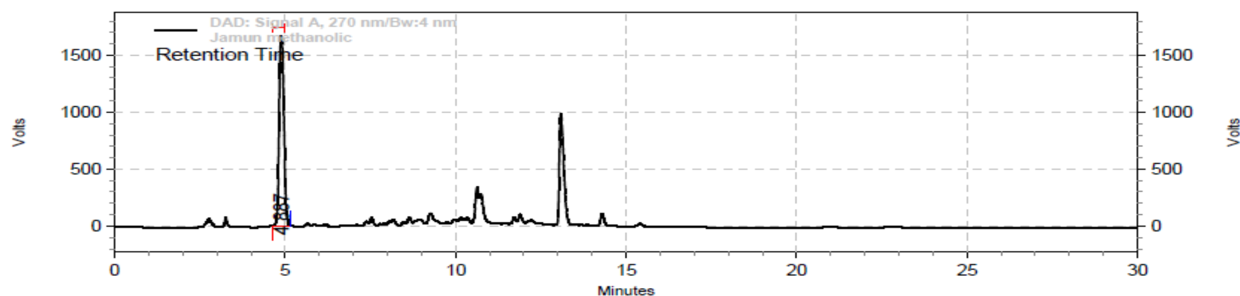


Fig 5.16: HPLC chromatogram of *Syzygium cumini L.* methanolic extracts with standard Gallic acid

Calculation:

Syzygium cumini L. ethanolic extracts analysis: based on fig 5.15 and fig 5.16 following observation are recorded

Retention time: 4.933 minute

Wavelength: 270nm.

Injection volume: 5µl

Assay: Area of test= 3087563

Area of Standard= 5816657

$$\frac{3206929}{5816657} \times \frac{11.35}{50} \times \frac{100}{454.56} \times 99\% = 2.72\%$$

Test solution =454.56 mg in 100ml of water

Syzyium cumini L. methanolic extracts analysis:

Retention time: 4.837 minute

Wavelength: 270nm.

Injection volume: 5µl

Assay: Area of test= 3444120

Area of Standard= 5816657

$$\frac{3444120}{5816657} \times \frac{11.35}{50} \times \frac{100}{218.01} \times 99\% = 6.10\%$$

Test solution =218.01 mg in 100ml of water

HPLC-DAD reveals the presence of gallic acid in seed extracts of *S. cumini* and *N. sativa* when compared with standard gallic acid analysis. Gallic acid also plays an important role as an antioxidant agent.

5.4.3: HPLC spectrum of seed extracts of *Nigella sativa* with Thymoquinone

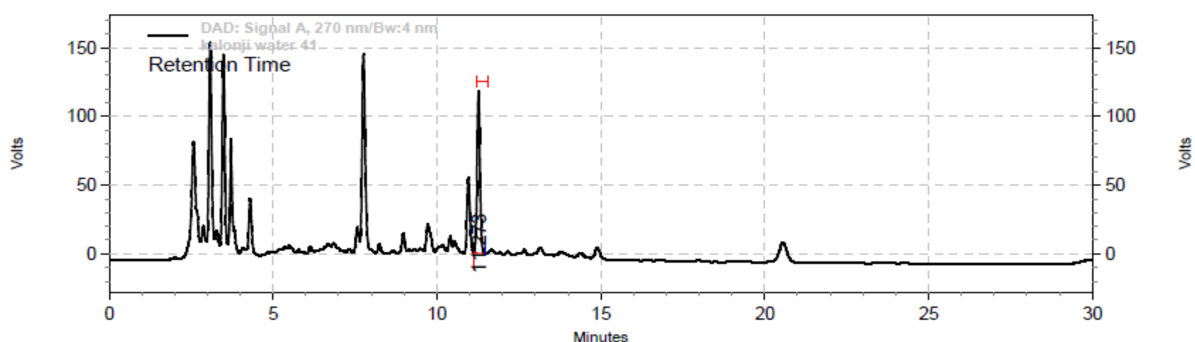


Fig 5.17: Water extract of *Nigella sativa* with Thymoquinone as standard

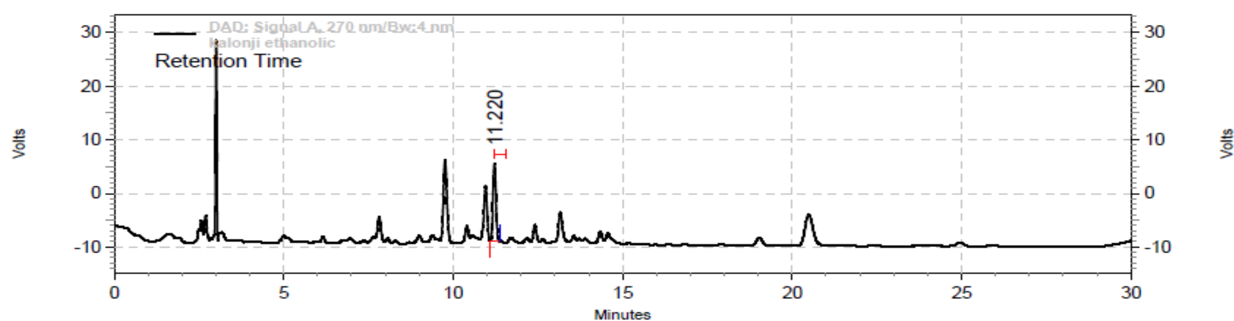


Fig 5.18: Ethanolic extract of *Nigella sativa* with Thymoquinone as standard

Calculation: based on peak value obtained in fig 5.17 and fig 5.18 calculation can be made in following way-

Water extract of *Nigella sativa* with Thymoquinone as standard

$1534232/2102843 \times 5.2/10 \times 1/10 \times 10/10.20 \times 99 = 3.68\%$

Ethanolic extract of *Nigella sativa* with Thymoquinone as standard as shown in fig.

$181336/2102843 \times 5.2/10 \times 1/10 \times 10/11.3 \times 99 = 3.93\%$

In HPLC results presence of thymoquinone was confirmed.

5.5: ANTIBACTERIAL ANALYSIS OF SEED EXTRACTS

5.5.1: Antibacterial effects of seed extracts of *N. sativa:* Table 5.8: *N. sativa* seed extracts showing zone of inhibition (Mean of triplicates) against bacteria with MTCC number and compared with standard antibiotics Chloramphenicol (CHL) and Gentamicin (GEN). MeOH- Methanolic extract, Acetone- Acetonic extract, EtOH – Ethanolic extract.

Bacteria (MTTC)	CHL	GEN	Water	MeOH	Acetone	EtOH
B. subtilis (121)	17.00	19.67	9.00	11.33	11.33	10.33
S. typhimurium (3231)	15.00	13.67	3.67	10.33	6.33	8.33
B. subtilis (441)	16.67	15.33	9.67	12.67	10.67	11.33
S. enteric (1164)	15.67	13.67	8.67	10.67	6.33	8.67
S. aureus (96)	17.00	15.33	4.67	10.33	6.67	7.33
B. subtilis (1305)	17.67	15.33	9.00	11.33	6.33	8.33
S. aureus (7443)	18.00	18.00	8.33	10.67	9.00	9.33
E. coli (40)	10.33	17.33	7.33	9.33	8.67	8.33
B. cereus (430)	13.00	15.33	7.33	8.67	8.67	7.33
B. cereus (2086)	15.67	16.33	8.00	9.67	8.67	8.33

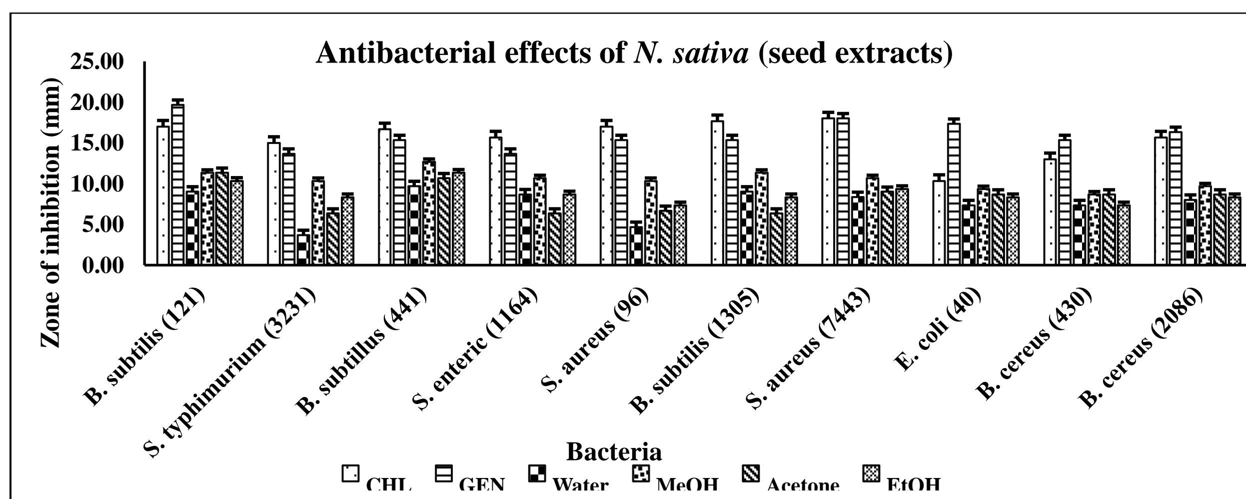


Fig 5.19: Antibacterial effects of seed extracts derived from *N. sativa* (CHL- Chloramphenicol, GEN – Gentamicin, MeOH- Methanolic extract, Acetone- Acetonic extract, EtOH – Ethanolic extract).

5.5.2: Antibacterial effects of seed extracts of *S. cumini*

Table 5.9: *S. cumini* seed extracts showing zone of inhibition (Mean of triplicates) against bacteria with MTCC number and compared with standard antibiotics. (CHL- Chloramphenicol, GEN – Gentamicin, MeOH- Methanolic extract, Acetone- Acetonic extract, EtOH – Ethanolic extract).

Bacteria (MTTC)	CHL	GEN	Water	MeOH	Acetone	EtOH
B. subtilis (121)	17.00	19.67	9.00	11.33	11.33	10.33
S. typhimurium (3231)	15.00	13.67	3.67	10.33	6.33	8.67
B. subtilis (441)	16.67	15.33	9.67	11.67	10.67	11.33
S. enteric (1164)	15.67	13.67	8.67	10.67	6.33	9.33
S. aureus (96)	17.00	15.33	4.67	10.33	6.67	9.67
B. subtilis (1305)	17.67	15.33	9.00	10.33	6.33	11.67
S. aureus (7443)	18.00	18.00	8.33	10.67	9.00	10.33
E. coli (40)	10.33	17.33	7.33	9.67	8.67	10.67
B. cereus (430)	13.00	15.33	7.33	8.67	8.67	9.33
B. cereus (2086)	15.67	16.33	8.00	9.67	8.67	9.67

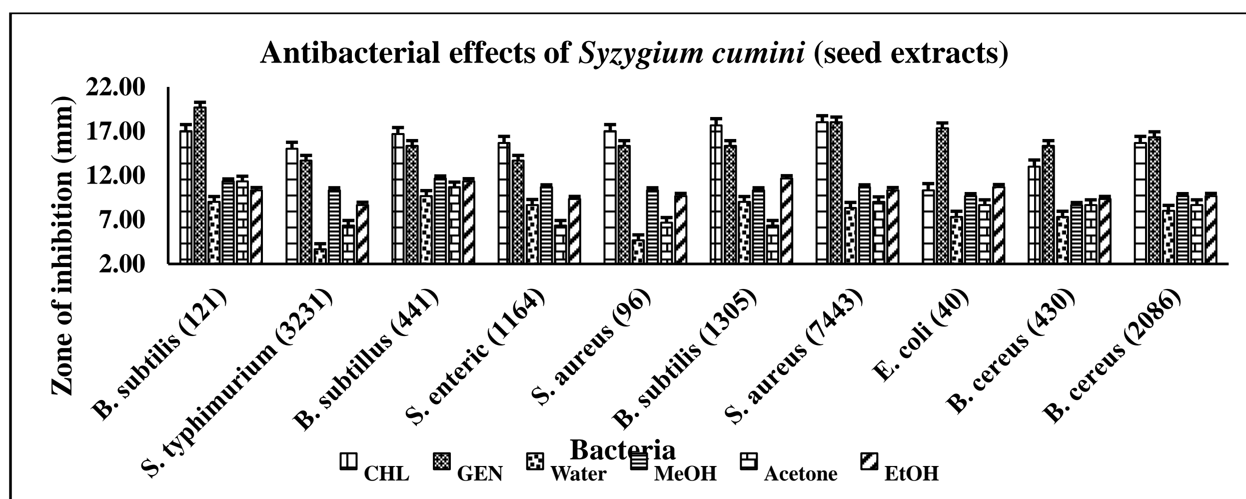


Fig 5.20: Antibacterial effects of seed extracts derived from *S. cumini* (CHL- Chloramphenicol, GEN – Gentamicin, MeOH- Methanolic extract, Acetone- Acetonic extract, EtOH – Ethanolic extract).

5.6: Antifungal effects of seed extracts

5.6.1: Antifungal effects of seed extracts of *Nigella sativa*

Table 5.10: Antifungal effects of seed extracts of *N. sativa* in mean of triplicates (NSE-*N. sativa* ethanolic extract, NSM- *N. sativa* methanolic extract, NSW- *N. sativa* water extract and NSA- *N. sativa* acetonic extract with 10, 20 and 30 µg concentration. Zone of inhibition shown in mm.

Fungi (MTCC)	NSE 10 µg	NSE 20 µg	NSE 30 µg	NSM 10 µg	NSM 20 µg	NSM 30 µg	NSW 10 µg	NSW 20 µg	NSW 30 µg	NSA 10 µg	NSA 20 µg	NSA 30 µg
S. cerevisiae (MTCC464)	9.07	10.57	11.63	6.03	7.20	8.29	2.40	3.40	5.17	3.40	4.87	5.80
C. albicans (MTCC183)	5.77	6.77	8.10	7.37	5.30	6.92	3.37	4.47	5.47	2.93	4.07	5.47
A. niger (MTCC281)	6.77	8.53	9.40	4.33	4.47	6.07	2.93	6.17	8.10	3.53	4.87	6.13
A. parasiticus (MTCC696)	4.23	5.57	8.47	5.37	6.37	6.73	2.67	4.07	6.73	3.33	4.47	6.93

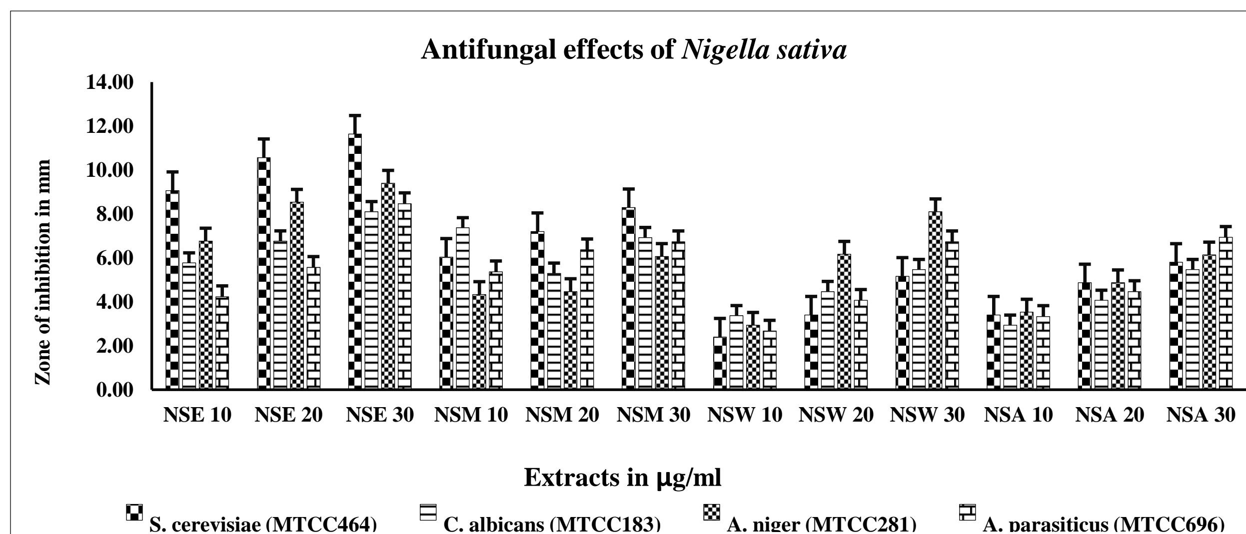


Fig 5.21: Antifungal effect of seed extract derived from *N. sativa* (Standard Error Mean). (NSE- *N. sativa* ethanolic extract, NSM- *N. sativa* methanolic extract, NSW- *N. sativa* water extract and NSA- *N. sativa* acetonic extract)

The observed antifungal activity is mentioned in table 5.10 and analysis is represented in fig 5.21. The ethanolic extracts of *N. sativa* has shown highest level of antifungal properties followed by methanolic extracts and acetonic extracts though, least antifungal effects were observed in aqueous extract of *N. sativa* against four strains of fungi. All four types of extracts shown antifungal activity against four test strains. *Candida albicans* shown more resistatnt towards ethanolic, acetonic and aqueous extract while *Aspergillus niger* has shown most resistant towards methanolic extract. The bioactive compounds present in seed extracts of *Nigella sativa* causes these antifungal activity. Forouzanfar mentioned the antimicrobial effects of *N. sativa* extracts in 2014 and similar findings have been reported by Emeka *et al*., in 2015. Rogozhin *et al*. in 2011 isolated two molecules from *N. sativa* extract Ns-D1 and Ns-D2 defensin molecues and shown antifungal activity against plant pathogenic fungi like, *Phytophthora infestans* and other oomycetes. These defensins are mainly bioactive molecues which shows the antifungal activity. Khan *et al*., reported in 2003 that aqueous extract of *N. sativa* can minimize the *Candida albicans* effects in in-vivo conditions. Aljabre *et al*, in 2005 and Mahmoudvand *et al*. in 2014, reported that thymoquinone the active molecule of *N. sativa* seeds and its extract has anti-dermatophyte potential against pathogenic dermal fungi like, *Trichophyton rubrum, Microsporum gypseum*, *Microsporum canis*, and *Trichophyton mentagrophytes* species. Bita *et al*. in 2012, found that extract of *N. sativa* has antifungal activity against pathogenic *Candida albicans* similarly our findings also confirms that the extract derived from seeds of *N. sativa* shown antifungal activity against *C. albicans*. Sitara *et al* in 2008 reported that extracts of *N. sativa* have antifungal activity against *Aspergillus niger*, and *A. flavus* species which are also pathogenic species for human beings, so *N. sativa* extracts can be utilized for antifungal activity and drug formulations too for affective treatment against these pathogenic fungi. Hader *et al*. reported in 1993 about hypoglycemic effects of *N. sativa* due to its phytoconstituents. Ghamdi in 2001 reported anti-inflammatory, analgesic and antipyretic activity of *N. sativa* extracts. Enazi *et al*. in 2007 reported that, thymoquinone present in seed extract of *N. sativa* can reduce the oxidative stress condition and improve the diabetic condition in mice model system. Alhebshi *et al*. reported neuroprotective effects of thymoquinone in cultured neurons of rats due to amyloid β-induced neurotoxicity in 2013.

5.6.2: Antifungal effects of seed extracts of *Syzygium cumini*

Table 5.11: Table: Antifungal effects of seed extracts of *S. cumini* in mean of triplicates (SCE- *S. cumini* ethanolic extract, SCM- *S. cumini* methanolic extract, SCW- *S. cumini* water extract and SCA- *S. cumini* acetonic extract with 10, 20 and 30 µg concentration). Zone of inhibition mentioned in table is in mm.

Fungi (MTCC)	SCE 10 µg	SCE 20 µg	SCE 30 µg	SCM 10 µg	SCM 20 µg	SCM 30 µg	SCW 10 µg	SCW 20 µg	SCW 30 µg	SCA 10 µg	SCA 20 µg	SCA 30 µg
S. cerevisiae (MTCC464)	3.87	4.40	6.93	8.43	9.67	11.00	3.93	5.90	7.73	5.73	7.43	11.40
C. albicans (MTCC183)	3.93	5.00	7.53	6.27	8.27	9.93	2.93	4.33	7.03	4.93	6.93	7.20
A. niger (MTCC281)	2.27	4.53	5.33	6.93	9.07	10.87	2.43	3.93	5.27	4.40	6.30	8.80
A. parasiticus (MTCC696)	2.47	5.10	7.33	5.60	8.47	10.40	3.13	4.47	5.67	3.73	7.27	8.60

In table 5.11 antifungal effects of *S. cumini* extracts is shown with three different concentration. The increased concentration of extracts not shown any significant increase in zone of inhibition so, increased concentration values are not shown. Zone of inhibition is shown in mm.

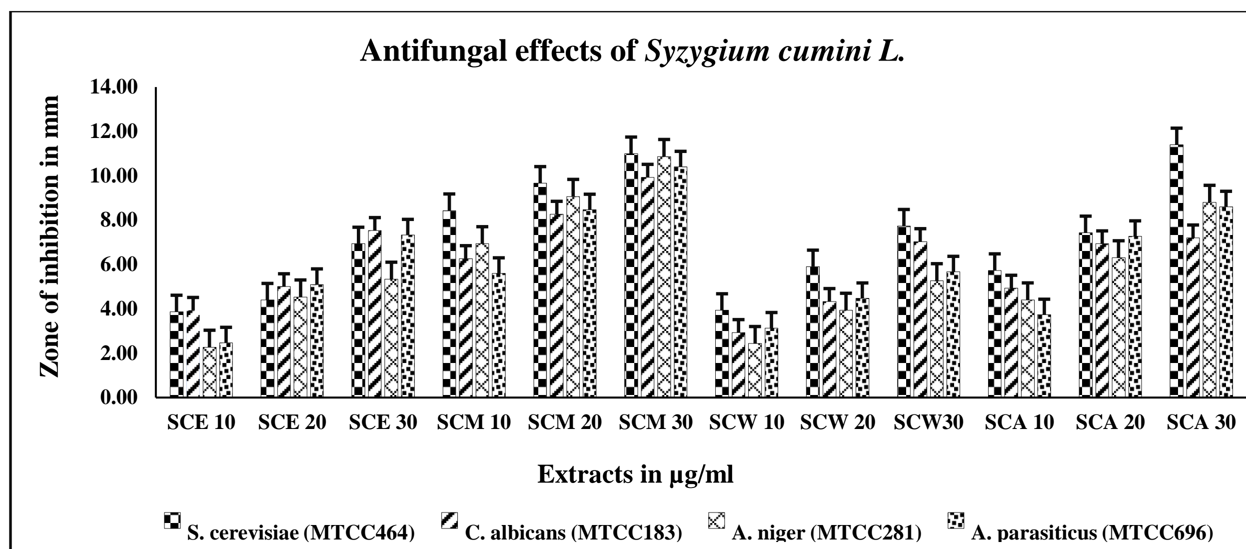


Fig 5.22: Antifungal effect analysis of seed extract derived from *S. cumini* (Standard Error Mean). (SCE- *S. cumini* ethanolic extract, SCM- *S. cumini* methanolic extract, SCW- *S. cumini* water extract and SCA- *S. cumini* acetonic extract with 10, 20 and 30 µg concentration).

The obtained value of antifingal activity mentioned in table 5.11 and analysis is represented in fig 5.22. Methanolic extracts derived fromm *S. cumini* L. have shown maximum antifungal effects in all four strains of fungi used in experiment, second most effective antifingal effects were observed in acetonic extracts followed by ethanolic extracts and least antifungal effects were observed in aqueous extract.

Arora and Kaur (1999) reported the antimicrobial activity of various spices against pathogenic bacteria and fungi. Benherlal and Arumughan in 2007 reported the chemical composition of *S. cumini* and its antioxidant activities. Bhuiyan also described the antibacterial activity of seed extracts of *S. cumini* against pathogenic bacteria. Kumar *et al*. in 2017 also reported the antibacterial effects of seed extracts of *S. cumini.* Many researchers also described the antibacterial and antifungal effects of seed extracts of *S. cumini* which supports our current research findings too.

OBJECTIVE 2: To investigate the cytotoxic effects of deltamethrin and seed extracts of *Nigella sativa* and *Syzygium cumini* in *in-vitro* state.

5.7: Results of invitro cytotoxic effects:

Table 5.12: Cytotoxic effects of Deltamethrin (DM), seed extract of *S. cumini L.* (SCM-*S. cumini* methanolic; SCE- *S. cumini* ethanolic) and *N. sativa L.* (NSM- *N. sativa* methanolic; NSE-*N. sativa* ethanolic). Results are shown in Average of triplicate i.e. Mean ± SD.

Sample	Concentration	SW480	A549	HeLa
Vinblastine (Pos Con)	**8.1098 µg/ml**	**95.4 ± 0.3**	**85.5 ± 3.4**	**79.7 ± 0.5**
DM	**100 µg/ml**	**14.4 ± 4.0**	**41.9 ± 3.1**	**6.2 ± 4.2**
DM	**200 µg/ml**	**38.4 ± 1.5**	**35.7 ± 1.7**	**10.4 ± 3.6**
NSM	**200 µg/ml**	**99.3 ± 0.8**	**99.3 ± 0.1**	**35.6 ± 1.2**
NSE	**200 µg/ml**	**34.5 ± 2.9**	**46.4 ± 1.3**	**18.6 ± 5.6**
SCM	**200 µg/ml**	**14.4 ± 2.9**	**36.5 ± 2.6**	**10.6 ± 2.0**
SCE	**200 µg/ml**	**12.6 ± 0.36**	**18.3 ± 2.5**	**6.8 ± 2.9**
DM+ NSM	**100 µg/ml + 200 µg/ml**	**98.2 ± 1.5**	**99.2 ± 0.3**	**34.6 ± 2.6**
DM + NSE	**100 µg/ml + 200 µg/ml**	**14.9 ± 4.9**	**17.9 ± 4.9**	**7.9 ± 4.3**
DM + SCM	**100 µg/ml + 200 µg/ml**	**31.7 ± 2.9**	**35.6 ± 3.1**	**10.2 ± 0.7**
DM +SCE	**100 µg/ml + 200 µg/ml**	**47.5 ± 5.3**	**58.6 ± 1.6**	**15.7 ± 2.0**
DM + SCE + NSE	**100 µg/ml + 100 µg/ml + 100 µg/ml**	**29.9 ± 4.8**	**54.6 ± 1.3**	**4.0 ± 3.4**
DM +SCM + NSM	**100 µg/ml + 100 µg/ml + 100 µg/ml**	**90.7 ± 2.3**	**98.4 ± 0.6**	**40.13 ± 0.3**

In the table the 5.12 the experimental protocol is described with obtained cytotoxicity on three cell lines- SW480, A549 and HeLa. Vinblastine was used as positive control and Deltamethrin was used as negative control with both the concentration used. As per the standard protocol of 48 hrs exposure time cytotoxicity was measured. The extracts effects on cytotoxicity or antiproliferative effects were also found in this experiment. The methanolic extract of *N. sativa* shown highly cytotoxic effects in all the three cell lines. The images obtained are shown in fig 5.23 to fig 5.25 for all three cell lines. The combitorial effects of deltamethrin and seed extracts also found significantly cytotoxic to all three cell lines used in experiment.

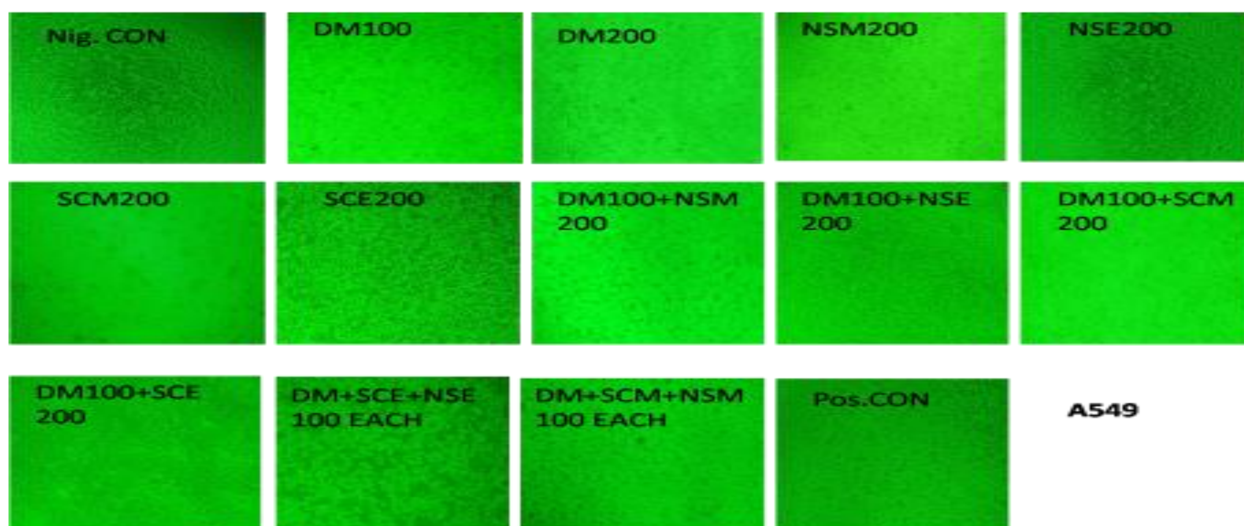


Fig 5.23: Cytotoxic effects on A549 cell line of DM- Deltamethrin, SCE- *S. cumini* ethanolic extract, SCM- *S. cumini* methanolic extract, NSE- *N. sativa* extract, NSM- *N. sativa* methanolic extract (alone and in combination).

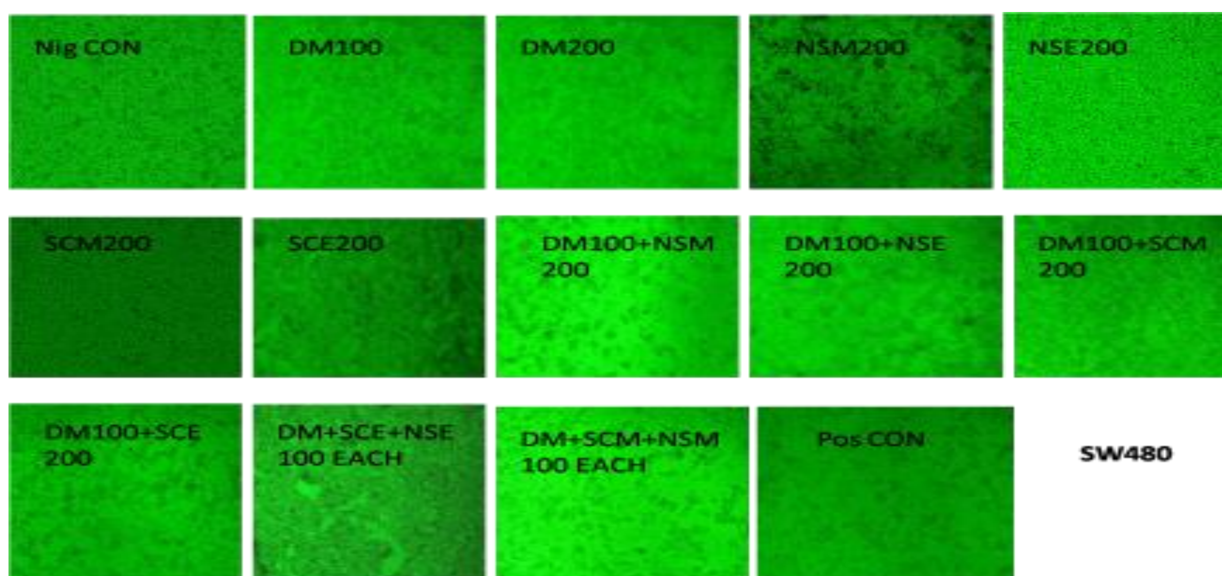


Fig 5.24: Cytotoxic effects on SW480 cell line of DM- Deltamethrin, SCE- *S. cumini* ethanolic extract, SCM- *S. cumini* methanolic extract, NSE- *N. sativa* extract, NSM- *N. sativa* methanolic extract (alone and in combination).

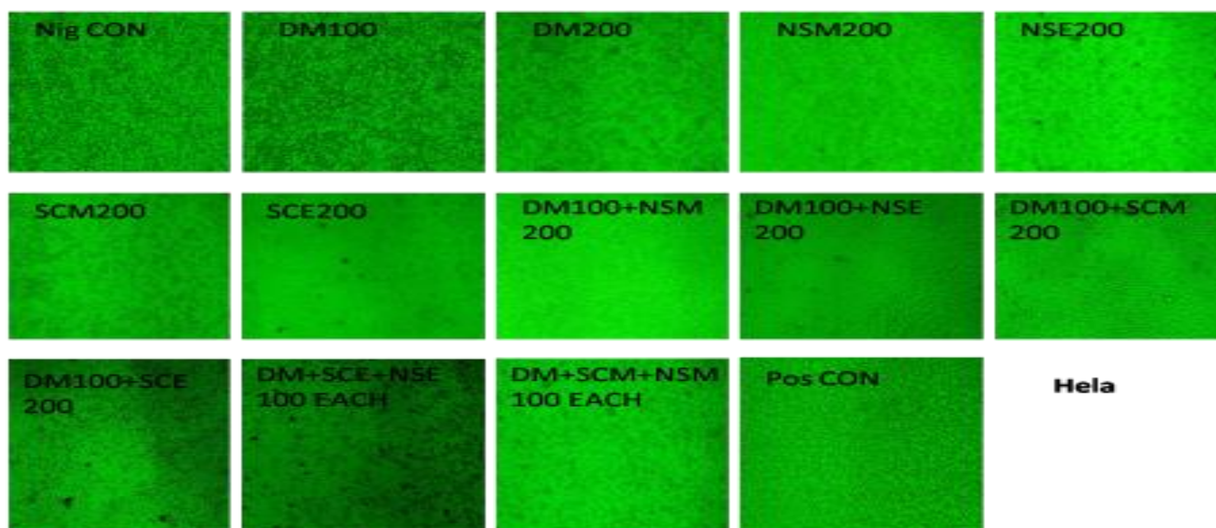


Fig 5.25: Cytotoxic effects on HeLa cell line of DM- Deltamethrin, SCE- *S. cumini* ethanolic extract, SCM- *S. cumini* methanolic extract, NSE- *N. sativa* extract, NSM- *N. sativa* methanolic extract (alone and in combination).

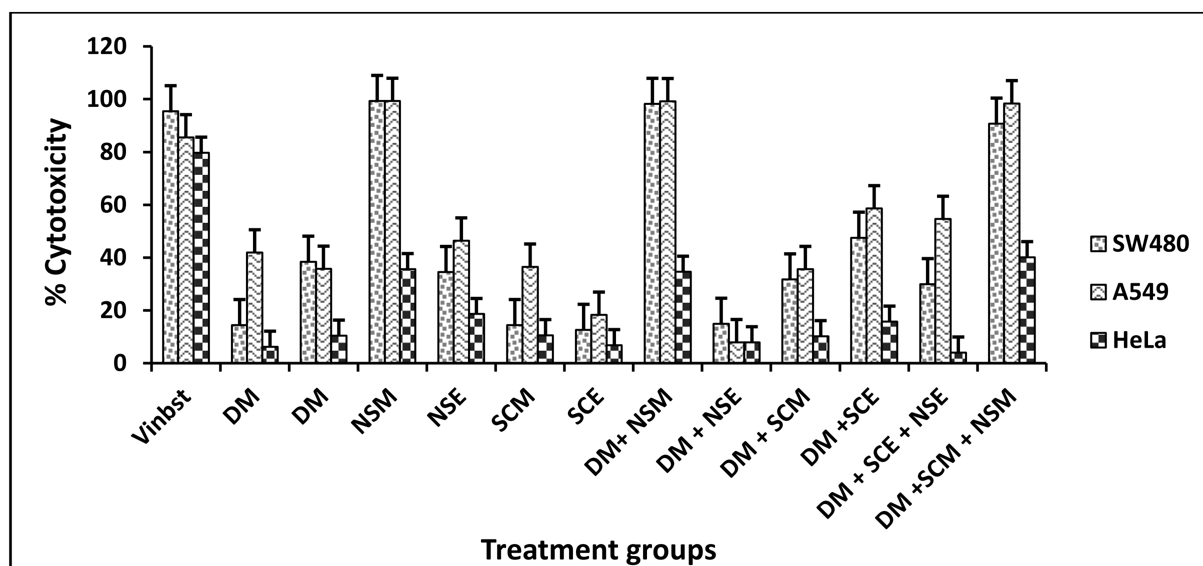


Fig 5.26: Cytotoxicity analysis of DM- Deltamethrin, SCE- *S. cumini* ethanolic extract, SCM- *S. cumini* methanolic extract, NSE- *N. sativa* extract, NSM- *N. sativa* methanolic extract (Values are expressed in Mean ±SD)

In table 5.12 cytotoxicity activity is mentioned for defined dose of deltamethrin, Vinblastine, and extracts derived from *N. sativa* and *S. cumini* L. In fig 5.23, fig 5.24 and fig 5.25 pictorial presentation of cell lines are shown for cytotoxicity effects. Analysis of cytotoxicity effects

mentioned in fig 5.26. Maximum cytotoxicity effects were observed in methanolic extracts of *N. sativa* for all three cell lines SW480, A549 and HeLa while ethanolic extracts shown less potential cytotoxicity effects cell lines. Moreover, *S. cumini* ethanolic as well as methanolic extracts have shown feebler antiproliferative and cytotoxic effects against all three cell lines. While deltamethrin alone with dose 100 µg/ml and 200 µg/ml shown the antiproliferative effects and cytotoxic effects against HeLa, SW480 and A549 cell line, but it has shown low cytotoxic effects against HeLa cell lines. In combinatorial effects of NSM and DM has shown effects against all three cell lines used in study, while NSE+DM has low cytotoxic effects against all three cell lines. SCM and DM combination shown the decent effects against two cell lines while against HeLa cell line cytotoxic effects were noted deprived. SCE and DM in combination shown effects against all three cell lines. DM, SCE and NSE combination shown against all three cell lines but least was recorded for HeLa cell lines while DM, SCM and NSM combination shown more cytotoxicity effects on all three cell lines than the previous combination.

All living organisms are having potential threats to different groups of pesticides due to its over exploitation in agriculture or disease control programs. To minimize the effects of pesticides various synthetic chemicals and phytochemicals are explored and used as various drugs and nutraceuticals. In the present research study, it has been established that deltamethrin pesticides can lead to cytotoxic condition and can lead to lethal effects for cell system. Findings of current research studies suggest that the seed extracts of *S. cumini* and *N. sativa* contains so many valuable bioactive molecules which have shown antioxidant, antifungal, antiproliferative as well as anti-toxicity against deltamethrin pesticide. Armella *et al*. observed the cytotoxicity of deltamethrin and DECIS on various cell lines. The antioxidant and cytotoxic activity obtained was like Banerjee & Narendhirakannan (2011) and Kumar *et al* (2017) findings for *S. cumini* and for *N. sativa.* The cytotoxicity effects on lung cancer cell line was reported by Al-Sheddi *et al* (2014). Selection of human cell lines in the current research studies considered for antiproliferative (SW480, A549) and anti-cancerous (HeLa) studies, deltamethrin can easily pass through membrane and can cause the lethal effects on cell survival or can turn cancerous cells more cancerous. Pei *et* al., (2016) shown that the thymoquinone inhibits the angiotensin II derived cell proliferation activity. Cell division rate may increase or decrease due to deltamethrin pesticide exposure, so to encounter this effect the phytochemicals may be effective force to minimize the damage to cell system in invitro conditions. Romero *et al.,* (2012) shown that deltamethrin induced cytotoxicity can be prohibited

by various antioxidants in SH-SY5Y cells, similarly the findings of Kumar *et al.,* (2018) disclose that piperine and curcumin can reduce the toxic effects of deltamethrin similarly the bioactive compounds present in seed extracts of *S. cumini* and *N. sativa* can be explored for in-vivo studies and our in-vitro findings also support that the whole seed extracts or bioactive molecules derived from seed extracts of *S. cumini* and *N. sativa* can also show the protective effects against deltamethrin pesticides toxicity. Ilboudo *et al.,* (2014) shown that deltamethrin cytotoxicity effects can be reduced by using some defined antioxidants like, vit C, vit E, and Trolox and our current research findings also suggests that phytochemicals obtained from seeds extracts of *N. sativa* and *S. cumini* L. also contains bioactive antioxidant molecules to reduce the deltamethrin induced cytotoxic effects in cell lines. Al-Attar *et al.,* (2010), reported the preventive effects of seed extracts of *N. sativa* in diazinon exposed rats and proposed to explore the effects against hepatotoxicity, nephrotoxicity, cardiotoxicity and immunotoxicity. The diazinon causes hazards to aquatic life forms, wild life and non-targeted invertebrates (Eisler, 1986).

Antioxidant properties analysis of seed extracts defines that these seed extracts can be exploited to minimize the oxidative stress conditions in invitro as well as in vivo state. In the current research, against deltamethrin toxicity first time *N. sativa* and *S. cumini* extracts were used in in-vitro conditions and shown favourable results and we strongly recommend that these extracts can be explored for various antioxidant analysis, antifungal activities, and anti-cancerous studies as well. In our research findings the toxicity effects due to deltamethrin can be minimized by seed extracts of *N. sativa* and *S. cumini.*

OBJECTIVE 3: To investigate the biochemical properties, immunotoxicity and immunomodulatory effects of deltamethrin and seed extracts of *Nigella sativa* and *Syzygium cumini* in Swiss albino mice.

5.8: Results of body weight and organ weight due to deltamethrin exposure

Table:5.13 Relative weight of different organs in mg obtained from mice after dissection; data is represented for 6 mice in each group with Mean ± SD. Where *** showing very significant difference, ** showing significant difference and * showing least difference than control group. NS is non-significant difference than control group ($p<0.05$). (Five groups: DM- Deltamethrin, DM+NS- Deltamethrin + *Nigella sativa* extract, DM+SC- Deltamethrin + *Syzygium cumini* extract, DM+NS+SC- Deltamethrin + *Nigella sativa* extract + *Syzygium cumini* extract, and Control group)

Organ Wt. (mg)	DM	DM+NS	DM+SC	DM+NS+SC	Control
Right Kidney	$519±3.6^{***}$	$533±3.7^{NS}$	$531±2.9^{NS}$	$529±3.4^{NS}$	534±4.2
Left Kidney	$431±4.6^{***}$	$459±3.2^{NS}$	$453±2.0^{*}$	$453±3.9^{*}$	460±5.1
Liver	$1731±43.5^{***}$	$2248±57.4^{NS}$	$2235±32.7^{NS}$	$2101±38.6^{***}$	2283±37.2
Heart	$114.5±2.9^{***}$	$142.1±5.5^{NS}$	$132.8±5.3^{*}$	$137.6±6.6^{NS}$	142.0±4.5
Spleen	$387.6±5.2^{***}$	$416.8±4.2^{***}$	$415.6±3.8^{***}$	$421.8±3.6^{*}$	427.3±4.3
Lungs	$146.5±2.5^{***}$	$178.6±3.0^{NS}$	$165.8±1.4^{***}$	$172.3±4.6^{**}$	180.0±6.8

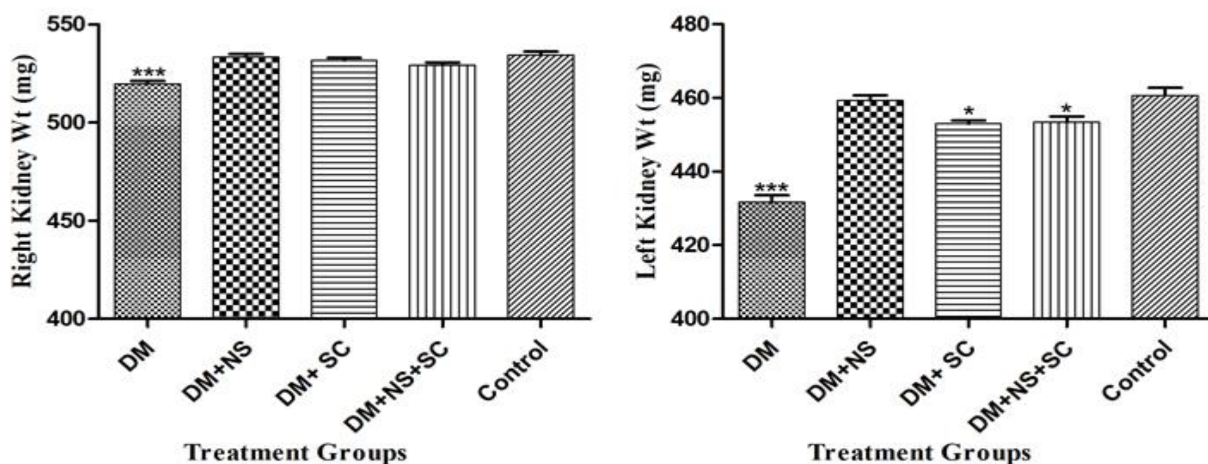


Fig.5.27: Effect of deltamethrin (DM) on weight of Right and Left Kidney in different groups and nephro-protective effect of seed extracts derived from *N. sativa* (NS) and *S. cumini* (SC), Values are represented with Mean with SEM. Comparison test by one-way ANOVA and "Newman-Keuls Multiple Comparison Test" suggest there are highly significant (***) difference among groups with p value ($p<0.01$).

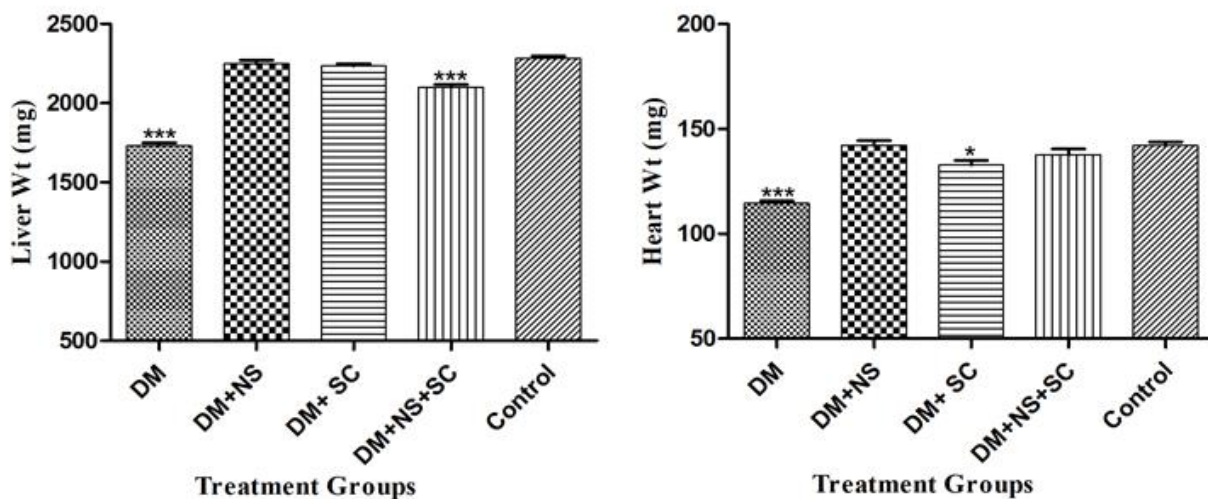


Fig. 5.28: Effect of Deltamethrin (DM) on Liver and Heart weight and hepatoprotective and cardioprotective effect of *N. sativa* (NS)extract and *S. cumini* (SC) extract. Highly Significant difference between control and treated group is represented with ***. Values are represented with Mean with SEM for 6 mice in each group with p value ($p<0.01$). Comparison was done with One-way ANOVA and "Newman-Keuls Multiple comparison Test".

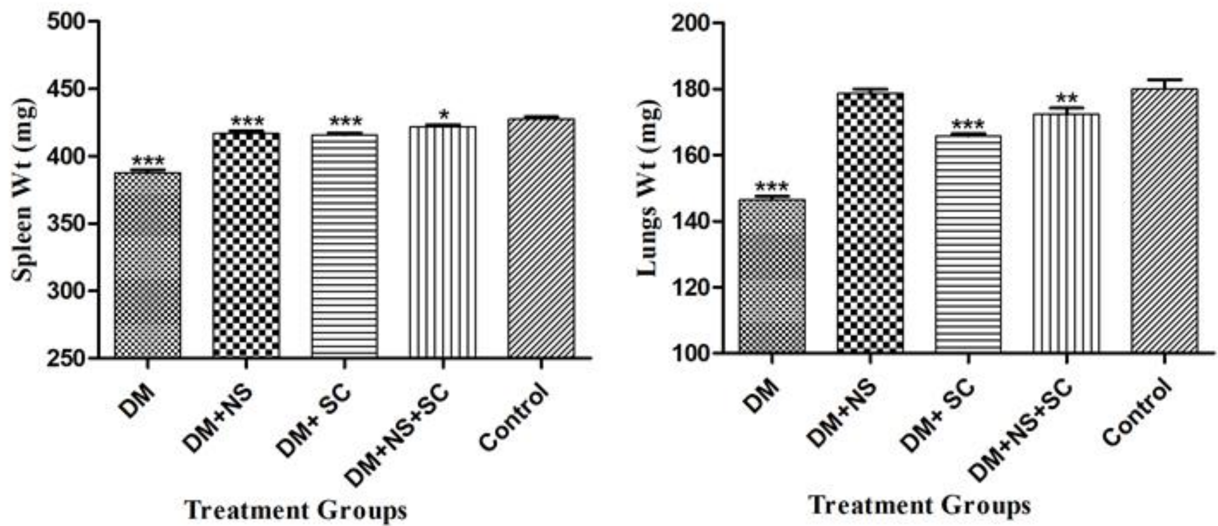


Fig.5.29: Effect of deltamethrin (DM) on weight of spleen and lungs, spleno-protective and lung-protective effects of seed extracts of *N. sativa* (NS) and *S. cumini* (SC), Values are represented with Mean with SEM. Comparison test by one-way ANOVA and "Newman-Keuls Multiple Comparison Test" suggest there are highly significant (***) difference among groups with p value ($p<0.01$).

In table 5.13 relative mean value of body weight of different organs of treatment groups and control group has shown, where there was significant difference in deltamethrin exposed group with control group was noted for all organs used in the current research study. Weight of right kidney was reduced by 2.81% while the weight of left kidney was reduced by 6.3%. There was no significant difference in weight of other treatment groups than control group for weight of right and left kidney. The mean value of liver weight was significantly decreased in deltamethrin (DM) and deltamethrin + *Nigella sativa* + *S. cumini* (DM+NS+SC) group by 24.18% and 7.97% than control group. The mean of heart weight in DM group was decreased by 19.72% than control group and rest of the groups were not recorded with significant difference. The mean value of spleen and lungs weight in DM group was reduced by 9.3% and 23.9% than mean weight of spleen and lungs of control group. Analysis of organs weight have been shown in fig 5.27, fig 5.28 and fig 5.29, graphs are made with GraphPad Prism-5.

Table 5.14: Relative body weight of mice in grams before and after experimental duration; weight is shown for 6 mice in Mean±SD. (Five groups: DM- Deltamethrin, DM+NS- Deltamethrin + *Nigella sativa* extract, DM+SC- Deltamethrin + *Syzygium cumini* extract, DM+NS+SC- Deltamethrin + *Nigella sativa* extract + *Syzygium cumini* extract, and Control group)

Weight	DM	DM+NS	DM+SC	DM+NS+SC	Control
Before Treatment	$26{\pm}1^{***}$	$24.8{\pm}0.75^{NS}$	$25.3{\pm}0.81^{*}$	$24.6{\pm}0.81^{NS}$	23.6±0.81
After Treatment	$23{\pm}0.63^{***}$	$23{\pm}0.89^{***}$	$22.6{\pm}0.51^{***}$	$22.1{\pm}0.40^{***}$	25±0.63

To compare the effect of oral dose administration of 18mg/kg deltamethrin on body weight of mice in each group was done, then obtained data was analysed statistically to find out the toxicity effect, simultaneously anti-toxic effects of extracts derived from seeds of *N. sativa* and *S. cumini*. The mean value of body weight before and after treatment was recorded and analysed by ANOVA and then followed by "Newman-Keuls Multiple Comparison Test" suggest there are highly significant (***) difference among groups with p value (p<0.01). Data is mentioned in table 5.14.

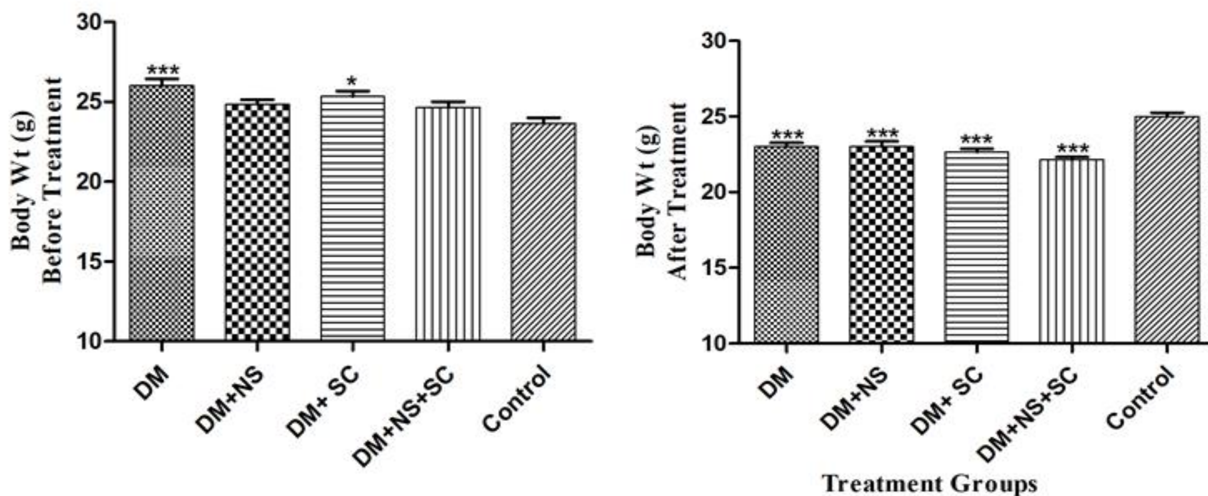


Fig. 5.30: Effect of deltamethrin (DM) on body weight of mice and protective effects of seed extracts of *N. sativa* (NS) and *S. cumini* (SC), Values are represented with Mean with SEM. Comparison test by one-way ANOVA and "Newman-Keuls Multiple Comparison Test" suggest

there are highly significant (***) difference between control group and treatment groups with p value (p<0.01).

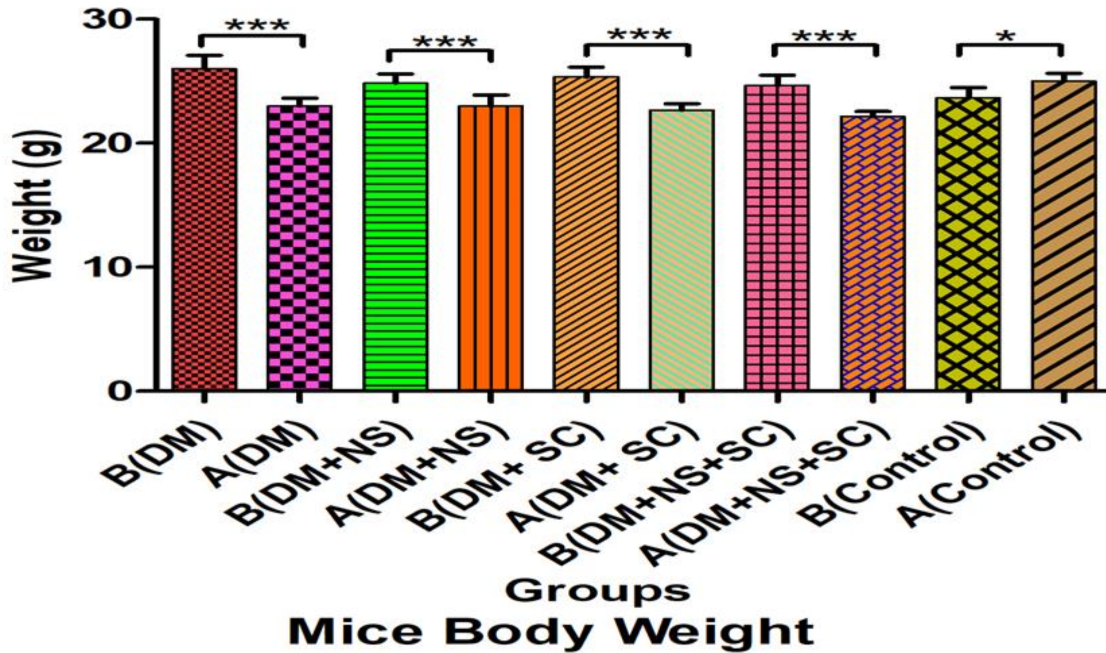


Fig. 5.31: Comparative analysis of mice body weight before and after treatment using one-way ANOVA and "Newman-Keuls Multiple Comparison Test", results suggest there are highly significant (***) difference among groups with p value (p<0.01). Values are represented with Mean with SEM. Group comparison between before (B) and after (A).

In table 5.14 mean value of body weight of treatment groups and control groups have been shown with Mean and SEM. There was highly significant difference was observed in body weight of mice in each group in pre and post treatment. Beside control group, body weight in each treatment group was reduced in comparison to before treatment body weight. The body weight of mice analysis pre and post treatment is represented in fig 5.30 which depicts there was high significant difference between body weight of DM group with control group. In pretreatment there was no significant difference in control group and other treatment group while in post treatment analysis it was noted that there was very high significant difference in body weight in all group was observed than the control group. When each group body weight was analysed pre and post then it has been observed that there was very high significant difference was observed between each group pre and post

treatment pattern, only in between pre and post treatment of control group body weight low significant difference was observed which is represented in fig 5.31. Above all analyses suggest that due to exposure of deltamethrin pesticide in each treatment group there was adverse effects on body weight of mice due to various stress conditions faced by vital organs of the body system and resulting into altered metabolic activities of mice in all treatment groups. The pre and post analysis of each group also suggest that the body weight gain was normal in control group where body weight was increased from pretreatment group and body weight of mice in rest of the group was reduced.

Moid *et al*., (2014) also reported the reduction of body weight and organs weight in mice treated with deltamethrin which supports the current research findings, the reduction in body weight may be due to less feeding efficiency and altered metabolic activities of vital organs in the mice resulting into affected absorption of digested food material. The reduced affect of body weight correspondingly causing the reduction of organs weight in deltamethrin treated mice. In one of the experiments carried out by Enan *et al*. in 1996, there was no significant difference was observed in body weight of mice exposed with dose of 25 mg/kg body weight for 24 hours, it may be due to less duration of deltamethrin exposure to animal group but if deltamethrin can be exposed to animals for longer duration then there might be reduction in body weight of animals exposed with deltamethrin. Arena *et al*. (2008) also reported that due to 40 mg/kg exposure of fenvalerate pesticide in rats body weight was reduced; while Poormoosavi *et al*. (2018) reported that due to exposure of bisphenol A, overall body weight of wistar rats was reduced but the weight of kidney and liver was increased. The increased weight of liver and kidney show that stress condition of liver and kidney was increased to neutralize the oxidative stress condition of bisphenol A on liver and kidney. Abbassy and Mossa (2012) also reported the decrease in relative body weight while there was increase in liver weight but kidney weight was reduced due to exposure of cypermethrin and deltamethrin pesticide. Maalej *et al*. (2017) reported the body weight gain in deltamethrin treated rats which shows difference than current findings. Orlu (2014) also reported that the 25 mg/kg dose of deltamethrin in sprague-dawley rats caused the decrease in body weight and liver weight. So, the current research findings have similarity with earlier research findings in terms of modification in body weight and organs weight due to deltamethrin pesticide exposure.

5.9: Results of hematological parameters

Table 5.15: Haematological parameters of various groups exposed to deltamethrin (DM) and protective effects of seed extracts of *N. sativa* (NS) and *S. cumini* (SC), Values are represented with Mean ± SD. *** shows highly significant difference, ** shows significant difference, * represents less significant difference and NS denotes non-significant difference than control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

Parameters	DM	DM+NS	DM+SC	DM+NS+SC	Control
RBC (×10^6/µl)	9.08±0.41***	7.95±0.32^{NS}	8.38±0.27^{NS}	8.58±0.30*	8.07±0.31
WBC (×10^3/µl)	11.02±0.50***	11.12±0.38***	11.17±0.29***	11.12±0.17***	7.93±0.39
Lymphocytes (%)	82.83±3.92***	76.50±2.59*	75.33±2.50*	76.17±2.04*	70.83±4.12
Neutrophil (%)	36.00±2.53**	37.33±1.63*	37.17±0.75^{NS}	36.17±1.17**	40.33±2.94
TLC (cells/mm^3)	4953±303***	4910±284***	4747±268***	4897±233***	4190±142
Hematocrit (%)	27.67±0.52***	29.50±1.52***	31.33±1.03**	30.67±0.82**	33.50±1.70
MCV (fL)	39.83±0.23***	41.33±0.16***	41.63±0.15***	42.07±0.23***	44.35±0.22
MCH (pg)	15.45±0.20***	16.27±0.10***	16.30±0.11***	16.40±0.18***	18.12±0.27
MCHC (g/dl)	34.37±0.43***	36.70±0.60***	37.38±0.97***	37.00±0.47***	40.00±0.36
PCV (%)	39.25±0.75***	41.12±0.40***	40.75±1.04***	40.25±0.70***	43.37±0.50
Hb (g/dl)	9.87±0.55***	12.02±0.34**	12.03±0.19**	11.75±0.37*	11.25±0.10

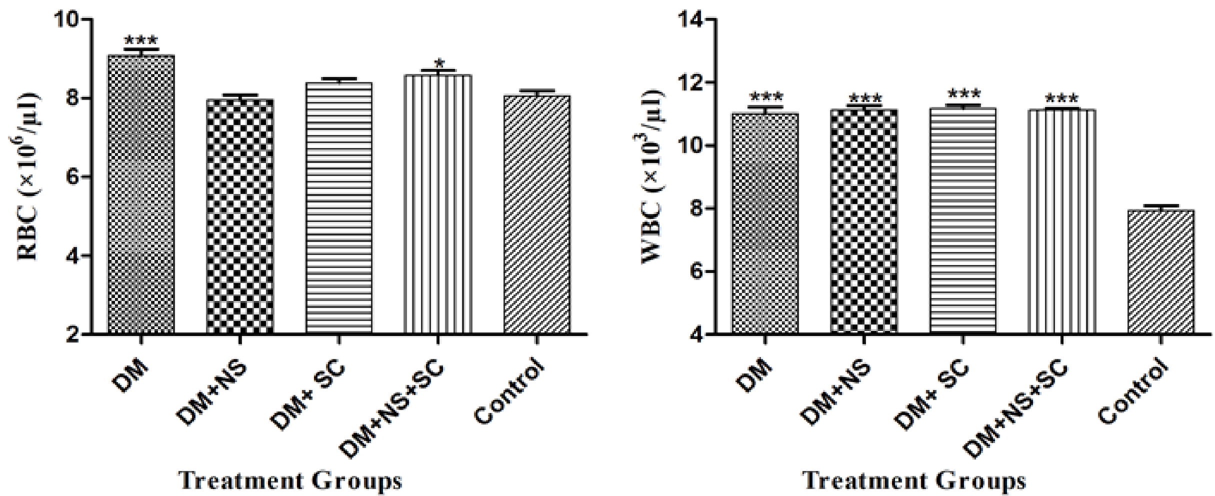


Fig. 5.32: Comparative analysis of RBC and WBC in different treatment groups using one-way ANOVA and "Newman-Keuls Multiple Comparison Test", results suggest there are highly significant (***) difference between control and treatment groups with p value ($p<0.01$). Values are represented with Mean with SEM.

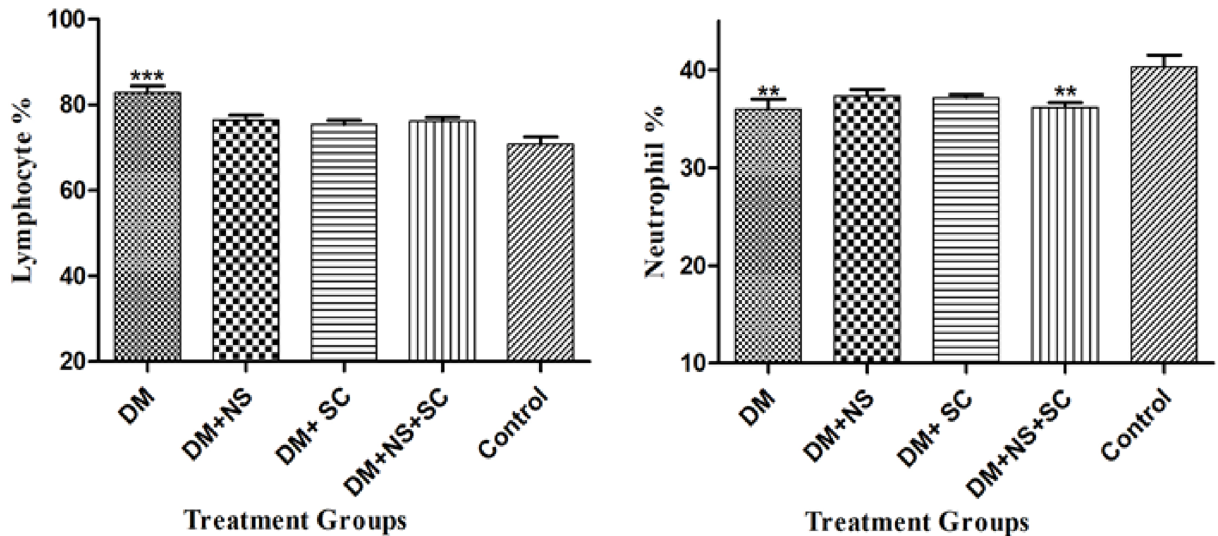


Fig. 5.33: Comparative analysis of Lymphocyte % and Neutrophil % in different treatment groups using one-way ANOVA and "Newman-Keuls Multiple Comparison Test", results suggest there is highly significant (***) difference and significant (**) difference between control and treatment groups and remaining are non-significant difference with control groups, with the p value ($p<0.01$). Values are represented with Mean with SEM.

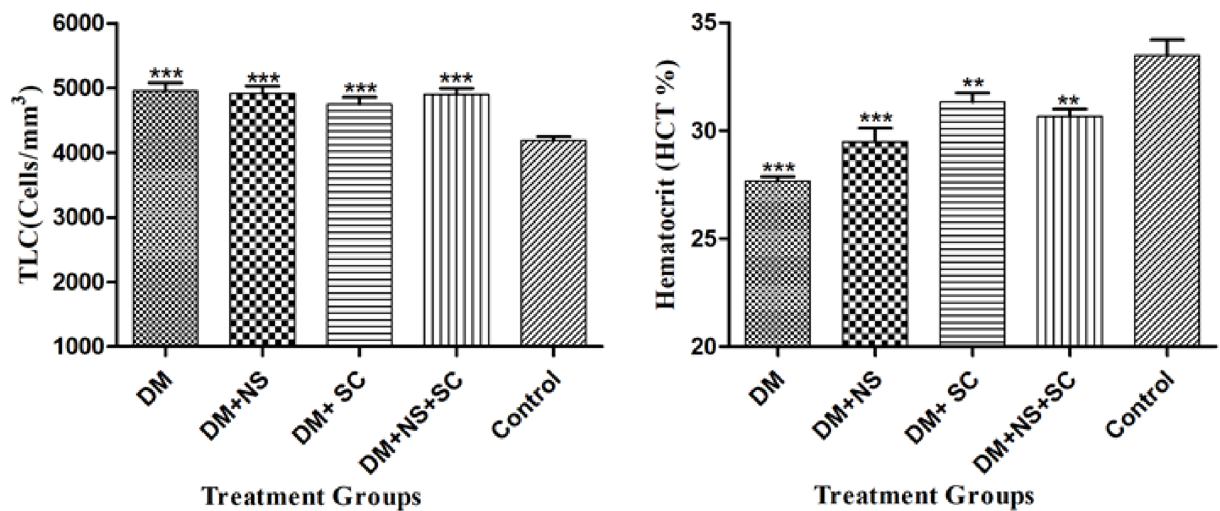


Fig. 5.34: Relative analysis of TLC (Cells/mm^3) and Hematocrit % in different treatment groups using one-way ANOVA and "Newman-Keuls Multiple Comparison Test", results suggest there is highly significant (***) difference and significant (**) difference between control and treatment groups with the p value (p<0.01). Values are represented with Mean with SEM.

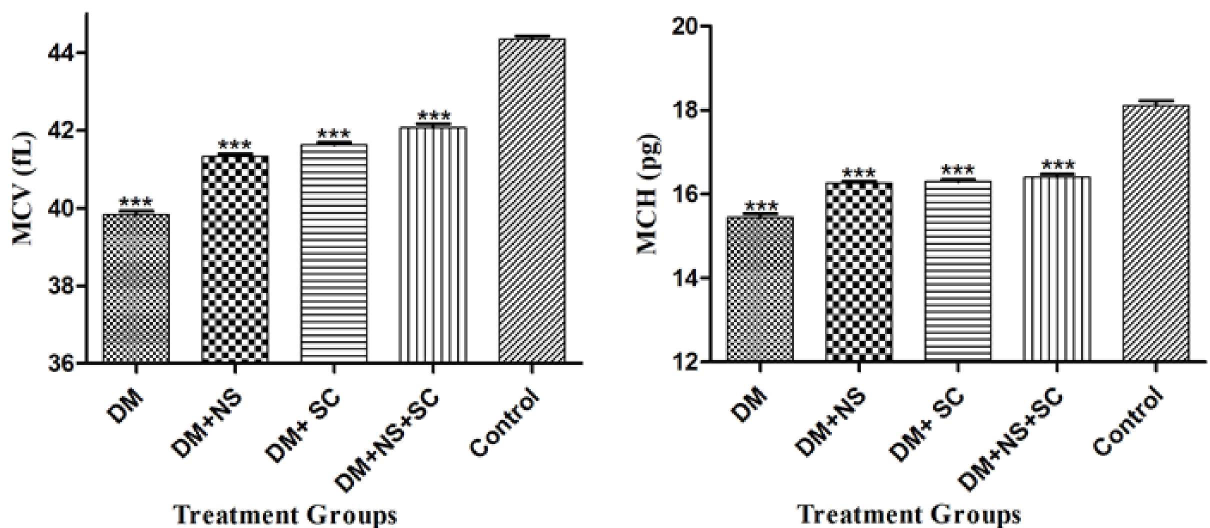


Fig. 5.35: Competent study of Mean corpuscular volume (fL) and Mean corpuscular hemoglobin (pg) in different treatment groups using one-way ANOVA and "Newman-Keuls Multiple Comparison Test", results suggest there is highly significant (***) difference between control and treatment groups with the p value (p<0.01). Values are represented with Mean with SEM.

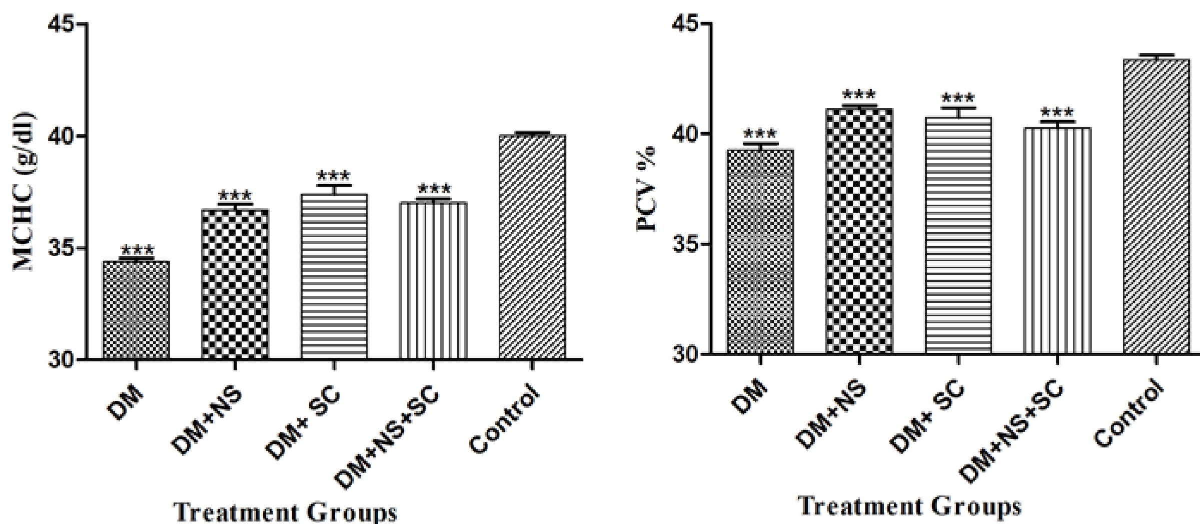


Fig. 5.36: Relative analysis of Mean corpuscular hemoglobin concentration (g/dl) and Packed cell volume (%) in different treatment groups using one-way ANOVA and "Newman-Keuls Multiple Comparison Test", results suggest there is highly significant (***) difference between control and treatment groups with the p value (p<0.01). Values are represented with Mean with SEM.

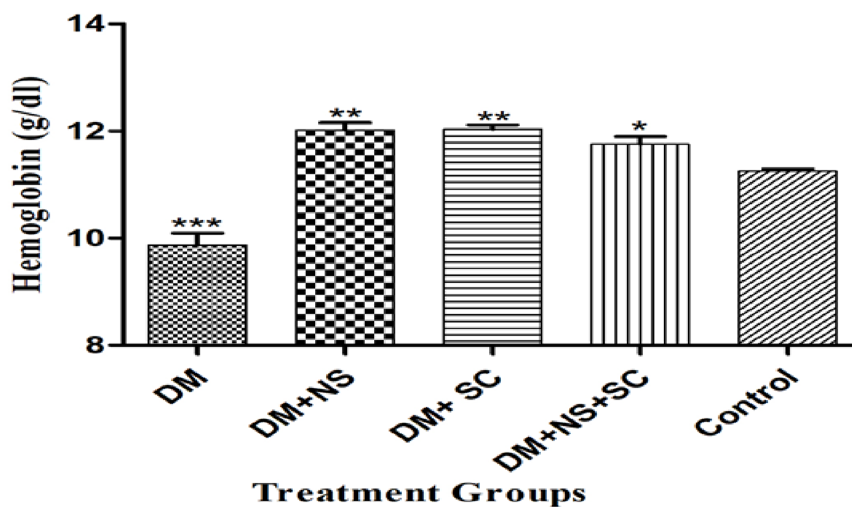


Fig. 5.37: Relative analysis of hemoglobin (g/dl) in different treatment groups using one-way ANOVA and "Newman-Keuls Multiple Comparison Test", results suggest there is highly significant (***) difference, significant (**) difference and less significant (*) difference between control and treatment groups with the p value (p<0.01). Values are represented with Mean with SEM.

In table 5.15 hematological parameters are shown. There was very significant difference observed between DM and control group in all parameters. Analysis of different parameters were shown in fig 5.32 to fig 5.37.

The RBC value was increased by 12.52% in DM group than control group, the increased value of RBC depicts the oxidative stress condition in mice of DM group. The WBC value also increased by 38.97% in DM group than control group. The increase in lymphocytes was also observed by 16.94% in DM group than control group. Neutrophil count was reduced by 10.74% in DM group and hemoglobin was reduced by 12.27%. The TLC value was increased by 18.21 %, while Hematocrit, MCV, MCH and PCV values were reduced by 22.06%, 10.19%, 14.74% and 9.5% respectively. The changes in the parameters depict the severe metabolic and biochemical changes due to exposure of deltamethrin pesticides. The protective effects of extracts of *N. sativa* and *S. cumini* has shown also significant difference between DM and DM+NS group and DM and DM+SC group. Synergistic effects of extracts of *N. sativa* and *S. cumini* also shown significant difference between DM and DM+NS+SC group. The various biochemical parameters of blood reflect the changes due to deltamethrin pesticides and its effects on oxidative stress in organs actively involved in metabolism.

Moid *et al*. (2014) findings also support the current research findings, they also reported the escalation in RBC, WBC, lymphocytes, MCV, HGB and other hematological parameters due to deltamethrin exposure in swiss mice. The hematological parameters like lymphocytes, monocytes and neutrophil were also decreased in Rehman *et al*. (2017) findings due to deltamethrin pesticide. Toś-Luty *et al*. (2001) also reported the significant changes in hematological parameters due to oral toxicity of deltamethrin and also provide the similarity with current research findings. Tiwari and Gill (2014) also reported that there was increase in hemoglobin in 0.1 mg dose in mice while Hb was decreased in 0.5 mg dose for 30 days. Lymphocytes were increased in both the dose while neutrophil was reduced in both the dose of deltamethrin. The escalated values of hematological parameters in Tiwari and gill findings stands with current research findings. Tiwari *et al*. (2018) reported the increase in TLC value and Lymphocytes while there was no significant difference was observed in neutrophil count in mice group treated with deltamethrin. Abbassy and Mossa (2012) also reported the increase in RBC and WBC while Hb was reduced due to deltamethrin exposure in rats which also confirms validation of current research findings.

5.10: Results of Biochemical parameters of blood serum

Table 5.16: Biochemical parameters of blood serum in various treatment groups exposed to deltamethrin and protective effects of seed extracts of *N. sativa* and *S. cumini*, Values are represented with Mean ± SD. *** shows highly significant difference, ** shows significant difference, * represents less significant difference and NS denotes non-significant difference than control group at p value (p<0.01) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test". (DM – Deltamethrin treated group, DM+NS – Deltamethrin followed by *N. sativa* extract, DM+SC – Deltamethrin followed by *S. cumini* extract, DM+NS+SC – Deltamethrin followed by combination of *N. sativa* and *S. cumini* extract).

Parameters	**DM**	**DM+NS**	**DM+SC**	**DM+NS+SC**	**Control**
Cholesterol (mg/dl)	199.3±7.2***	144.1±5.8***	89.3±3.7***	109.6±7.1^{NS}	112.8±5.1
Triglyceride (mg/dl)	170.3±7.1***	105.1±3.2***	71.1±3.7***	74.3±2.2***	88.8±2.0
HDL (mg/dl)	45.3±2.4***	55.0±2.7***	36.0±1.7*	52.6±2.0***	32.8±1.3
LDL (mg/dl)	86.3±3.4***	69.3±3.0***	61.6±3.2***	62.3±4.6***	43.3±2.73
VLDL (mg/dl)	27.0±2.0***	18.0±2.1***	15.0±0.89*	21.0±2.75***	12.6±1.0
Glucose (mg/dl)	206.6±6.8***	99.1±3.9*	80.3±3.8***	102.1±2.9**	93.3±5.1
Total Protein (mg/dl)	5.25±0.34***	4.1±0.19 ^{NS}	3.8±0.13 ^{NS}	3.9±0.10 ^{NS}	4.0±0.17
Albumin (g/dl)	4.0±0.23***	3.1±0.16^{NS}	2.8±0.07^{NS}	2.9±0.10^{NS}	3.0±0.17
Blood urea (mg/dl)	29.05±1.7***	23.61±0.63***	24.23±1.01***	24.23±0.55***	20.78±0.77
TBIL (mg/dl)	1.08±0.14***	0.68±0.07**	0.85±0.05***	0.66±0.08**	0.46±0.08
DBL (mg/dl)	0.35±0.05***	0.25±0.05 ^{NS}	0.21±0.04 ^{NS}	0.21±0.04^{NS}	0.20±0.06
Ser. Creatinine (mg/dl)	2.0±0.18***	1.5±0.08***	1.6±0.16***	1.38±0.07***	0.86±0.12
ALKP (IU/L)	186.6±5.5***	155.8±10.4***	149.1±6.9***	117.6±4.9***	94.3±2.8
AST (IU/L)	801.6±52.3***	723.3±55.3***	710.0±66.3***	346.6±23.5***	107.5±16.0
ALT (IU/L)	185.3±4.2***	157.3±5.9***	150.0±7.0***	112.5±5.2***	70.8±2.7

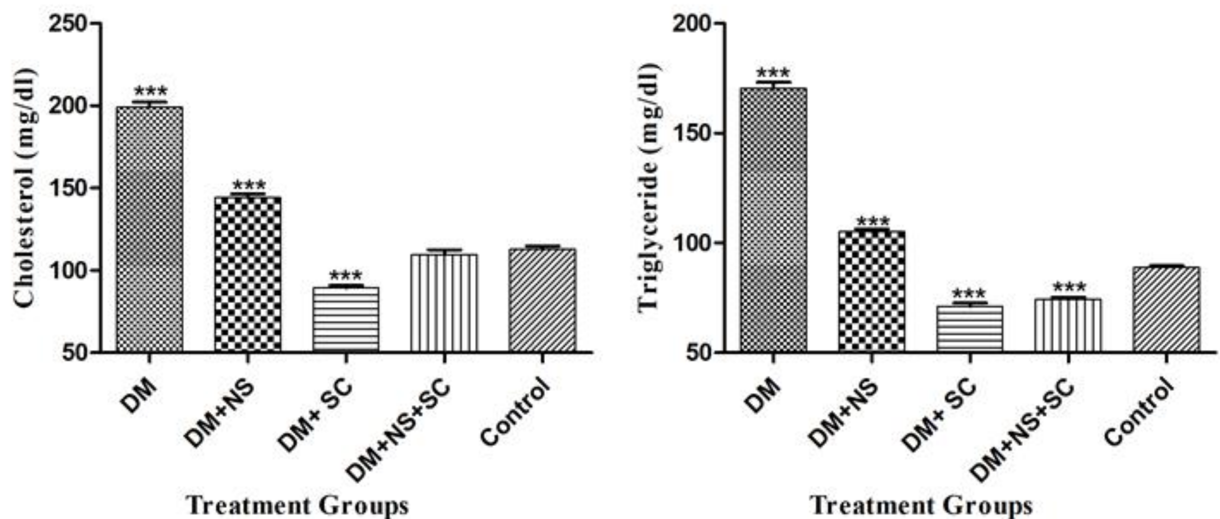


Fig. 5.38: Comparative analysis of Cholesterol (mg/dl) and Triglyceride (mg/dl) in different treatment groups using one-way ANOVA and "Newman-Keuls Multiple Comparison Test", results suggest there is highly significant (***) difference between control and treatment groups with the p value (p<0.01). Values are represented with Mean with SEM.

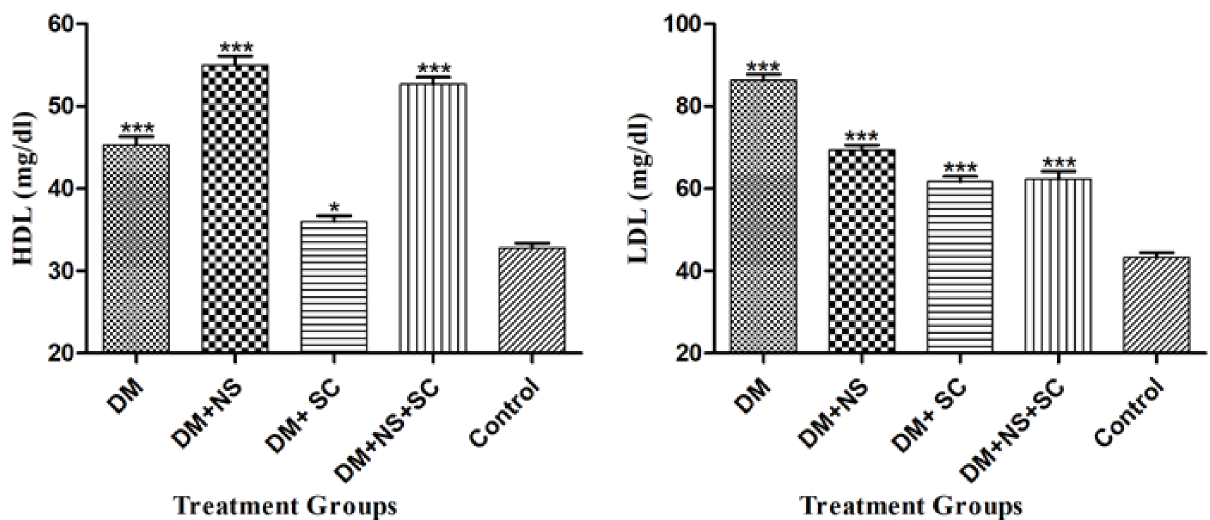


Fig. 5.39: Comparative analysis of HDL (mg/dl) and LDL (mg/dl) in different treatment groups using one-way ANOVA and "Newman-Keuls Multiple Comparison Test", results suggest there is highly significant (***) difference significant (*) difference between control and treatment groups with the p value (p<0.01). Values are represented with Mean with SEM.

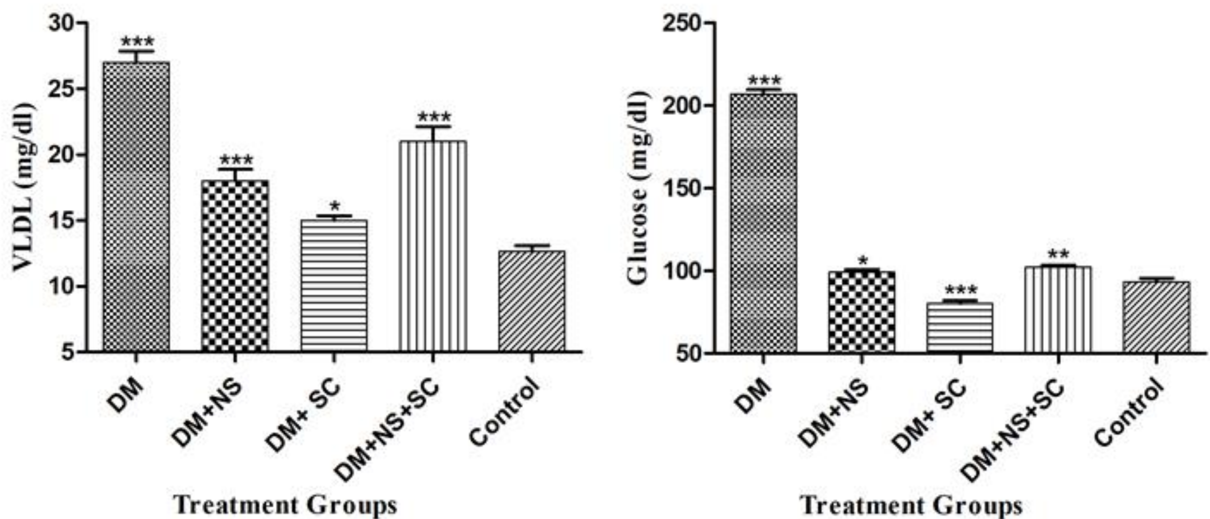


Fig. 5.40: Comparative analysis of VLDL (mg/dl) and Glucose (mg/dl) in different treatment groups using one-way ANOVA and "Newman-Keuls Multiple Comparison Test", results suggest there is highly significant (***) difference, (**) significant difference and (*) less significant difference between control and treatment groups with the p value ($p<0.01$). Values are represented with Mean with SEM.

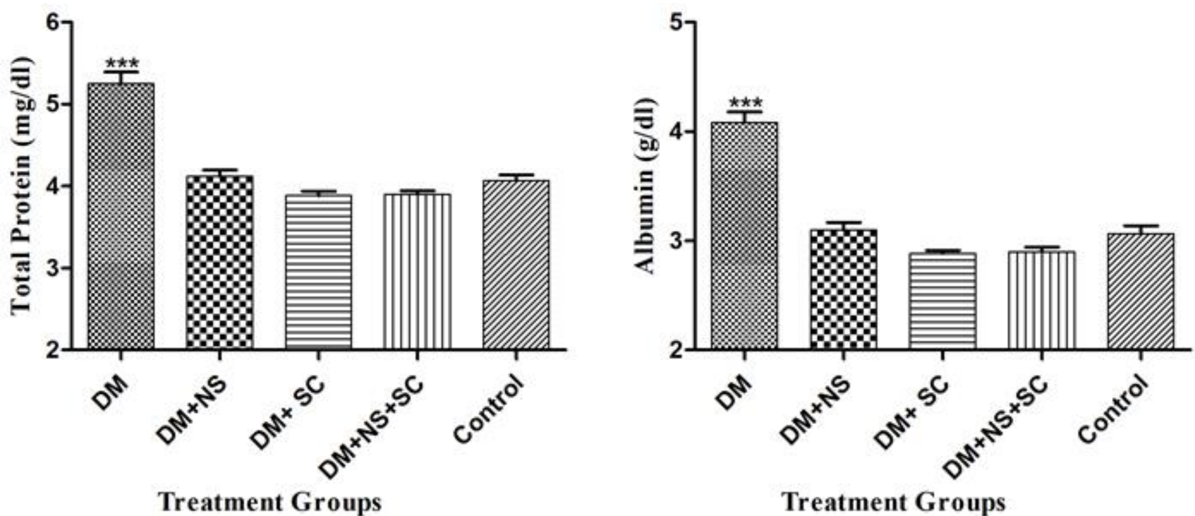


Fig. 5.41: Comparative analysis of Total Protein (mg/dl) and Albumin (mg/dl) in different treatment groups using one-way ANOVA and "Newman-Keuls Multiple Comparison Test",

results suggest there is highly significant (***) difference between control and treatment groups with the p value (p<0.01). Values are represented with Mean with SEM.

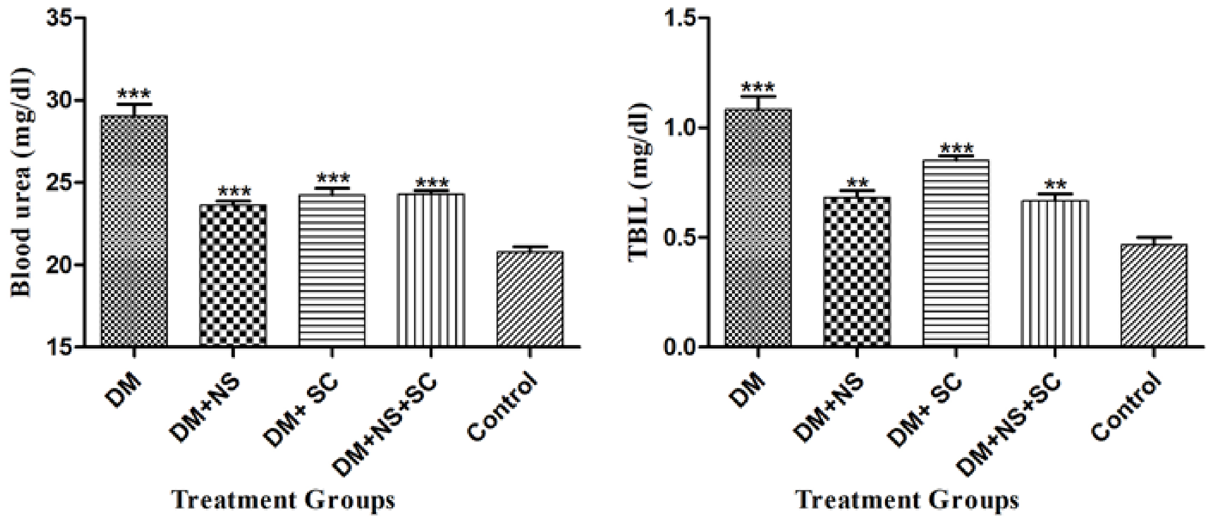


Fig. 5.42: Comparative analysis of Blood Urea (mg/dl) and TBIL (mg/dl) in different treatment groups using one-way ANOVA and "Newman-Keuls Multiple Comparison Test", results suggest there is highly significant (***) difference between control and treatment groups with the p value (p<0.01). Values are represented with Mean with SEM.

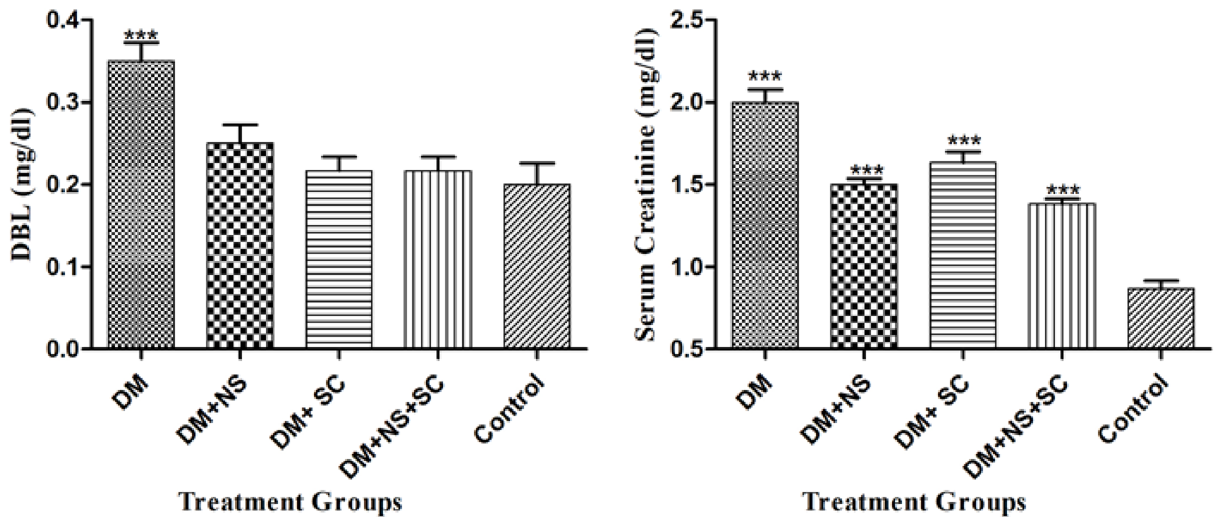


Fig. 5.43: Comparative analysis of DBL (mg/dl) and Serum creatinine (mg/dl) in different treatment groups using one-way ANOVA and "Newman-Keuls Multiple Comparison Test",

results suggest there is highly significant (***) difference between control and treatment groups with the p value (p<0.01). Values are represented with Mean with SEM.

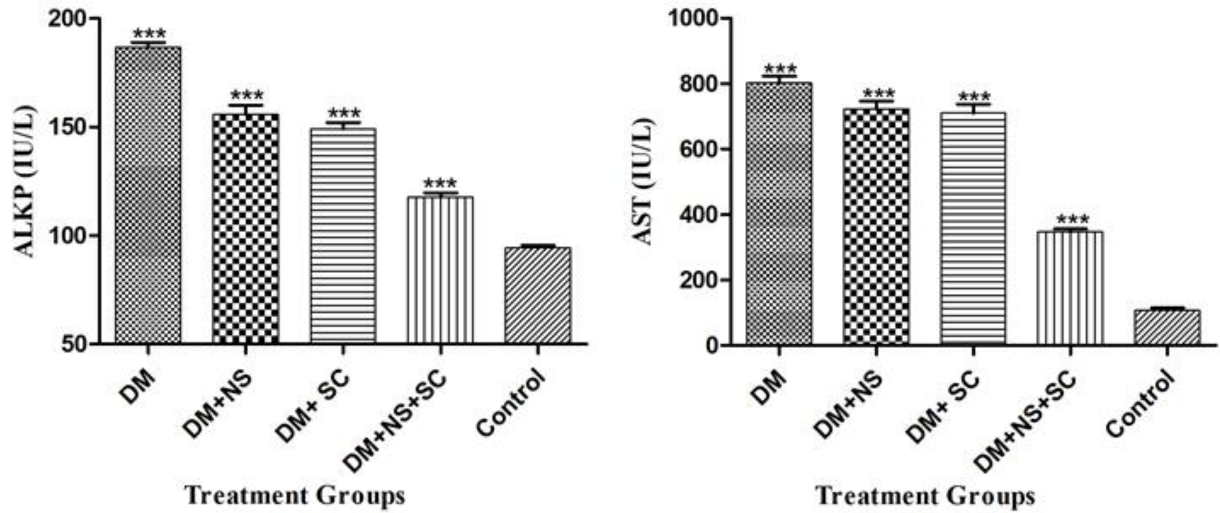


Fig. 5.44: Comparative analysis of ALKP (IU/L) and AST (IU/L) in different treatment groups using one-way ANOVA and "Newman-Keuls Multiple Comparison Test", results suggest there is highly significant (***) difference between control and treatment groups with the p value (p<0.01). Values are represented with Mean with SEM.

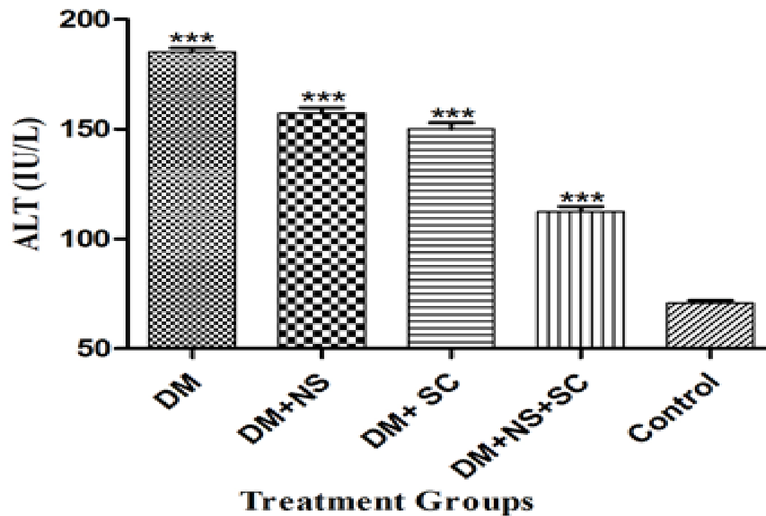


Fig. 5.45: Comparative analysis of ALT (IU/L) in different treatment groups using one-way ANOVA and "Newman-Keuls Multiple Comparison Test", results suggest there is highly

significant (***) difference between control and treatment groups with the p value ($p<0.01$). Values are represented with Mean with SEM.

In table 5.16 the biochemical parameters of blood serum have shown and all parameters mentioned in table shown highly significant difference between DM group and control group. Analysis of parameters are shown in fig 5.38 to fig 5.45. All parameters of lipid profile of blood serum shown significance difference in DM group than control group. There was an increase in DM group was observed in lipid profile, 76.68% in cholesterol, 91.78% increase in triglyceride, HDL was increased by 38.11%, LDL was increased by 99.31% and VLDL was increased by 114.29%. There was increase in DM group than control group in others parameters like, blood glucose by 121.11%, total protein by 31.25%, albumin by 33.33%, blood urea was increased by 39.8%, total bilirubin (TBIL) by 134.78%, differential bilirubin (DBL) by 75%, serum creatinine by 132.56%. There was significant difference was observed between ALKP, AST and ALT enzyme in DM group than control group. The overexpression of these enzymes was observed in DM treated group than control group, the ALKP expression was increased by 97.88%, AST was increased by 645.67% and ALT by 161.72%. Overexpression of these enzymes, altered lipid profile values and high protein expression suggest that there was very high oxidative stress condition was originated in most vital organ liver in deltamethrin treated group of mice. But when extracts were used then there was significant fall in overexpressed value of these parameters were recorded i.e. the extracts derived from *N. sativa* and *S. cumini* alone and synergistically reducing the oxidative stress condition caused due to deltamethrin exposure. Manna *et al*. (2005) reported that deltamethrin exposure to wistar rats causing the over expression of ALT, AST, ALP, LDH and glucose level in blood, while there was decrease in the value of Hb and PCV. Their findings also suggest the oxidative stress caused by deltamethrin and supports the current research results. Lamfon (2007) reported that raise in hepatic marker enzymes ALT and AST enzymes in hepatocytes of deltamethrin treated rats and due to use of Silymarin extracts reduced the expression of ALT and AST enzymes. The protective effect of Silymarin extracts recovers the oxidative stress condition due to deltamethrin pesticide, similarly in current research findings seed extracts of *N. sativa* and *S. cumini* reduces the hepatotoxicity condition due to deltamethrin. The findings of blood serum parameters when compared with the findings of Eraslan *et al* (2007) it can be said that the increase in expression of lipid profile, glucose level, protein and the three liver enzymes were observed in both 7.5 mg and 30 mg dose at 15th, 30th , 45th and 60th day mostly but some parameters have

shown reduced level of expression as well which not support with our findings, it may be due short duration of exposure and dose variation i.e. 18 mg adopted for this current study. Mongi *et al*. (2011) reported the modification in RBC, WBC, MCV, MCHC, Ht and Hb values while they observed the upsurge in activity of hepatic biomarker enzymes ALT, AST, ALP, LDH and γ-GT, their findings also support the current research findings. Abdel-Damin *et al*. (2016) findings of blood serum parameters support our findings, the expression of ALKP, AST, ALT, Lipid profile were increased in mice due to deltamethrin exposure while the extracts of *Spirulina platensis* shown significant decrease in the expression of these parameters like in our findings of *N. sativa* and *S. cumini* extracts. Findings of Aydin (2011) also shows the similarity with our findings in terms of over expression of AST, ALT, urea and serum creatinine in wistar rat blood parameters i.e. deltamethrin was causing the oxidative stress in rat also and causing the damage of liver functioning. Rehman *et al*. (2017) also shown the overexpression of liver marker enzymes ALKP (ALP), AST, ALT and GGTP in deltamethrin treated groups than control group and also supports our current research findings where these liver marker enzymes were over expressed. Maalej *et al*. (2017) reported that elevation of MDA in deltamethrin treated group while there was reduction in MDA expression when oleuropein and extracts derived from olive fruit was implemented, these findings also stands with the current research findings. Rjeibi *et al*. (2016) reported the modification in ALP, AST and LDH activity in wistar rats treated with deltamethrin. Tewari *et al*. (2018) exposed the variation in various biochemical activities due to deltamethrin exposure in 30 days and 60 days treatment with two defined doses. Tewari *et al*. revealed the change in Hb, RBC, WBC, MCV, MCHC, and biomarker enzymes values of ALT, AST, ALP due to deltamethrin exposure which supports the outcomes in current research.

5.11: Results of biochemical parameters of organs

5.11.1: Results of biochemical parameters of hepatic tissues:

Table 5.17: Biochemical parameters of **Hepatic** tissues in various treatment groups exposed to deltamethrin and protective effects of seed extracts of *N. sativa* and *S. cumini*, Values are represented with Mean ± SD. *** shows highly significant difference, ** shows significant difference, * represents less significant difference and NS denotes non-significant difference than control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple

Comparison Test". (DM – Deltamethrin treated group, DM+NS – Deltamethrin followed by *N. sativa* extract, DM+SC – Deltamethrin followed by *S. cumini* extract, DM+NS+SC – Deltamethrin followed by combination of *N. sativa* and *S. cumini* extract).

Parameters	DM	DM+NS	DM+SC	DM+NS+SC	Control
Catalase	48.5±4.7***	63.0±5.5***	59.1±4.8***	61.4±5.3***	115.4±7.4
Glutathione peroxidase	50.4±4.7***	63.1±3.4***	57.0±3.3***	58.5±3.3***	79.8±3.5
Superoxide dismutase	6.6±0.34***	10.5±0.54***	9.3±0.34***	10.7±0.44***	15.2±0.5
Glutathione s-transferase	119.1±4.4***	153.4±3.7***	134.7±2.2***	144.6±2.7***	182.7±8.2
Lipid peroxidase (LPO)	231.6±11.9***	143.0±6.8***	184.3±7.0***	158.3±5.5***	104.0±6.0
Succinate dehydrogenase	12.7±0.3***	15.6±0.4***	14.5±0.4***	15.2±0.6***	18.3±0.2
Reduced Glutathione	0.20±0.01***	0.30±0.03 ***	0.25±0.02 ***	0.27±0.02***	0.52±0.02
ALPase	0.69±0.20***	0.55±0.27***	0.64±0.02***	0.58±0.04***	0.44±0.02
ACPase	1.40±0.05***	1.20±0.06***	1.13±0.02***	1.15±0.02***	0.91±0.03
ATPase	2.75±0.12***	3.11±0.05***	3.10±0.05***	3.22±0.11***	3.75±0.08
Malondialdehyde (MDA)	91.53±4.5***	55.0±1.29***	72.3±2.80 ***	62.5±2.5***	42.5±2.45
NO	90.2±2.76***	73.5±1.71***	83.9±2.3***	76.7±1.16***	54.0±2.8
Cholesterol	1.74±0.01***	1.53±0.03***	1.28±0.05***	1.44±0.02***	1.16±0.04
Total Protein	13.8±0.42***	15.4±0.22***	14.65±0.26***	15.65±0.29***	16.9±0.40

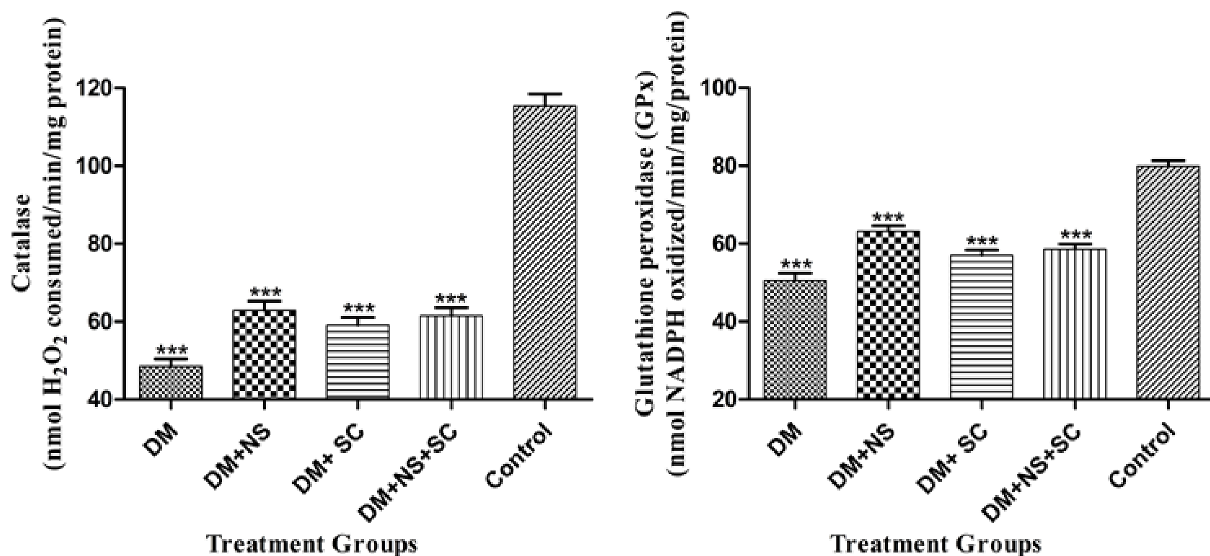


Fig 5.46: Catalase and Glutathione peroxidase analysis in hepatic tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group relative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

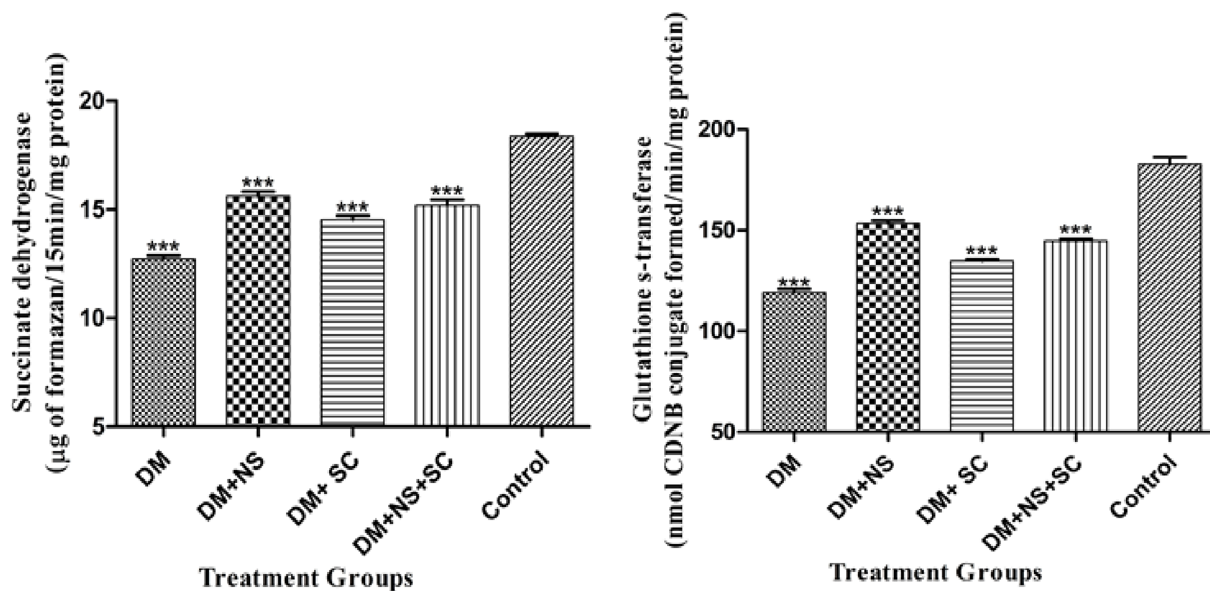


Fig 5.47: Superoxide dismutase and Glutathione S-transferase analysis in hepatic tissues in treatment groups and compared with control group. Values are represented with Mean with SEM.

Sign *** shows highly significant difference between treatment group relative to control group at p value (p<0.01) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

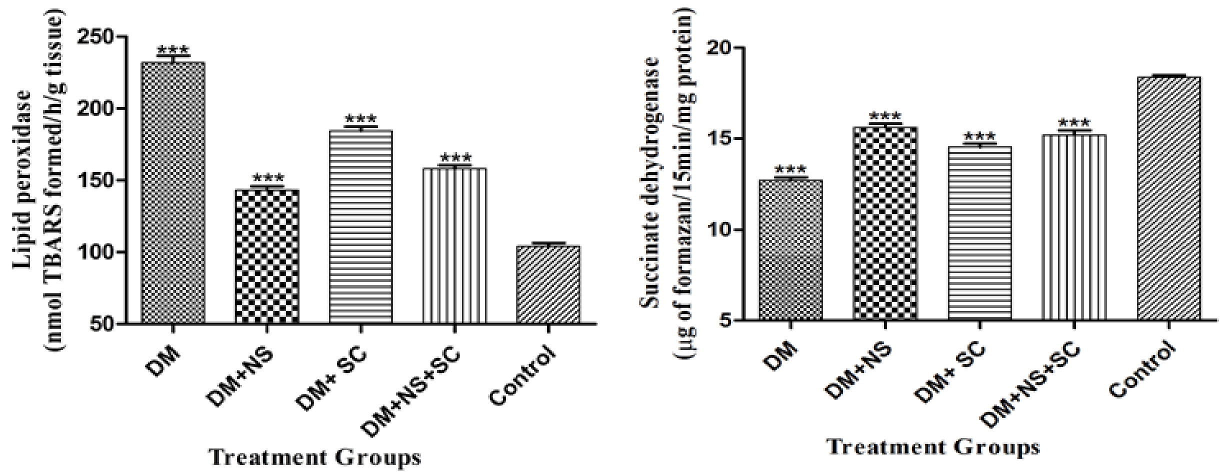


Fig 5.48: Analysis of activity of Lipid peroxidase and Succinate dehydrogenase in hepatic tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group relative to control group at p value (p<0.01) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

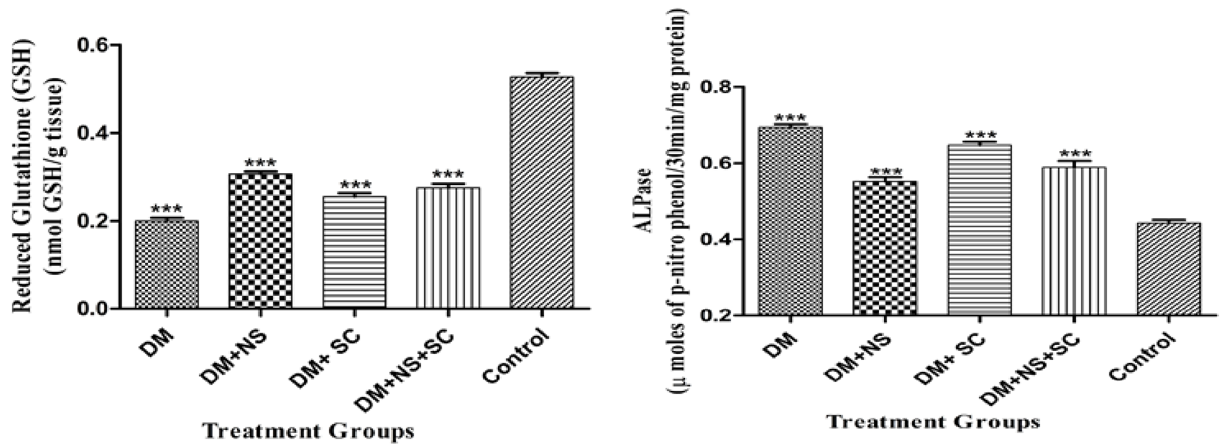


Fig 5.49: Analysis of activity of Reduced glutathione activity and ALPase in hepatic tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group relative to control group at p value (p<0.01) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

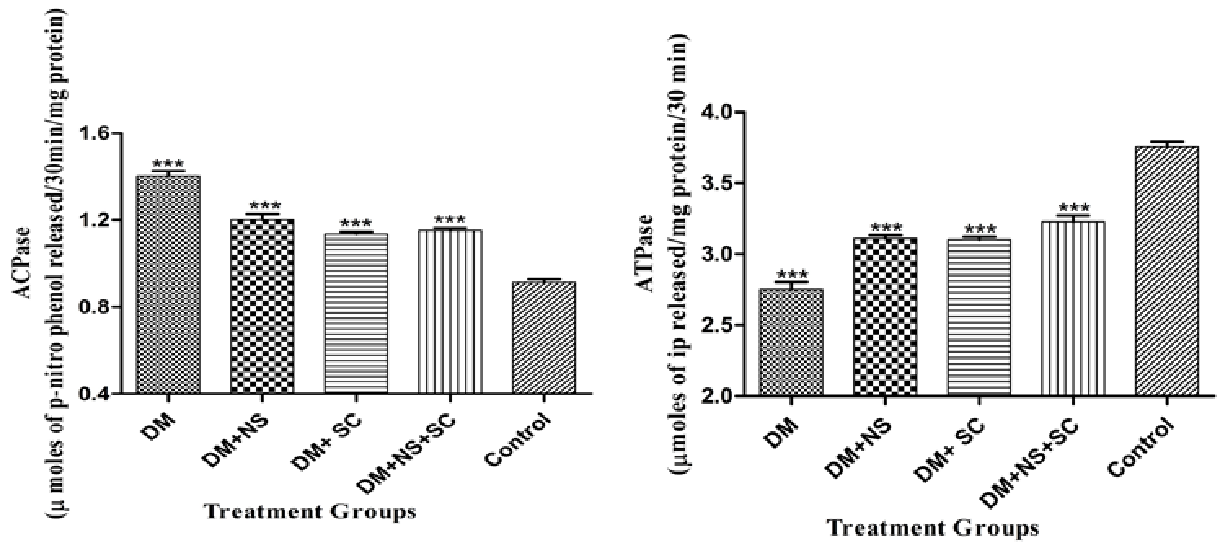


Fig 5.50: Analysis of activity of ACPase activity and ATPase in hepatic tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group relative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

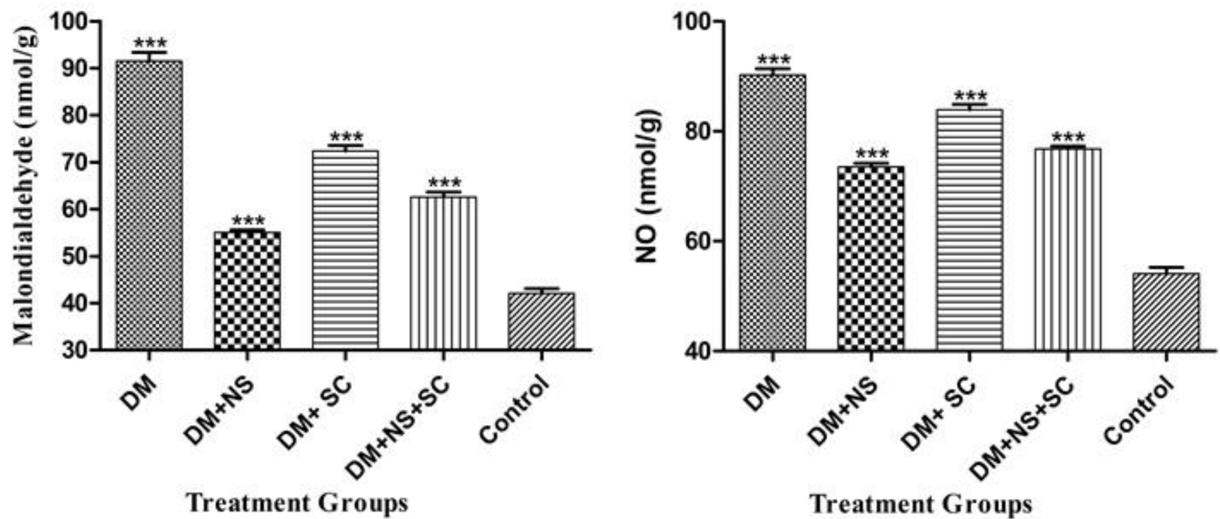


Fig 5.51: Analysis of activity of MDA activity and NO in hepatic tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group relative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

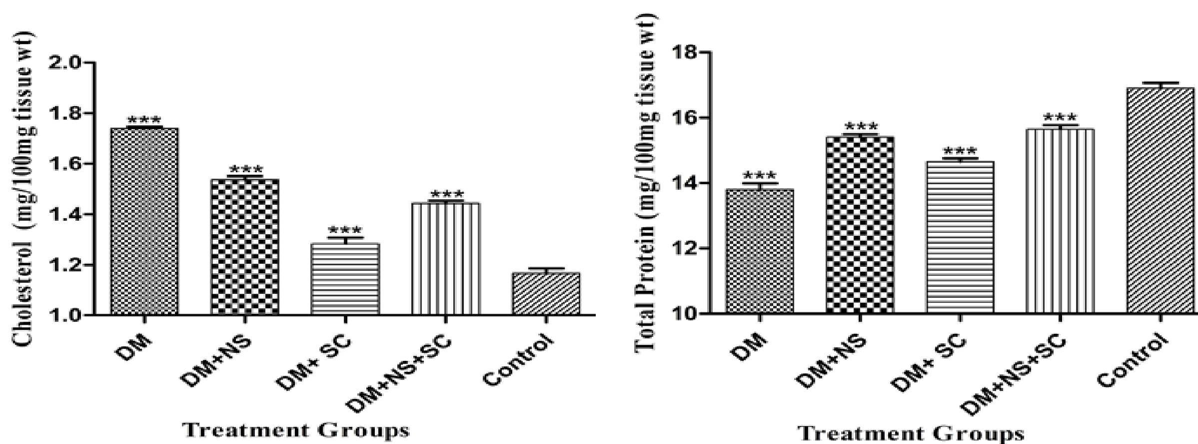


Fig 5.52: Analysis of presence of Cholesterol and Total protein in hepatic tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group relative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

The results of hepatic tissues parameters are shown in table 5.17 and analysis of results are represented in fig 5.46 to fig 5.52. The obtained results shown the decreased value of DM group relative to control group are catalase by 57.97%, GPx by 36.84%, SOD by 56.58%, GST by 34.81%, SDH 30.6%, GSH 61.54%, ATPase 26.67%, and total protein content in hepatic tissues were reduced by 18.34% while the parameters which were recorded as increased value in DM group than control group are LPO by 122.69%, ALPase by 56.82%, ACPase by 53.85%, MDA by 115.36%, NO 67.04% and Cholesterol was increased by 50%. The difference in expression of these oxidative markers in DM groups comparative to control group reflects the stress condition originated due to deltamethrin pesticide and liver is the first vital organ to face the stress of this pesticide. The significant changes in these marker enzymes reflects the damage in liver tissues. The other group consisting extracts (DM+NS, DM+SC and DM+NS+SC) shown the protective effects in current research findings in comparison to DM group. The overexpression of marker enzymes in DM group was reduced due to extracts of *N. sativa* and *S. cumini*.

Manna *et al*. (2005) reported the decrease in liver cytochrome 450 due to deltamethrin exposure in wistar rats, they also revealed the decrease in in the expression of catalase, GST, GSH, and glycogen content in hepatic tissues. Their findings stand with current research consequences. Mongi *et al*. (2011) revealed that deltamethrin causes the modification in

hematological as well as biochemical changes in wistar rats. Moid *et al*. (2014) also reported that decrease in total protein, cholesterol, SDH, and ATPase in both low and high dose of deltamethrin, while they reported the upsurge in ALPase and ACPase value in deltamethrin treated group. Their findings suggest the positive value with current research findings. Rehman *et al*. (2006) have reported that reduction in the enzymatic activity of catalase, GPx and GST in hepatic tissues which stands with the current research findings, the activity of LPO in hepatic tissues was increased in their research findings similarly in current research finding also LPO was overexpressed in hepatic tissues due to consociate of deltamethrin pesticide. Maalej *et al*. (2017) reported that deltamethrin causes the damage to hepatic tissues and resulting into alteration of liver marker enzymes SOD and catalase while fruit extracts of olive or oleuropein was used these marker enzymes expression improved which also stands with current research findings. Mani and Sadiq (2014) also reported about the reduction of acetylcholinesterase, ATPase, and antioxidant marker enzymes in deltamethrin treated wistar rats while treatment of naringin modulated the activity of marker enzymes. Arora *et al*. (2016) described the expression of significant changes in six liver originated proteins-apolipoproteins IV, apolipoproteins E, haptoglobin, hemopexin, vitamin D binding proteins and fibrinogen gamma chain in wistar rats due to deltamethrin pesticides. Apolipoproteins are involved in transport of cholesterol, triglyceride and fat-soluble vitamins from intestine to liver, change in expression of apolipoproteins modifies the transportation of these important molecules to liver and causing the source of stress condition. Change in expression of haptoglobin protein resulting into increased rate of binding with free hemoglobin released from lysis of erythrocytes. Rjeibi *et al*. (2016) described that due to exposure of deltamethrin there was modification in oxidative stress marker enzymes. The expression of ALT, AST, LDH, SOD, Catalase, GPx in liver tissues were changed due to deltamethrin pesticide but when seed extracts of *Amaranthus spinosus* reversed the expression of these oxidative marker enzymes. Their findings suggest that seed extracts of *Amaranthus spinosus* had potent antioxidant activity as well as hepatoprotective effect in deltamethrin induced rats. Similarly, in the current research findings *N. sativa* and *S. cumini* extracts shown their antioxidant activity and revealed the potent hepatoprotective effects in deltamethrin induced Swiss albino mice. Tewari *et al*. (2014; 2018) reported the alteration in oxidative marker enzymes due to exposure of deltamethrin and lindane pesticide and caused the damage to pulmonary tissues, liver tissues and nephritic tissues.

5.11.2: Results of biochemical parameters of nephritic tissues

Table 5.18: Biochemical parameters of **Nephritic** tissues in various treatment groups exposed to deltamethrin and protective effects of seed extracts of *N. sativa* and *S. cumini*, Values are represented with Mean ± SD. *** shows highly significant difference, ** shows significant difference, * represents less significant difference and NS denotes non-significant difference than control group at p value (p<0.01) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test". (DM – Deltamethrin treated group, DM+NS – Deltamethrin followed by *N. sativa* extract, DM+SC – Deltamethrin followed by *S. cumini* extract, DM+NS+SC – Deltamethrin followed by combination of *N. sativa* and *S. cumini* extract).

Parameters	DM	DM+NS	DM+SC	DM+NS+SC	Control
Catalase	57.7±2.6***	126.5±3.9***	92.9±5.0***	114.4±6.1***	160.2±4.6
Glutathione peroxidase	114.7±5.1***	135.0±3.1***	126.9±2.7***	128.5±2.5***	145.8±4.3
Superoxide dismutase	5.1±0.23***	8.6±0.26***	6.5±0.38***	7.6±0.31***	13.1±0.35
Glutathione s-transferase	91.9±3.1***	167.2±4.3***	126.6±4.2***	146.8±2.9***	244.8±4.8
Lipid peroxidation	284.3±8.1***	199.2±6.4***	224.9±6.5***	212.7±4.7***	176.0±6.8
Succinate dehydrogenase	13.1±0.23***	14.6±0.33***	16.3±0.33***	15.0±0.44***	17.4±0.32
Reduced Glutathione	0.78±0.05***	0.29±0.03***	0.20±0.02***	0.31±0.01***	0.41±0.03
ALPase	0.71±0.02***	0.50±0.15***	0.62±0.00***	0.61±0.01***	0.40±0.03
ACPase	1.31±0.05***	1.17±0.04***	1.21±0.03***	1.19±0.03***	0.81±0.02
ATPase	2.76±0.07***	3.20±0.05***	3.0±0.11***	2.86±0.06***	3.41±0.03
MDA	257.0±2.8***	161.3±4.3***	169.0±2.1***	172.2±5.0***	138.9±2.5
NO	189.6±4.07***	114.7±3.67***	137.5±3.72***	128.8±4.35***	101.7±3.6
Cholesterol	1.20±0.05***	1.03±0.07**	1.10±0.05***	1.11±0.04***	0.93±0.02
Total Protein	12.76±0.3***	13.51±0.21**	13.08±0.19***	13.35±0.18***	13.98±0.26

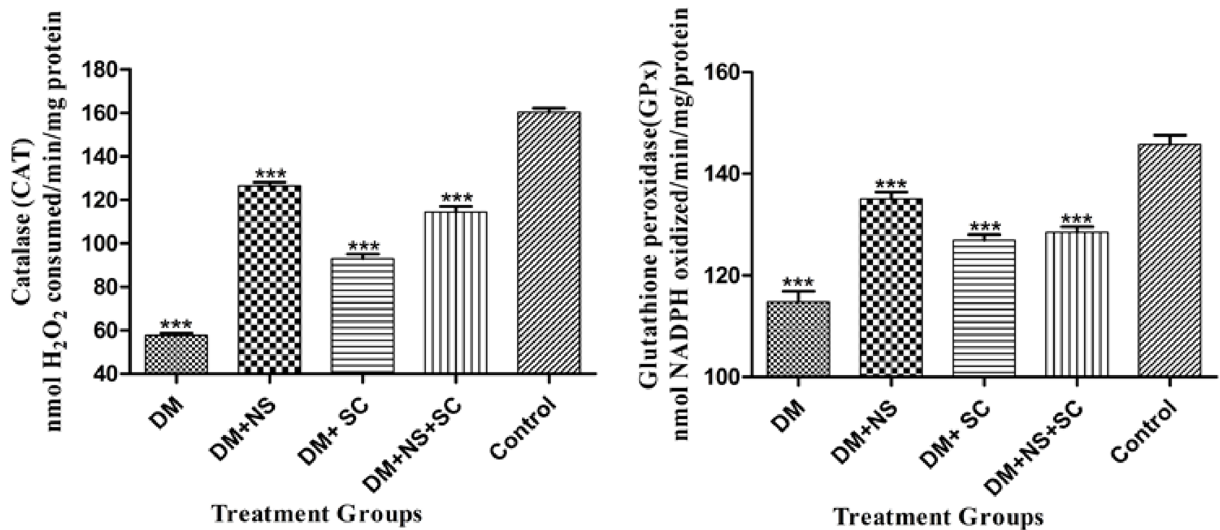


Fig 5.53: Catalase and Glutathione peroxidase analysis in nephritic tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group relative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

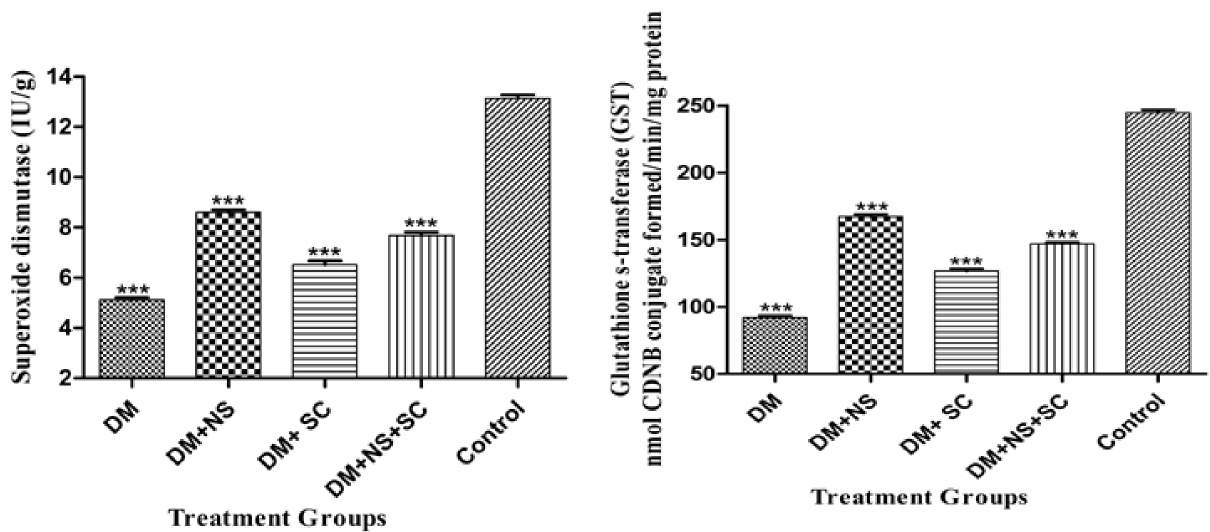


Fig 5.54: Superoxide dismutase and Glutathione S-transferase analysis in nephritic. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group relative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

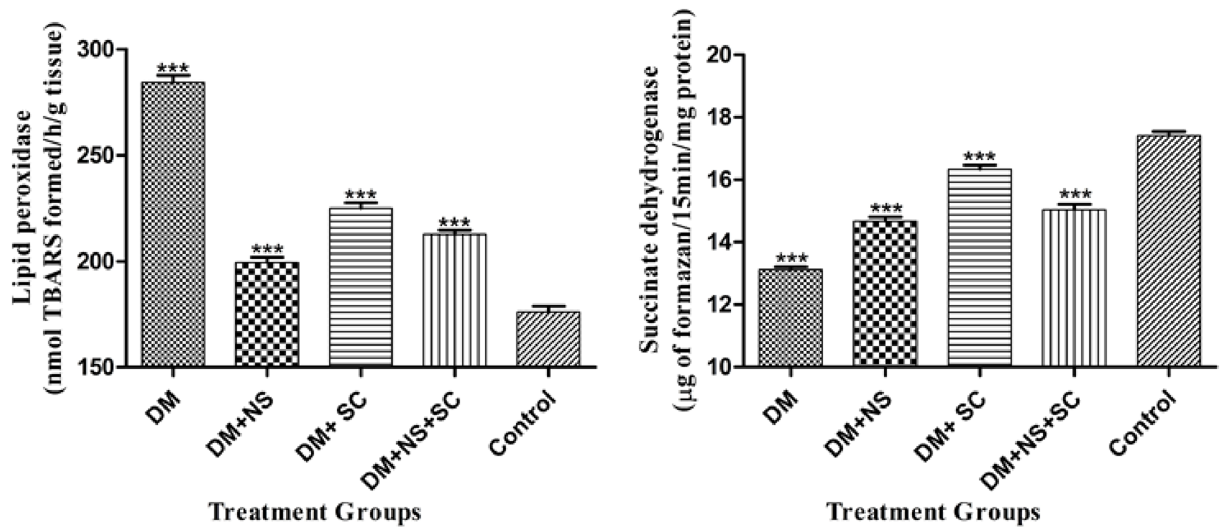


Fig 5.55: Lipid peroxidase and Succinate dehydrogenase analysis in nephritic tissues. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group relative to control group at p value (p<0.01) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

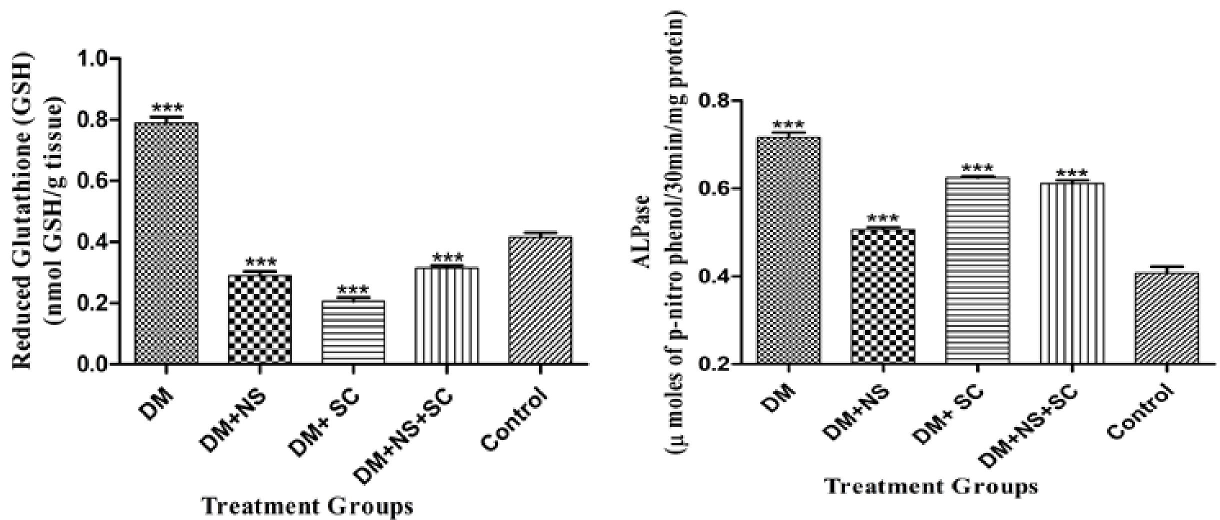


Fig 5.56: Reduced glutathione and ALPase analysis in nephritic tissues. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group relative to control group at p value (p<0.01) using one-way ANOVA.

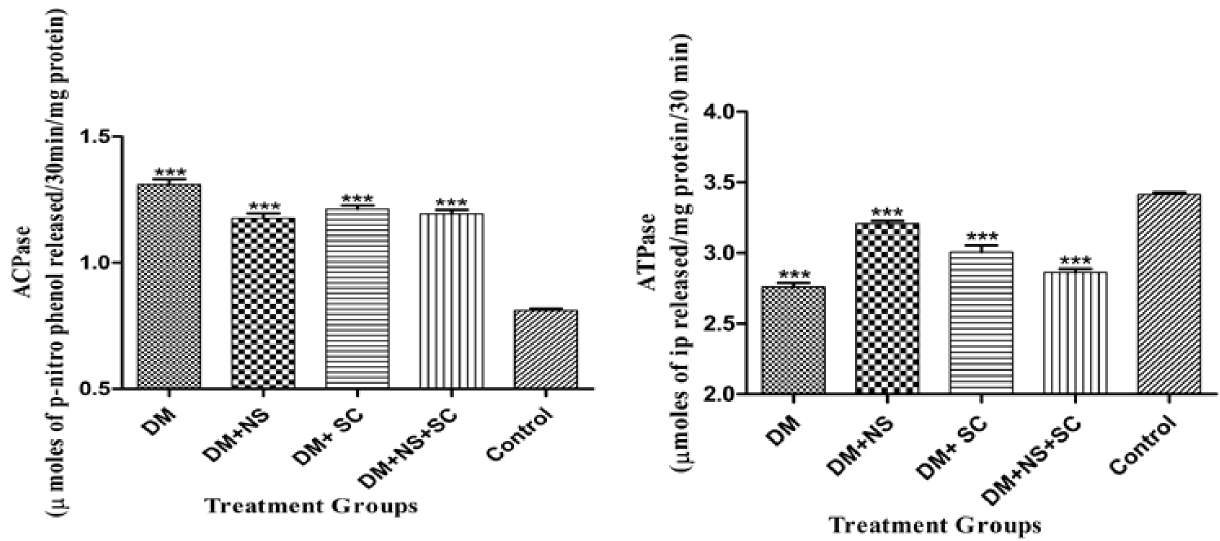


Fig 5.57: ACPase and ATPase analysis in nephritic tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group relative to control group at p value (p<0.01) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

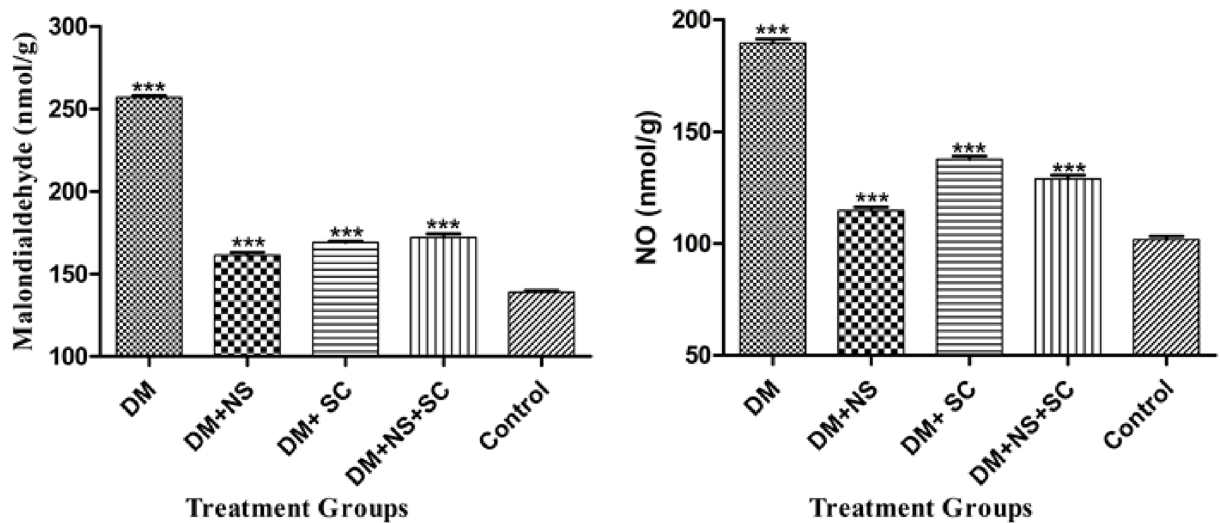


Fig 5.58: MDA and NO analysis in nephritic tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group relative to control group at p value (p<0.01) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

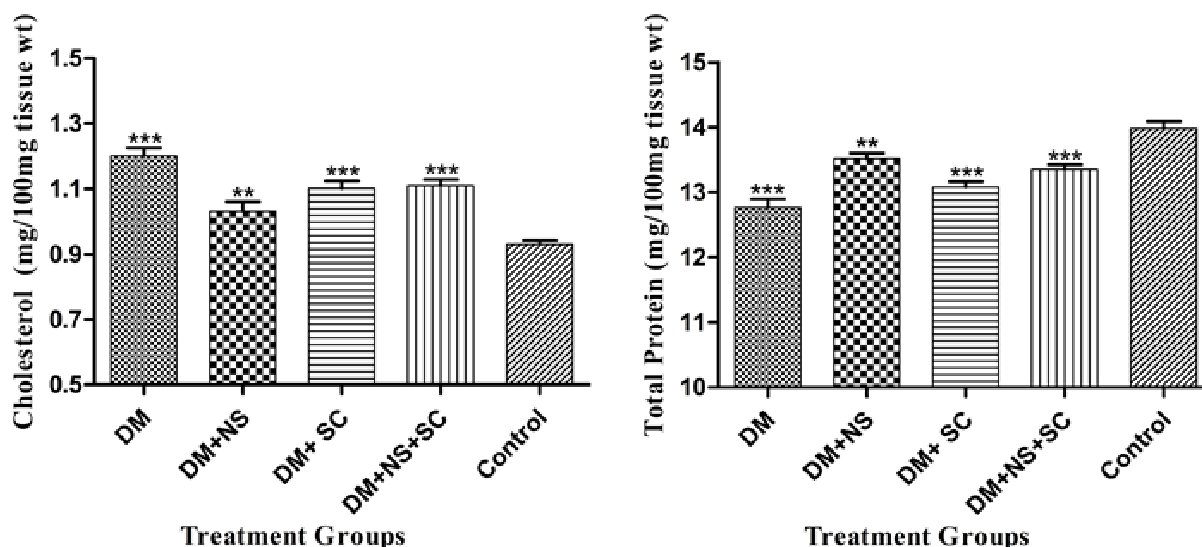


Fig 5.59: Cholesterol and Total protein analysis in nephritic tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference and ** shows significant difference between treatment group relative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

The results of nephritic tissue parameters are shown in table 5.18 and analysis of results are characterized in fig 5.53 to fig 5.59. There was demotion in expression of markers enzymes noted in DM group relative to control group. The decrease in expression of enzyme catalase by 63.98%, GPx by 21.33%, SOD by 61.07%, GST by 62.46%, SDH by 24.71%, ATPase by 19.06% and Total protein content in nephritic tissues was reduced by 9.16% while there was elevation in activity of LPO by 61.53, GSH by 90.24%, ALPase by 61.73%, MDA by 85.03%, NO by 86.43% and cholesterol content was increased by 29.03%. The seed extracts treated group improved the activity in all parameters significantly than DM group.

Rehman *et al*. (2006) shown the changes in kidney marker enzymes induced by deltamethrin. The changes in expression of these enzymes suggest the oxidative stress condition generated due to deltamethrin pesticide. Maalej *et al*. (2017) shown the decrease in the activity of catalase, SOD and MDA values in deltamethrin treated groups than the control group, these values supports the current results. They also reported the histological changes in nephritic tissues. Chargui *et al*. (2012) reported the alteration in oxidative parameters in nephritic tissues due to low dose of

deltamethrin pesticide exposure in female rats. Tewari and Gill (2014) also reported the modification in kidney marker enzymes due to deltamethrin pesticide in swiss albino mice with two defined doses for 30 days and 60 days. Some of enzymes were over expressing in 30 days exposure than 60 days exposure. Sharma *et al*. (2014) stated the alteration in expression of antioxidant enzymes in nephritic tissues, alteration of GST, CAT, SOD, GPx, and GSH values, changes in these enzymes due to defined dose of deltamethrin in wistar rats. El-Gerbed (2014) described the histological alteration in kidney due to deltamethrin exposure while a antioxidant agent lycopene was used then recovery was observed in deltamethrin induced histological changes in kidney. Sakr and Al-Amoudi (2012) described the expression of antioxidant marker enzymes in kidney tissues were altered due to deltamethrin exposure but when leaves extracts of *Ocmium basilicum* was shown protective effects on histological condition of kidney tissues as well as recovery in marker enzymes expression. Above findings and results validate the current research findings where deltamethrin causes the alteration in expression of marker enzymes and repossession of histological status of kidney tissues and marker enzymes due to seed extracts of *N. sativa* and *S. cumini*.

5.11.3: Results of biochemical parameters of cardiac tissues

Table 5.19: Biochemical parameters of **Cardiac** tissues in various treatment groups exposed to deltamethrin and protective effects of seed extracts of *N. sativa* and *S. cumini*, Values are represented with Mean ± SD. *** shows highly significant difference, ** shows significant difference, * represents less significant difference and NS denotes non-significant difference than control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test". (DM – Deltamethrin treated group, DM+NS – Deltamethrin followed by *N. sativa* extract, DM+SC – Deltamethrin followed by *S. cumini* extract, DM+NS+SC – Deltamethrin followed by combination of *N. sativa* and *S. cumini* extract).

Parameters	DM	DM+NS	DM+SC	DM+NS+SC	Control
Catalase	73.9±2.85***	105.3±1.95***	77.4±1.87***	97.4±4.34***	123.6±5.76
Glutathione peroxidase	54.8±1.82***	69.1±1.79***	61.3±1.97***	64.9±2.8***	83.5±3.89
Superoxide dismutase	8.9±0.19***	12.5±0.28***	9.9±0.25***	11.9±0.29***	14.3±0.30

Glutathione s-transferase	93.9±2.53***	139.2±3.60***	111.8±4.08***	127.8±2.93***	155.9±2.92
Lipid peroxidation	232.5±6.6***	173.7±4.97***	213.2±3.5***	191.8±3.4***	146.0±3.7
Succinate dehydrogenase	12.4±0.3***	15.7±0.4***	13.1±0.4***	14.4±0.3***	16.7±0.4
Reduced Glutathione	0.25±0.00***	0.44±0.02***	0.31±0.00***	0.38±0.01***	0.50±0.03
ALPase	0.93±0.01***	0.58±0.04 NS	0.86±0.01***	0.60±0.05 NS	0.62±0.03
ACPase	1.65±0.02***	1.36±0.06 NS	1.48±0.05***	1.36±0.05 NS	1.3±0.05
ATPase	1.81±0.05***	3.44±0.07***	2.80±0.04***	3.06±0.07***	4.11±0.04
MDA	207.23±3.41***	161.37±2.74**	183.50±3.93***	160.90±6.20**	153.41±2.15
NO	135.2±3.20***	94.75±3.37***	115.95±2.77***	108.71±2.44***	72.78±2.01
Cholesterol	1.93±0.03***	1.44±0.04***	1.81±0.04***	1.51±0.05***	1.33±0.04
Total Protein	10.83±0.20***	13.65±0.18***	11.65±0.18***	13.15±0.27***	14.95±0.45

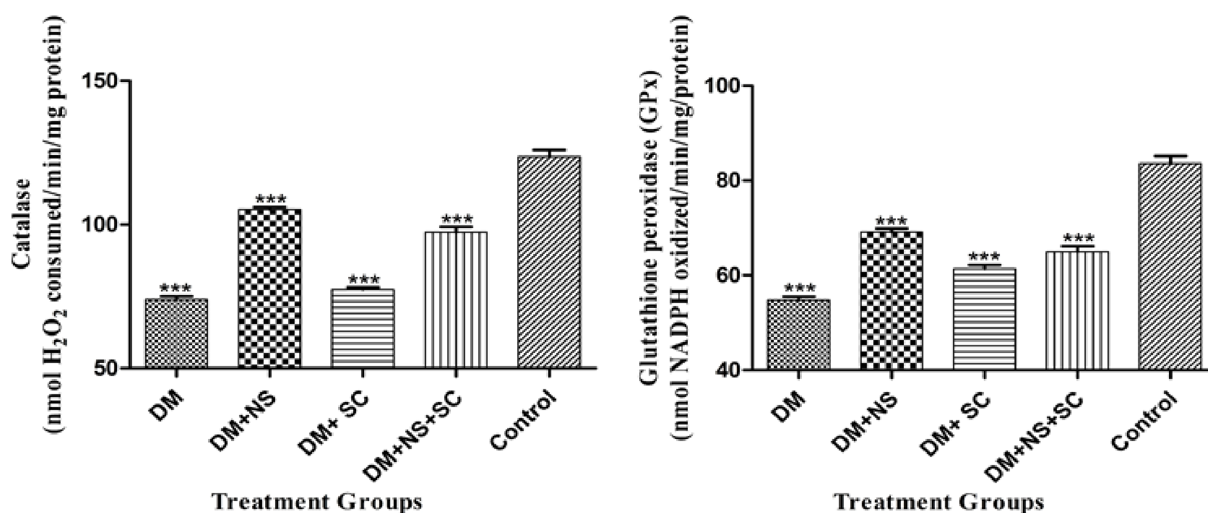


Fig 5.60: Catalase and Glutathione peroxidase analysis in cardiac tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference and ** shows significant difference between treatment group relative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

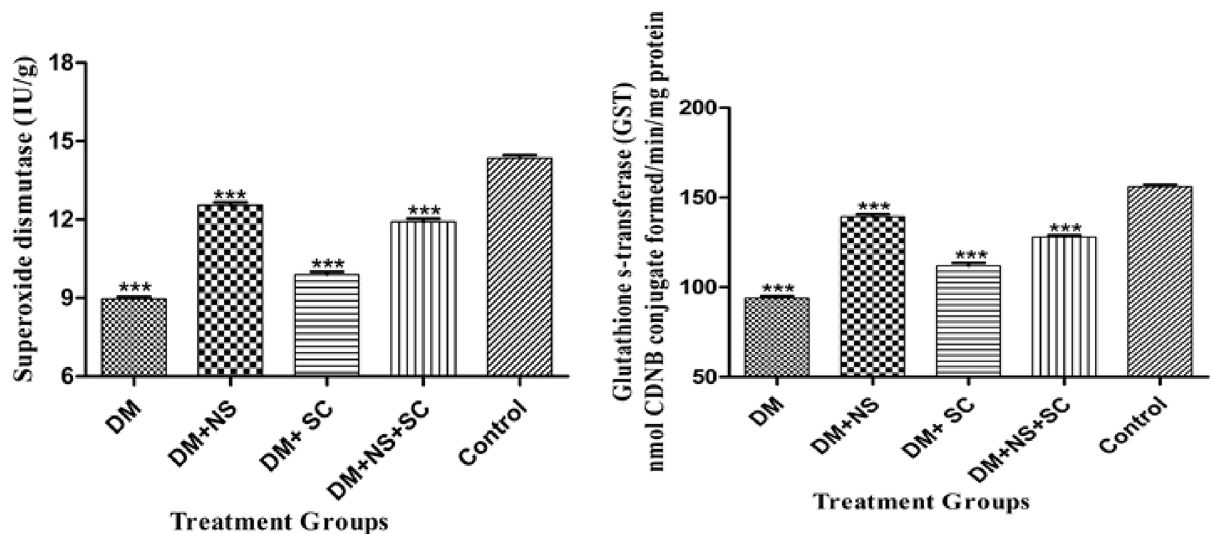


Fig 5.61: Superoxide dismutase and Glutathione S-transferase analysis in cardiac tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group relative to control group at p value (p<0.01) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

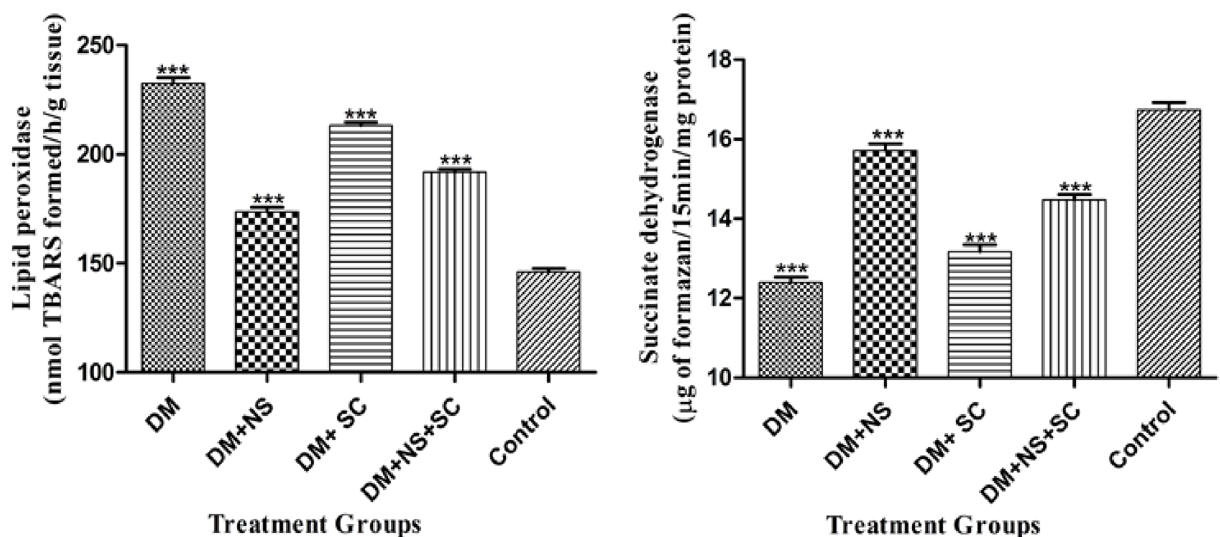


Fig 5.62: Lipid peroxidase and Succinate dehydrogenase analysis in cardiac tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group relative to control group at p value (p<0.01) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

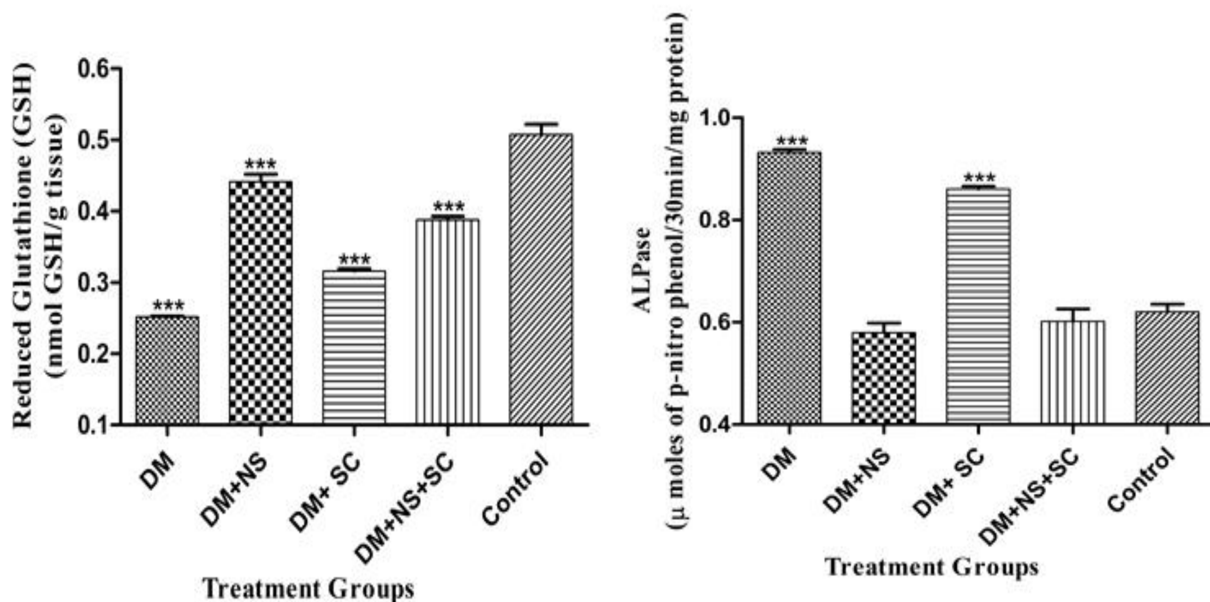


Fig 5.63: Reduced glutathione and ALPase analysis in cardiac tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group relative to control group at p value (p<0.01) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

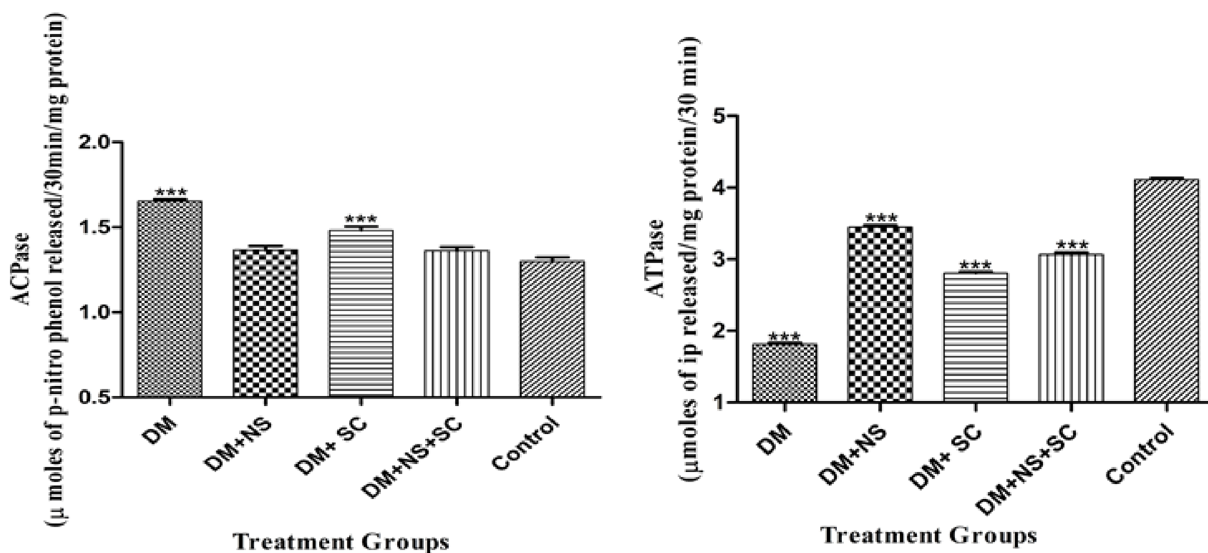


Fig 5.64: ACPase and ATPase analysis in cardiac tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group relative to control group at p value (p<0.01) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

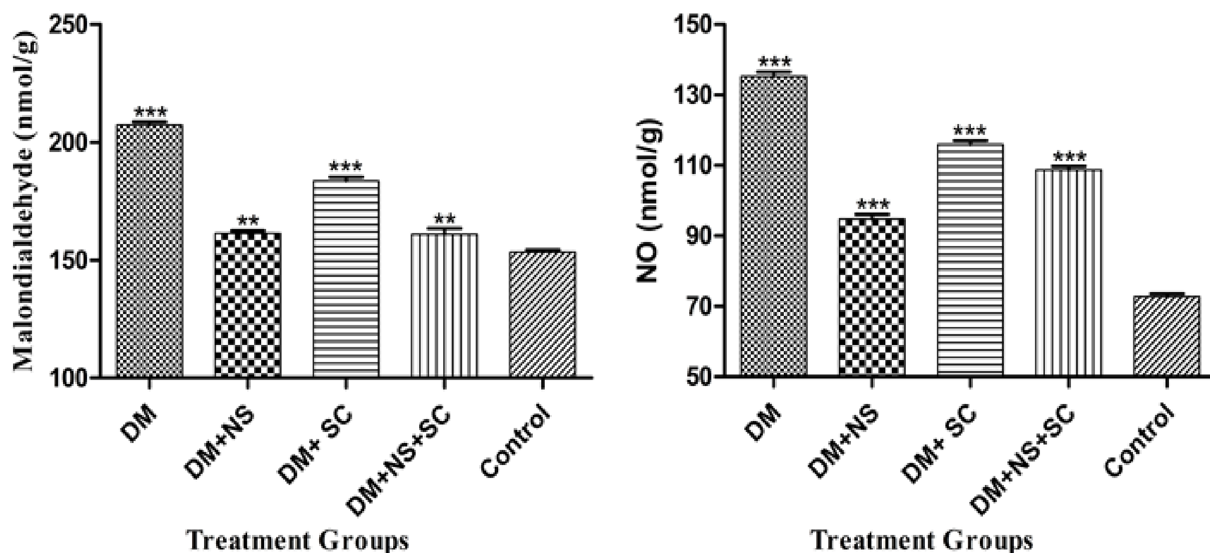


Fig 5.65: MDA and NO analysis in cardiac tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference and ** shows significant difference between treatment group relative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

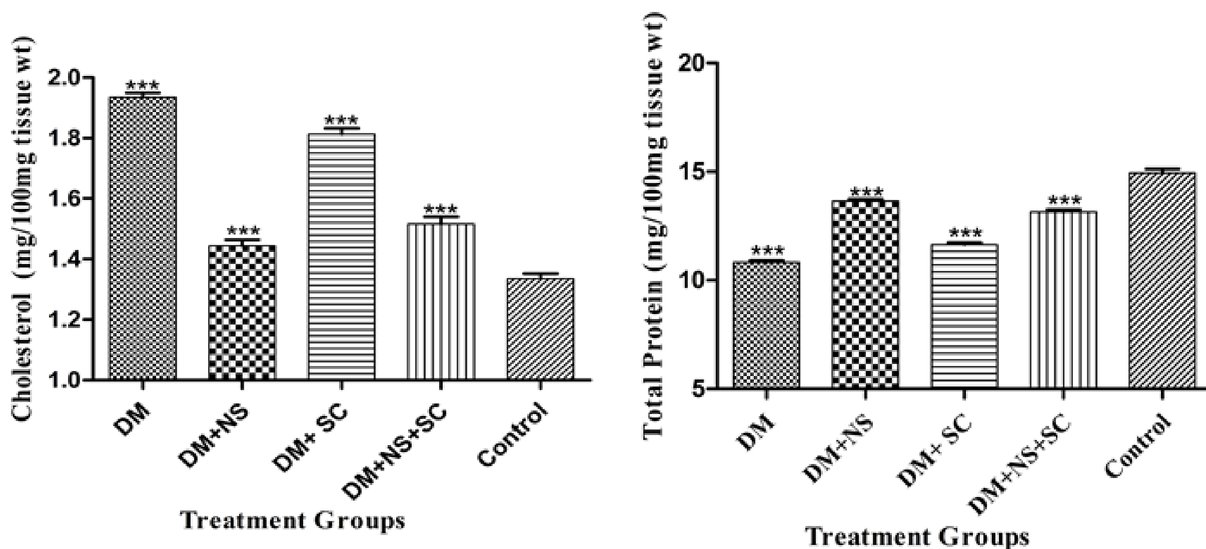


Fig 5.66: Cholesterol and Total protein analysis in cardiac tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group relative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

The results of nephritic tissues parameters are mentioned in table 5.19 and analysis of results are mentioned in fig 5.60 to fig 5.66. There was decrease in the value of enzymatic parameters in DM group than control group was observed, catalase by 40.21%, GPx by 34.37%, SOD by 34.76%, GST by 39.77%, SDH by 25.75%, GSH by 50%, ATPase by 55.96%, and total protein content was reduced by 27.56%. Some of the parameters where elevation was distinguished are, LPO by 59.25%, ALPase by 50%, ACPase by 26.92%, ATPase by 55.96%, MDA by 35.08%, NO by 85.77% and cholesterol value was elevated by 45.11%.

Papaefthimiou and Theophilidis (2001) shown the cardiotoxicity effects of deltamethrin pesticide in honey bee, *Apis mellifera macedonica*. Yonar and Sakin (2011) described the cardiotoxicity effects due to deltamethrin in *Cyprinus carpio* fish, the alteration in antioxidant marker enzymes were recorded while lycopene recovered the oxidative stress condition in fish. Yalin *et al*. (2012) stated the adverse effects of deltamethrin pesticide heart tissues of rats, but *Funalia trogii* extracts can improve the adverse effects. Haverinen and Vornanen (2014) reported the cardiotoxic effects of deltamethrin in rainbow trout fishes due to dysfunctioning of Na^+ channels in cardiac tissues. Haverinen and Vornanen (2016) also shown the cardiotoxicity effects of deltamethrin in crucian carp fish due to cardiac Na^+ channels dysfunction. Sirenko *et al*. (2017) reported the invitro analysis of cardiotoxicity in induced pluripotent stem cell due to environmental chemicals. Abdel-Daim (2016) reported that sub-acute dose of deltamethrin causes the cardiotoxicity effects in mice, these adverse effects can be overcome with extracts of *Spirulina platensis* in mice. Above all findings recommend that deltamethrin is causing the oxidative stress condition in cardiac functioning which support the current research findings.

5.11.4: Results of biochemical parameters of lung tissues

Table 5.20: Biochemical parameters of **Lung** tissues in various treatment groups exposed to deltamethrin and protective effects of seed extracts of *N. sativa* and *S. cumini*, Values are represented with Mean ± SD. *** shows highly significant difference, ** shows significant difference, * represents less significant difference and NS denotes non-significant difference than control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test". (DM – Deltamethrin treated group, DM+NS – Deltamethrin followed by *N.*

sativa extract, DM+SC – Deltamethrin followed by *S. cumini* extract, DM+NS+SC – Deltamethrin followed by combination of *N. sativa* and *S. cumini* extract).

Parameters	DM	DM+NS	DM+SC	DM+NS+SC	Control
Catalase	82.21±2.14***	109.15±2.16***	94.1±4.24***	104.6±5.70***	151.4±4.80
Glutathione peroxidase	60.3±3.31***	72.8±4.18**	65.9±1.76***	69.6±2.40***	79.1±4.79
Superoxide dismutase	11.2±0.47***	13.3±0.44***	11.8±0.27***	12.9±0.42***	15.0±0.41
Glutathione s-transferase	116.0±3.83***	131.3±3.37*	111.8±4.08***	129.5±2.70**	136.8±3.61
Lipid peroxidation	245.7±4.84***	186.9±2.50***	229.5±3.57***	193.2±8.14***	160.4±5.12
Succinate dehydrogenase	10.7±0.49***	16.0±0.53***	12.4±0.23***	15.4±0.4***	18.7±0.69
Reduced Glutathione	0.22±0.00***	0.42±0.00***	0.31±0.00***	0.40±0.01***	0.57±0.00
ALPase	1.93±0.04***	1.60±0.06**	1.81±0.05***	1.64±0.03***	1.51±0.05
ACPase	1.55±0.04***	1.16±0.03*	1.42±0.04***	1.16±0.03*	1.10±0.05
ATPase	1.42±0.04***	2.06±0.12***	1.55±0.05***	2.11±0.08***	2.37±0.11
MDA	214.2±3.63***	148.8±4.22***	193.5±3.84***	158.9±3.10***	130.0±5.57
NO	114.4±3.53***	76.8±2.68***	95.5±2.42***	76.9±3.05***	68.1±2.93
Cholesterol	1.68±0.05***	1.27±0.02NS	1.45±0.04***	1.33±0.03NS	1.30±0.04
Total Protein	10.2±0.24***	12.8±0.37***	11.1±0.32***	12.6±0.35***	14.2±0.26

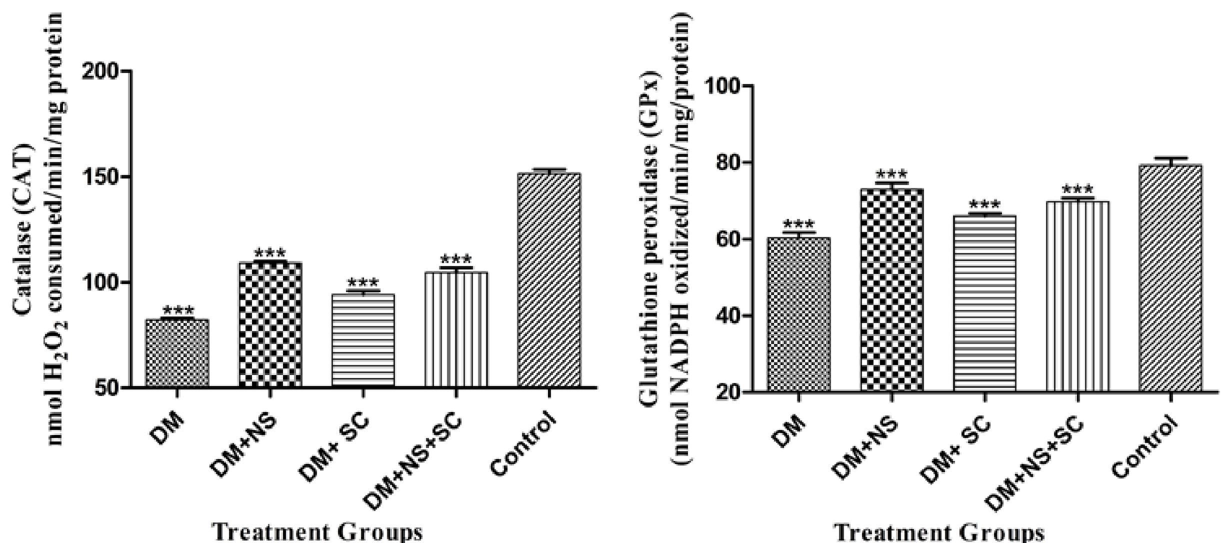


Fig 5.67: Catalase and Glutathione peroxidase analysis in Lung tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group relative to control group at p value (p<0.01) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

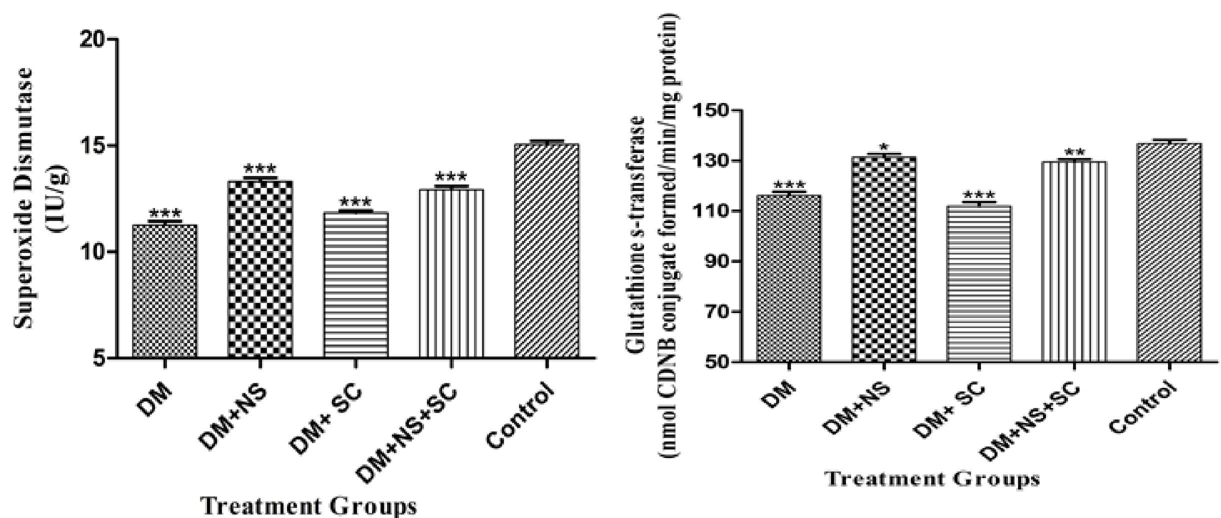


Fig 5.68: Superoxide dismutase and Glutathione S-transferase analysis in Lung tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference, ** shows significant difference and * shows least significant difference between treatment group relative to control group at p value (p<0.01) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

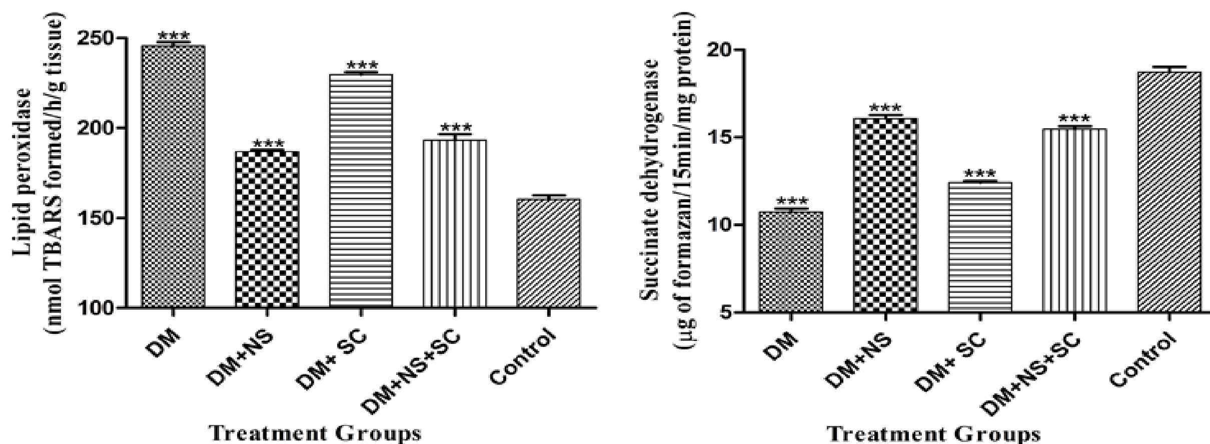


Fig 5.69: Lipid peroxidase and Succinate dehydrogenase analysis in Lung tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group comparative to control group at p value (p<0.01) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

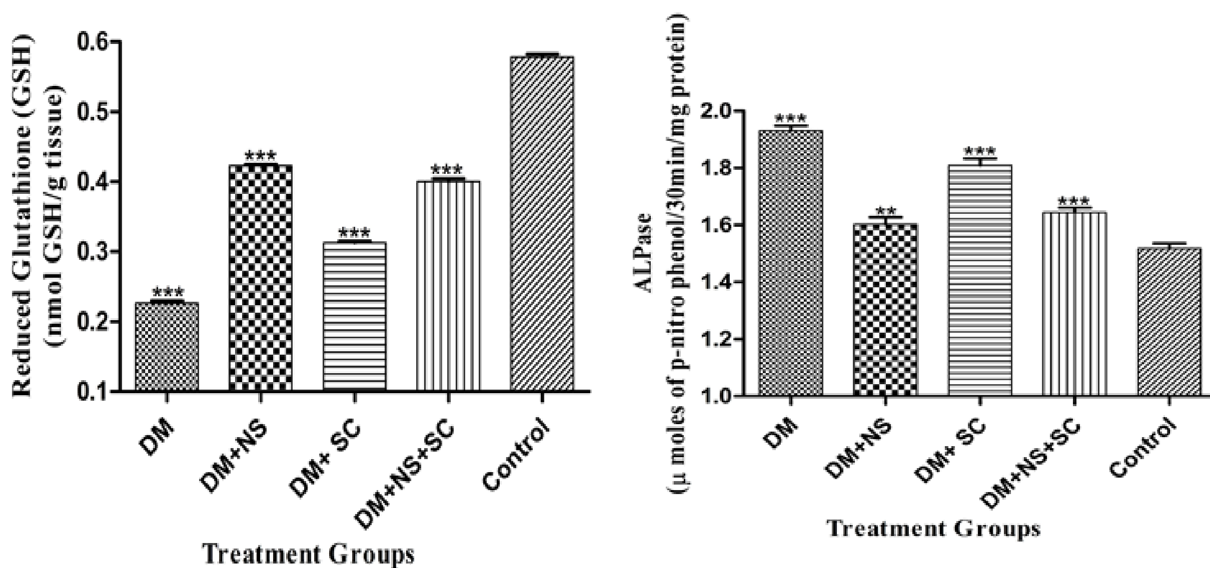


Fig 5.70: Reduced Glutathione and ALPase analysis in Lung tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference and ** shows significant difference between treatment group comparative to control group at p value (p<0.01) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

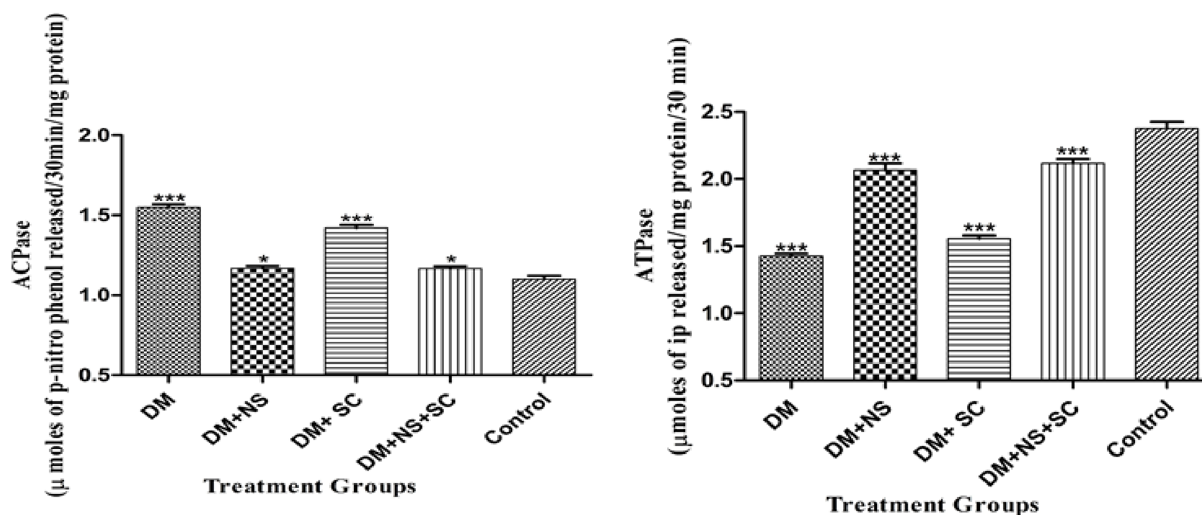


Fig 5.71: ACPase and ATPase analysis in Lung tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference and * shows significant difference between treatment group comparative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

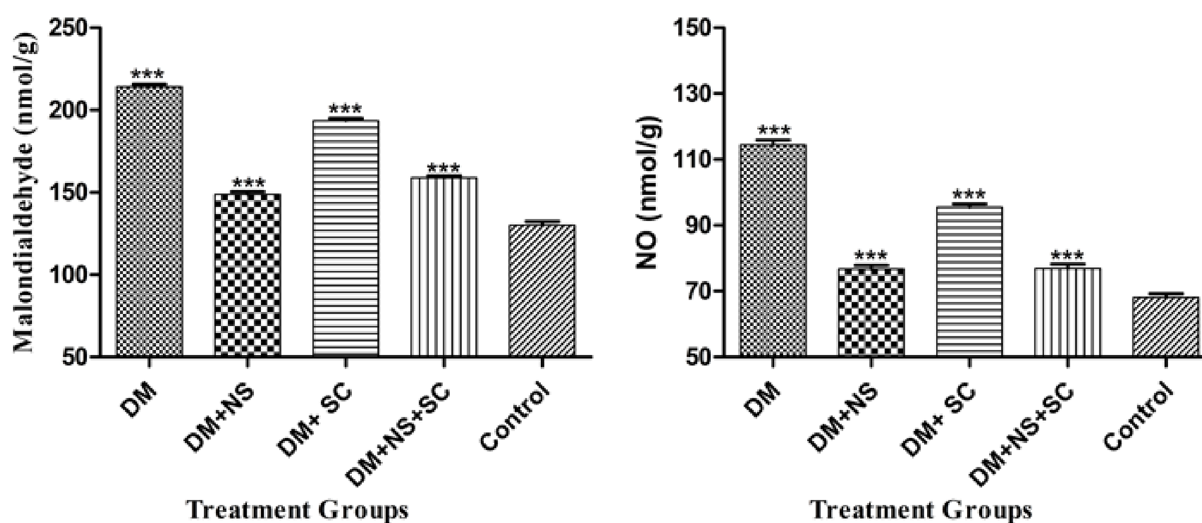


Fig 5.72: MDA and NO analysis in Lung tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group comparative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

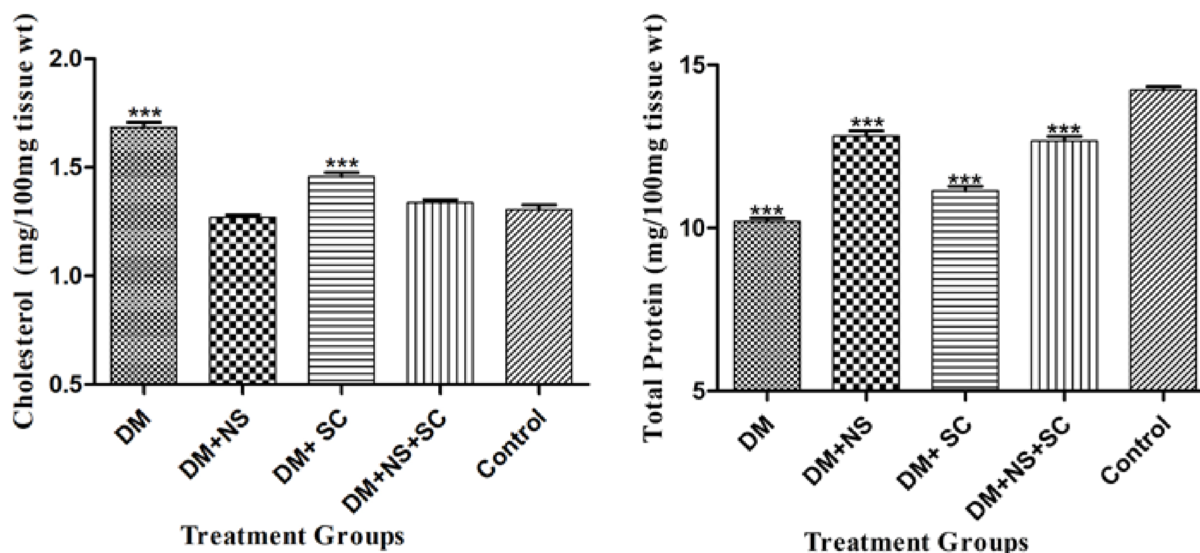


Fig 5.73: Cholesterol and Total Protein analysis in Lung tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group comparative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

The results of different parameters of lung tissues are mentioned in table 5.20 and analysis of results are represented in fig 5.67 to fig 6.73. The various parameters shown significant decrease in their values in DM group than control group, catalase by 45.7%, GPx by 23.77%, SOD by 25.33%, GST by 25.2%, SDH by 42.78%, GSH by 61.4%, ATPase by 40.08% and total protein content was reduced by 28.17%. Some of the parameters shown significant elevation in DM group than control group are; LPO by 53.18%, ALPase by 27.81%, MDA by 64.77%, NO by 67.99% and there was also increase in cholesterol value by 28.17%.

Manna *et al*. (2005) described the histological changes in lung tissues in rats due to 15 mg/kg deltamethrin dose for 30 days. They reported also the biochemical changes in lung tissues specially declined activity of cytochrome P450. Erdogan *et al*. (2006) reported that morphologic changes in lung tissues due to deltamethrin inhalation in rats. Wu *et al*. (2013) described the distribution of deltamethrin in acute dose (512 mg/kg) was mostly analysed in lungs tissues and causing the morphological and histological changes. The deposition of deltamethrin in lungs tissues depicts the oxidative stress condition leading to altered biochemical metabolism. El-Tahir (1993) also described the improvement in respiratory system due to volatile compounds present in

oil of *N. sativa* in guinea pigs. The deposition of deltamethrin in lungs tissues was followed by hepatic tissues, cardiac tissues, nephritic tissues, blood and brain tissues in 512 mg/kg dose of deltamethrin in rats while with 1024 mg/kg dose of deltamethrin the deposition pattern was recorded as, lungs tissues followed by blood, cardiac tissues, nephritic tissues, brain tissues and hepatic tissues. The above all findings suggest that deltamethrin is causing damage the functioning of vital organs of the individual and resulting into change in expression of biochemical markers of the organ system. The current research parameters related to deltamethrin induced lungs toxicity also leads to supports the above findings. Oxidative stress conditions were minimized or reversed by plant extracts and improved lungs morphology and histology were observed.

5.11.5: Results of biochemical parameters of splenic tissues

Table 5.21: Biochemical parameters of **Splenic** tissues in various treatment groups exposed to deltamethrin and protective effects of seed extracts of *N. sativa* and *S. cumini*, Values are represented with Mean ± SD. *** shows highly significant difference, ** shows significant difference, * represents less significant difference and NS denotes non-significant difference than control group at p value (p<0.01) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test". (DM – Deltamethrin treated group, DM+NS – Deltamethrin followed by *N. sativa* extract, DM+SC – Deltamethrin followed by *S. cumini* extract, DM+NS+SC – Deltamethrin followed by combination of *N. sativa* and *S. cumini* extract).

Parameters	DM	DM+NS	DM+SC	DM+NS+SC	Control
Catalase	82.65±1.39***	97.40±1.68***	85.93±1.92***	99.58±5.11 ***	137.55±2.45
Glutathione peroxidase	43.85±1.38***	65.25±1.25***	48.65±1.84***	63.26±1.37***	92.08±2.08
Superoxide dismutase	8.53±0.21***	11.56±0.25***	9.45±0.23***	10.68±0.19***	13.93±0.35
Glutathione s-transferase	130.25±2.40***	160.51±2.46***	139.08±2.99***	149.11±3.62***	175.38±2.26
Lipid peroxidation	233.5±3.13***	194.4±4.73***	220.1±3.40***	201.2±2.92***	137.9±3.05
Succinate dehydrogenase	11.79±0.06***	16.19±0.05***	13.59±0.05***	15.85±0.05***	18.0±0.12
Reduced Glutathione	0.28±0.00***	0.36±0.00***	0.32±0.00***	0.35±0.00***	0.48±0.01

ALPase	0.94±0.02***	0.62±0.04***	0.81±0.02***	0.64±0.03***	0.51±0.04
ACPase	1.93±0.03***	1.65±0.02***	1.74±0.02***	1.81±0.04***	1.47±0.05
ATPase	1.62±0.03***	2.20±0.06***	1.87±0.04***	1.84±0.04***	3.00±0.05
MDA	227.06±3.89***	181.38±6.23***	194.3±3.9***	183.1±4.07***	130.3±3.30
NO	174.65±4.02***	108.1±4.66***	153.3±3.67***	122.8±5.07***	90.38±2.45
Cholesterol	1.86±0.03***	1.48±0.04***	1.74±0.03***	1.63±0.04***	1.31±0.04
Total Protein	9.27±0.05***	12.65±0.07***	10.52±0.10***	11.95±0.11***	14.5±0.06

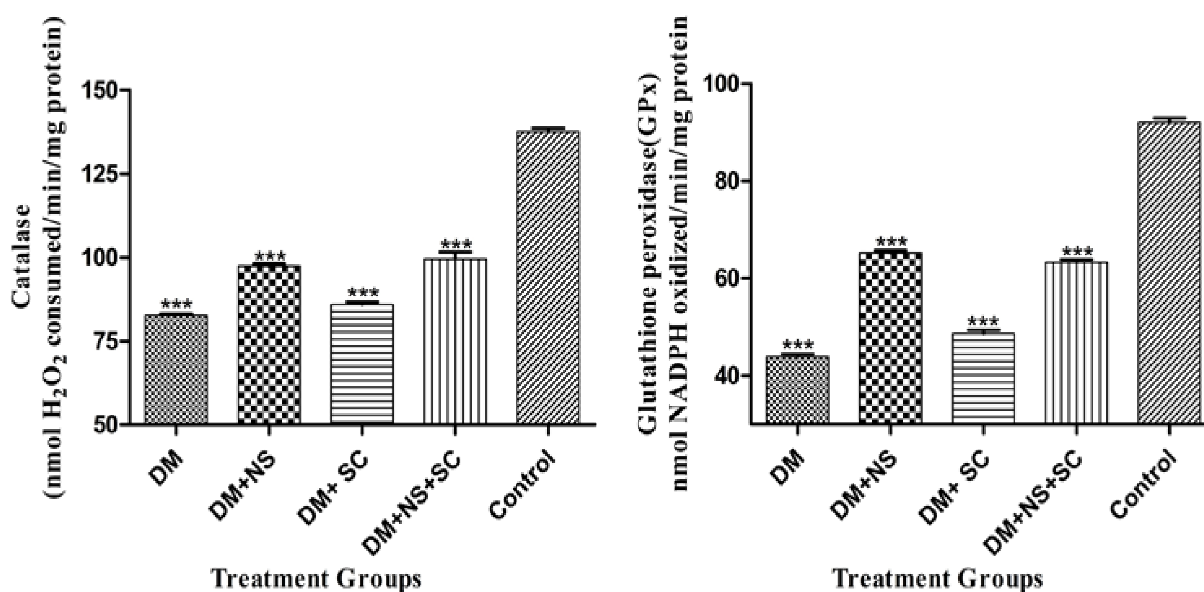


Fig 5.74: Catalase and Glutathione peroxidase analysis in Spleen tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group comparative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

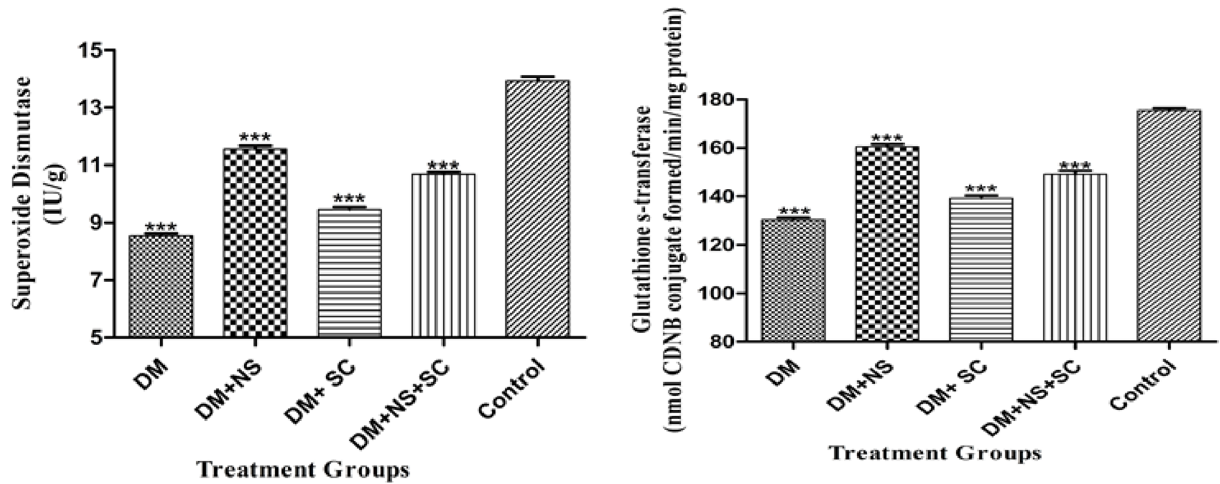


Fig 5.75: Superoxide dismutase and Glutathione S-transferase analysis in Spleen tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group comparative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

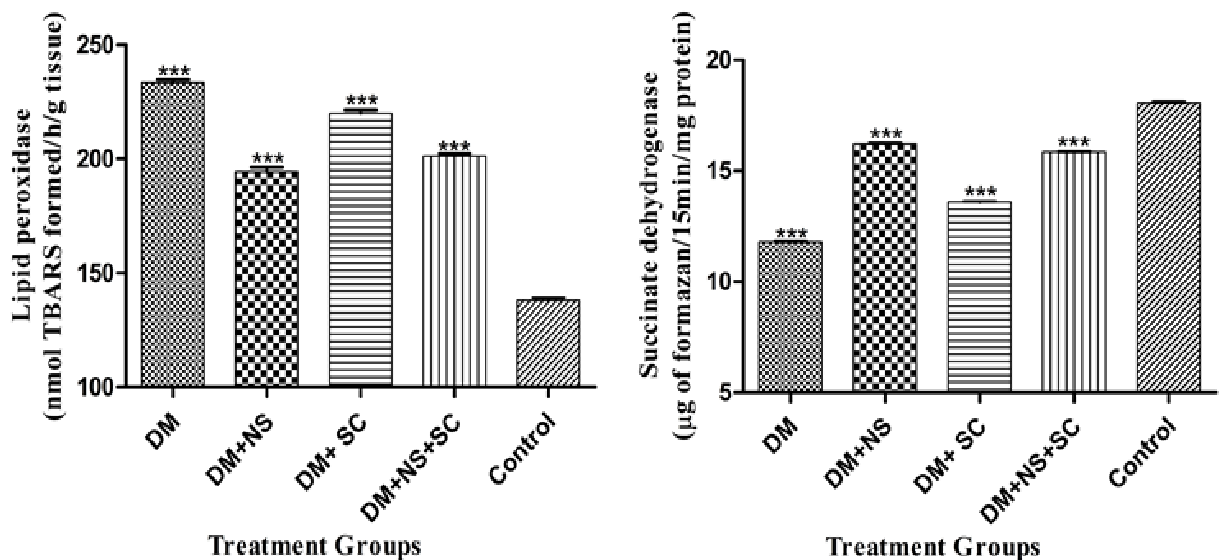


Fig 5.76: Lipid peroxidase and Succinate dehydrogenase analysis in Spleen tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group comparative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

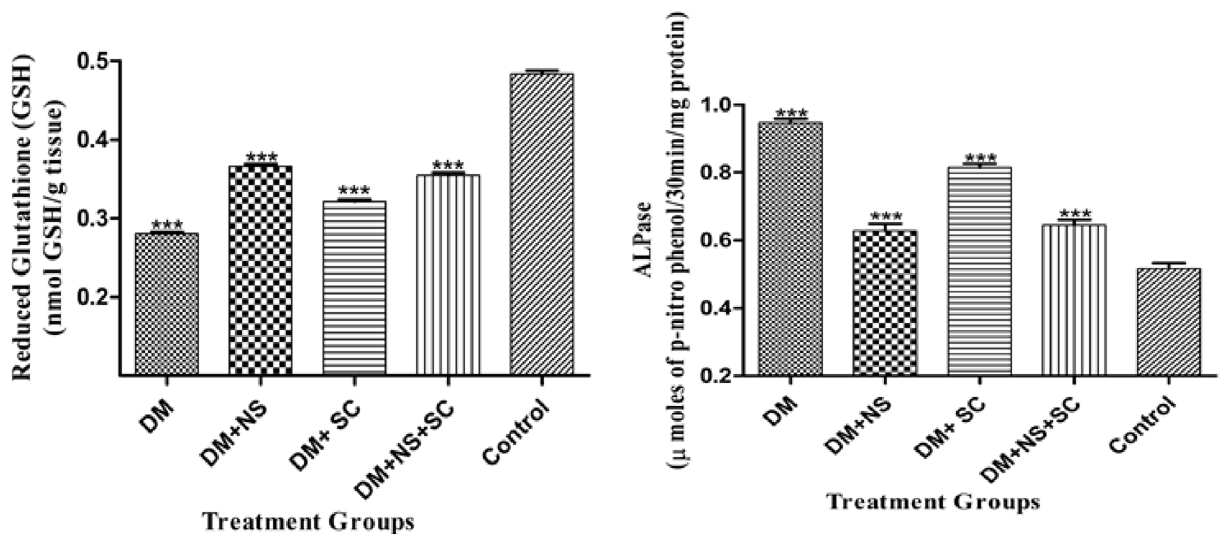


Fig 5.77: Reduced glutathione and ALPase analysis in Spleen tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group comparative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

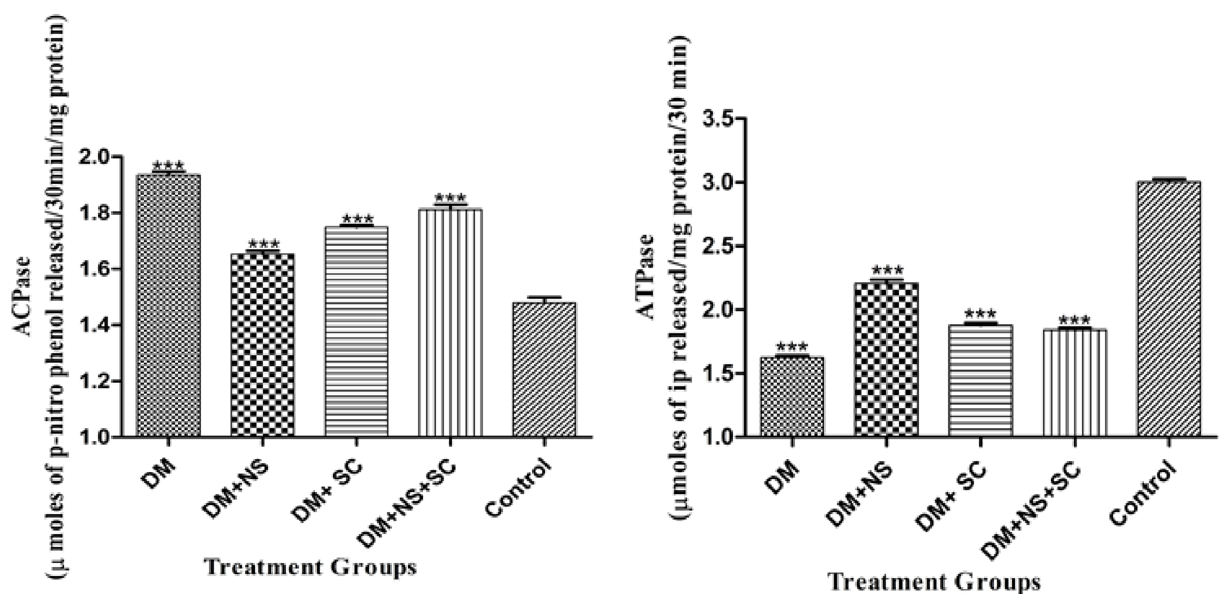


Fig 5.78: ACPase and ATPase analysis in Spleen tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group comparative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

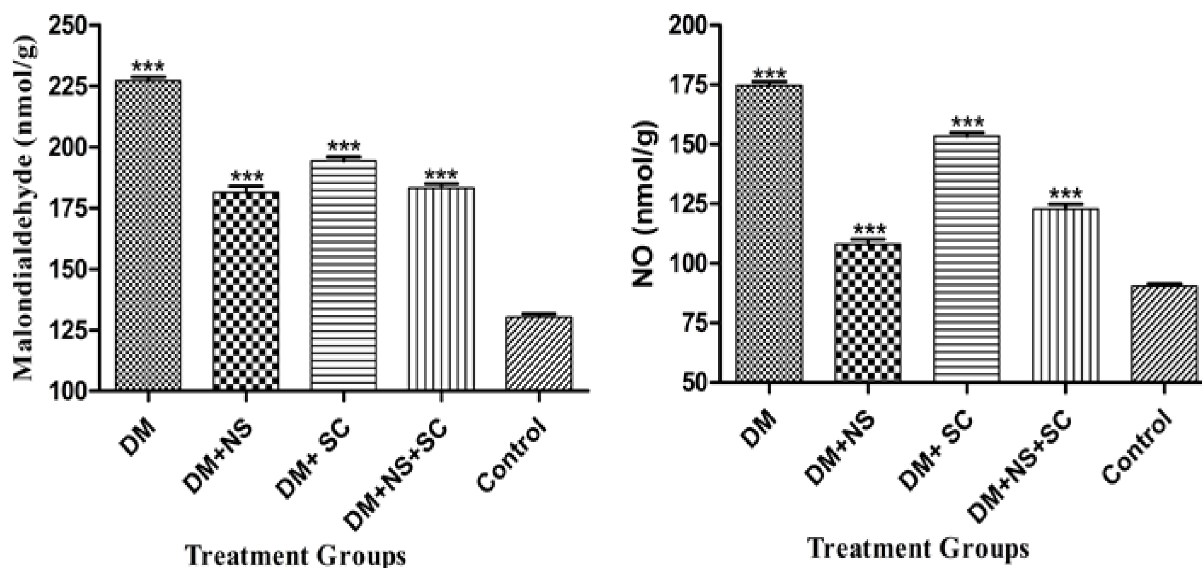


Fig 5.79: MDA and NO analysis in Spleen tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group comparative to control group at p value (p<0.01) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

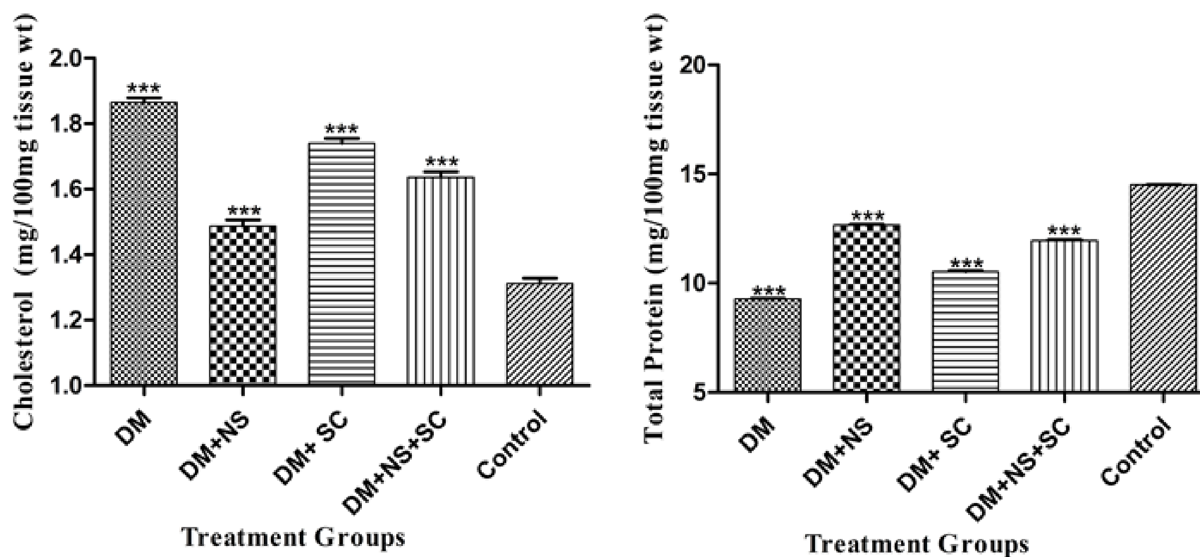


Fig 5.80: Cholesterol and Total protein analysis in Spleen tissues in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group comparative to control group at p value (p<0.01) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

The results of splenic tissues parameters are mentioned in table 5.21 and analysis of results are characterized in fig 5.74 to fig 5.80. There was significant changes in antioxidant parameters and other biochemical markers have been identified in DM group than control group, the decreased value of different parameters are, catalase by 39.91%, GPx by 52.38%, SOD by 38.77%, GST by 25.73%, SDH by 34.5%, GSH by 41.67%, ATPase by 46 % and total protein content value was reduced by 36.07%. There were some parameters where raise in values were recorded are, LPO by 69.33%, ALPase by 84.31%, ACPase by 31.29%, MDA by 74.26%, NO by 93.24% and cholesterol content was increased by 41.98% in DM group relative to control group. These parameters were reversed to some extent in recovery mode when seed extracts of *N. sativa* and *S. cumini* was used alone and in combination in deltamethrin treated groups. These changes in parameters reflects the antioxidant properties of seed extracts of *N. sativa* and *S. cumini* and causing the protective effect in deltamethrin induced splenic toxicity.

Łukowicz-Ratajczak and Krechniak (1992) revealed the adverse effects of deltamethrin on spleen resulting into abnormal functioning of immune system. Yildirim *et al*. (2006) described the deltamethrin effects in spleen of *Nile tilapia* fish and resulting into morphological, histological, and biochemical modifications in spleen tissues. Tewari and Gill (2014) also reported the effects on splenocytes due to deltamethrin (0.5 mg/kg) exposure for 30 days, they described the neutropenia and lymphocytosis was significantly increased due to deltamethrin pesticides in mice similar findings were also observed in current research results. Kumar and Sharma (2015) pronounced the splenic apoptosis due to deltamethrin exposure and recovery due to piperine and curcumin antioxidants which also supports the current research explanations. Rehman *et al*. (2011) characterized the adverse effects of deltamethrin in splenic tissues resulting into immune compromised state development in mice and causing the increase infection due to pathogenic fungi *Candida albicans*. Aydin (2011) also described the contrary effects on spleen tissues due to deltamethrin and causing the immune compromised state development in rats. The above all findings supports and stands with current research observations where deltamethrin is causing the damage to splenic tissues and causing the change in biochemical metabolic process due to change in expression of marker enzymes due to oxidative stress caused by deltamethrin pesticides.

5.12: Results of Immunological Parameters

Table 5.22: Immunological parameters of **Blood** in various treatment groups exposed to deltamethrin and protective effects of seed extracts of *N. sativa* and *S. cumini*, Values are represented with Mean ± SD. *** shows highly significant difference than control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test". (DM – Deltamethrin treated group, DM+NS – Deltamethrin followed by *N. sativa* extract, DM+SC – Deltamethrin followed by *S. cumini* extract, DM+NS+SC – Deltamethrin followed by combination of *N. sativa* and *S. cumini* extract).

Parameters	DM	DM+NS	DM+SC	DM+NS+SC	Control
PFC assay (PFC/10^6 cells)	88.3±2.3***	287.8±2.7***	224.3±4.6***	308.0±1.7***	384.5±1.5
DTH (Foot Pad) assay (mm)	0.29±0.02***	0.61±0.03***	0.65±0.01***	0.62±0.02***	0.77±0.01
HT assay	66.0±5.2***	530.0±4.0***	381.0±5.5***	476.0±5.2***	576.0±4.0
Oxidative burst assay	0.12±0.01***	0.15±0.01***	0.14±0.00***	0.14±0.01***	0.23±0.01
Macrophage phagocytosis (%)	17.3±0.9***	43.7±0.8***	42.1±1.0***	38.7±0.6***	47.0±1.0
TNF-α (pg/ml)	200.3±16.9***	101.4±5.4***	153.5±5.5***	123.5±4.5***	25.9±2.0
IL-6 (pg/ml)	4.25±0.30***	6.9±0.21***	4.9±0.26***	6.3±0.21***	10.7±0.62

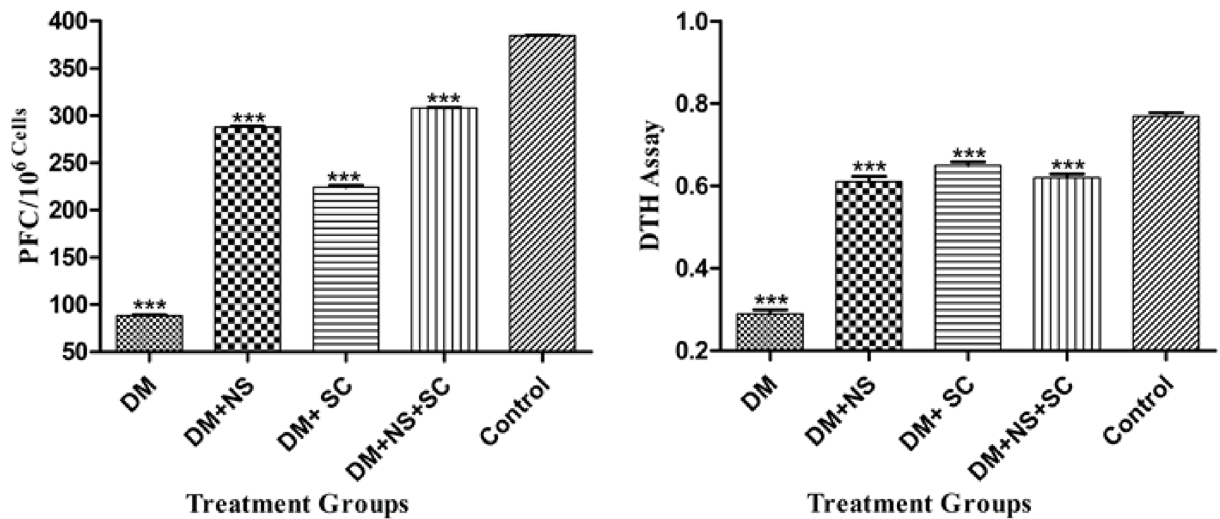


Fig 5.81: PFC assay and DTH assay (Foot pad thickness) analysis in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group comparative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

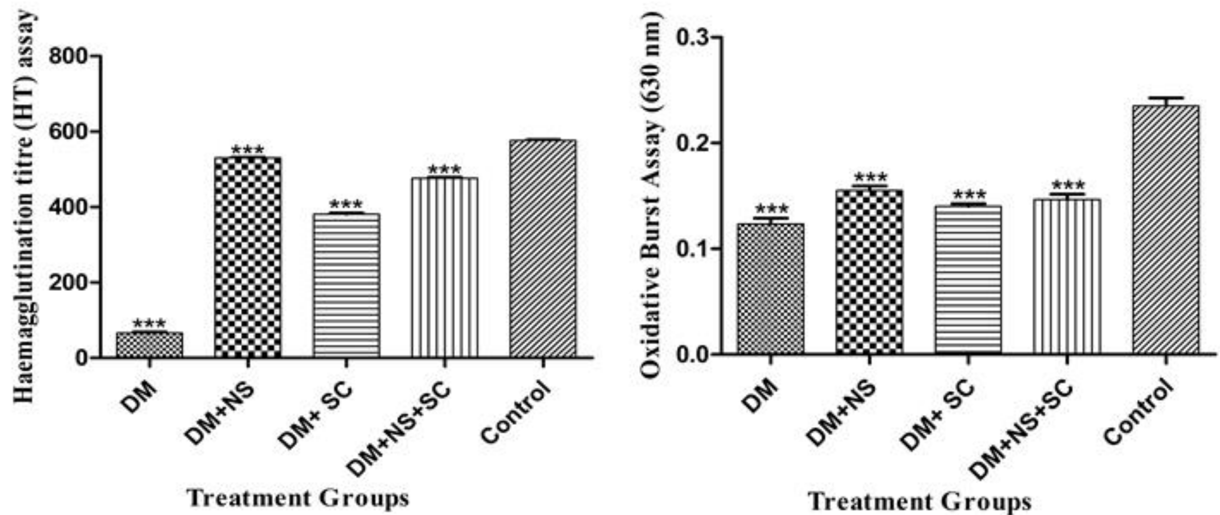


Fig 5.82: HT assay and Oxidative burst assay analysis in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group comparative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

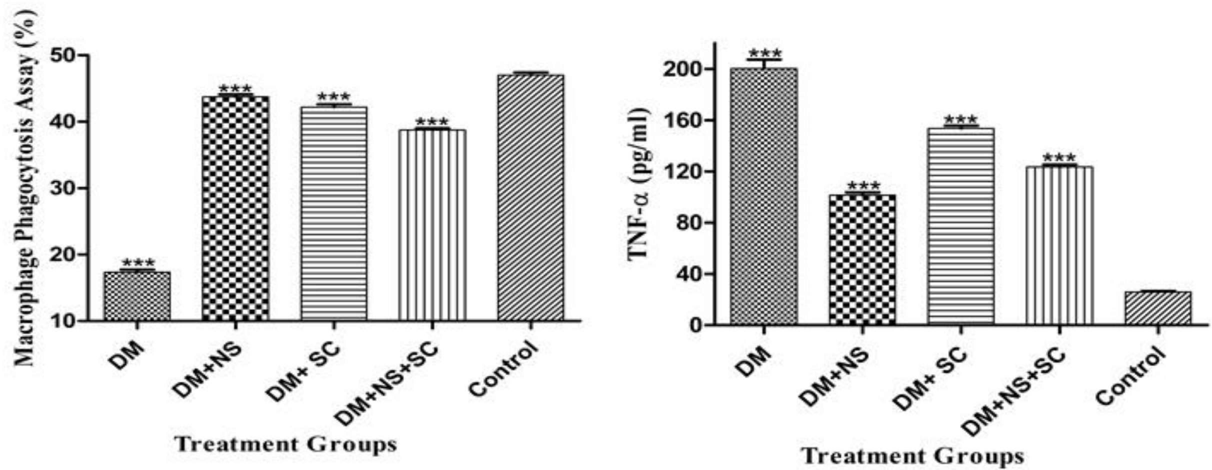


Fig 5.83: Macrophage phagocytosis assay and TNF-α analysis in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group comparative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

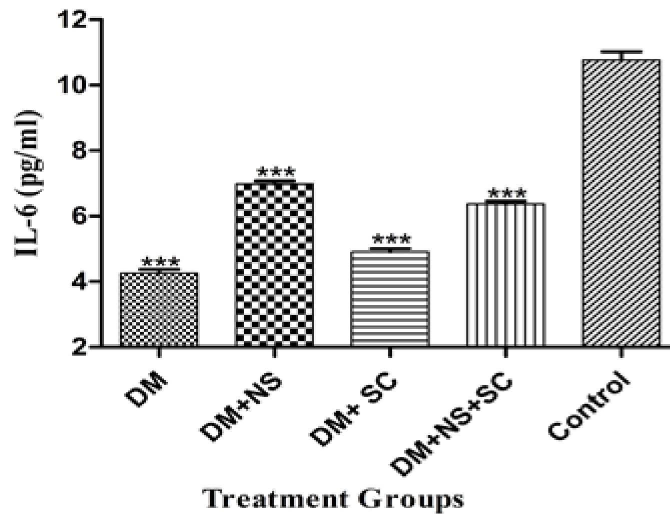


Fig 5.84: IL-6 analysis in treatment groups and compared with control group. Values are represented with Mean with SEM. Sign *** shows highly significant difference between treatment group comparative to control group at p value ($p<0.01$) using one-way ANOVA and "Newman-Keuls Multiple Comparison Test".

The results of immunological parameters are shown in table 5.22 and analysis of results are portrayed in fig 5.81 to fig 6.84. The current immunological outcomes are suggesting that deltamethrin causing the severe effects on lymphoid organs and resulting into immune compromise state development and seed extracts derived from *N. sativa* and *S. cumini* reversed the effects by improving the values. There was significant decrease in parameters in DM group relative to control group, PFC by 77.04%, DTH by 62.34%, Hemagglutinin titer by 88.54%, oxidative burst assay value by 47.83%, macrophage percentage by 63.19%, and Interleukin-6 value was recorded to decrease by 60.28%. There was elevation in TNF-α expression in DM group by 673.36% than control group. Oanh *et al*., in 2014 also established the negative effects of deltamethrin pesticides on immune response.

OBJECTIVE 4: To study the histopathological deterioration due to exposure of deltamethrin and protective effects of seed extracts of *Nigella sativa* and *Syzygium cumini* in Swiss albino mice.

5.13: Results of histopathological investigation

Due to deltamethrin exposure in group of mice histological changes have been observed in all targeted organs after HE-Staining process under microscope. Histological changes in organs Lungs, Heart, Liver, Spleen and kidney were performed and for all organs different parameters were followed as per standard method and also compared with earlier findings.

LUNGS:

Following parameters included for histological evaluation of lung tissues-

Table 5.23: Parameters for Histological examination of lungs due to DM exposure

Parameters	Gr1	Gr2	Gr3	Gr4	Gr5
Hemorrhage	2	1	1	1	0
Desquamation of bronchial epithelium	2	0	0	1	0
Peribronchial infiltration	3-4	1	1	1	1
Perivascular infiltration	2-3	1	1	1	0
Thickening of alveolar septa	1	0	0	0	0
Congestion in alveolar capillaries	1	0	0	0	0
Increase in perivascular space	3	1	1	1	1
Gr1-Deltamethrin (DM)exposed group, Gr2-DM for 10 days + NS extract for 10 days, Gr3-DM for 10 days + SC extract for 10 days, Gr4-DM for 10 days + NS & SC extract for 10 days, and Gr5-Control group treated with technical grade corn oil					
0 = No change, 1 = Mild change, 2 = Moderate change, 3 = Severe change, 4 = Very severe changes					

The pesticide Deltamethrin (DM) was administered in high dose of 18 mg/kg through gavage in Group 1 to 5, six mice in each group, till 10 days causes the persuaded peri-bronchial as well as peri-vascular infiltration of mononuclear cells along with septal cell infiltrations and thickening of septal wall as compared to control group 5 and shown in fig 5.85. In the second group of mice which was treated with DM for 10 days and followed by treatment of NS extract have shown recovery in Peri-bronchial and peri-vascular infiltration of mononuclear cells. Similarly, in group 3, there was recovery in status of lung tissues were observed as compare to group 1 but it was less effective than group 2. There was mild increase in perivascular space in Gr2-Gr5 but in Gr1 the change was observed as severe due to DM exposure. There was moderate hemorrhage observed in Gr1, while this was of mild level in Gr2-Gr4 and in Gr5 there was no hemorrhage in lung tissue observed.

Similar findings and alterations in lung tissues have been reported by Manna *et al.,* 2005; Erdogan *et al.,* 2006; Lamfon, 2007; Emara and Draz 2007 and Shona *et al.,* 2010. These microscopic observations can be elucidated through cytotoxic properties of deltamethrin and its direct effects on endothelial and alveolar cells of lung tissues. In other way the metabolites of deltamethrin can also lead the indirect injuries to endothelial and alveolar cells of lung tissues. The number of different metabolites during the metabolism of deltamethrin is 20 as per findings of Ruzo *et. al,* 1978 while in one of the other research studies by Anadon *et al,* in 1996 reported that 4′-HO- is a metabolite produced during deltamethrin metabolism, which may lead to high risk of toxicity effects and cause the injuries of various tissues of vital organs.

In one of the research studies of deltamethrin with defined dose of 15 mg/kg of deltamethrin organization orally in rats for 30 days prompts different neurotic changes, gentle to direct histological alterations in lungs, liver, stomach, kidney, testicles and cerebellum which related well with extensive sum in tissue buildups. The repeated deltamethrin portion stimuli poisonous quality and shows decline cytochrome P450 content, antioxidant agent status in rats (Manna *et al*., 2005). Erdogan *et al*. in 2006 reported that there were swollen ciliated cells and with a couple of strange cilia alongside gentle breaking down of alveolar coating cells and edematous alveolar septa of lung tissues during ultrastructural microscopic observation. So, with above all findings and reports it can be concluded that deltamethrin in high dose i.e. 18 mg/kg is causing damage to lung tissues and which can be recovered by seed extracts of *Nigella sativa* and *Syzygium cumini* alone as well as in combination of these two extracts.

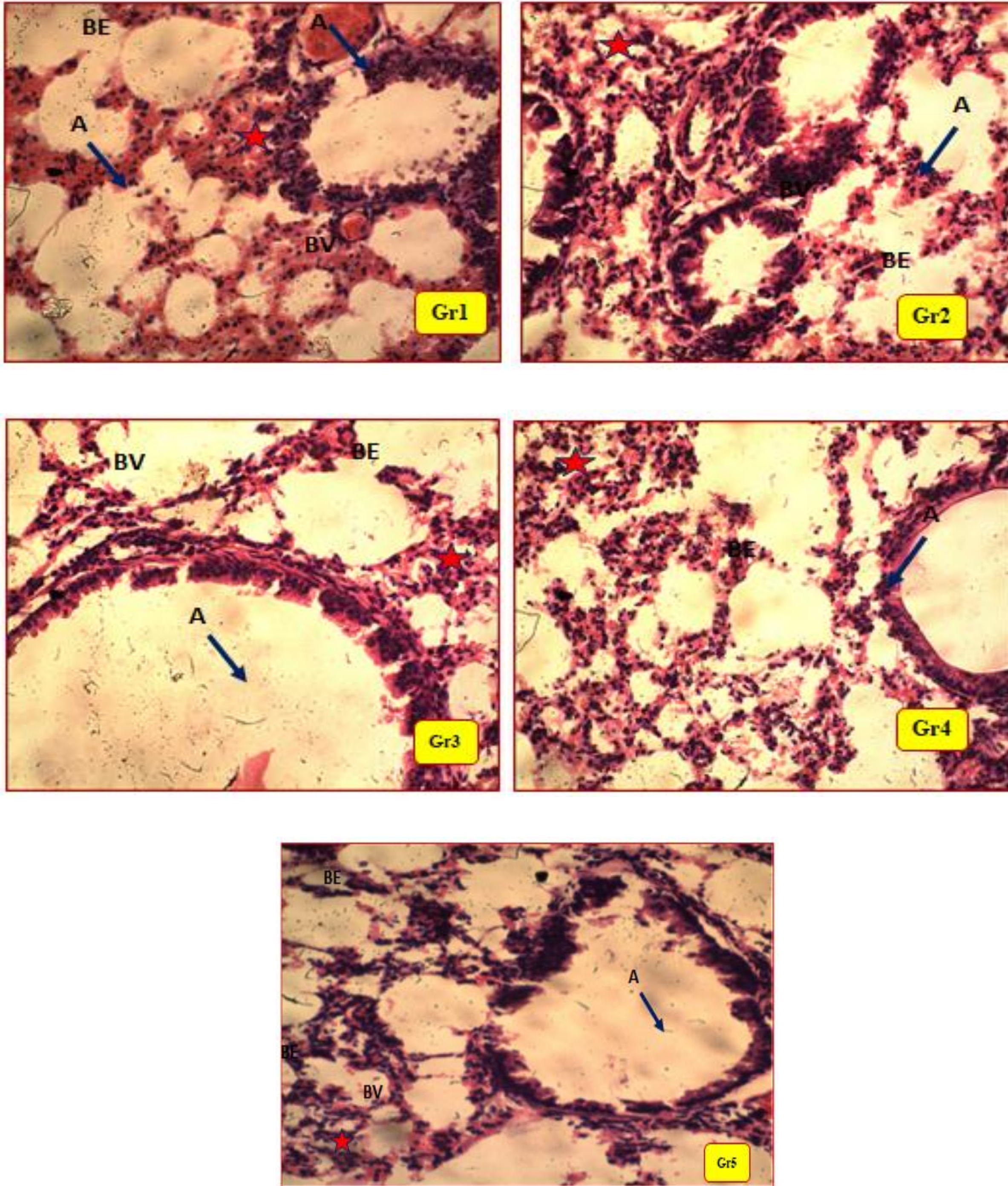


Fig 5.85: Histological observation under microscope at 40X magnification of lung tissues of five groups of mice. Gr1- Deltamethrin exposed mice, Gr2- DM for 10 days + NS extracts for 10 days, Gr3- DM for 10 days + SC extracts for 10 days, Gr4- DM for 10 days + NS and SC extracts for 10 days, and Gr5-Control group treated with corn oil. A- alveoli, BV-Blood Vessel, BE-Bronchial epithelium, and Star is showing infiltration.

HEART:

Following parameters included for histological evaluation of cardiac tissues-

Table 5.24: Parameters for Histological examination of heart tissues due to DM exposure

Parameters	Gr1	Gr2	Gr3	Gr4	Gr5
Heterogenous cell size	**2**	**0**	**1**	**0**	**0**
Vacuole formation	**2**	**1**	**1**	**0**	**0**
Cardiomyocytes architecture	**3**	**1**	**1**	**1**	**0**
Cardiac dilation	**2**	**1**	**1**	**1**	**0**
Ventricular failure	**1**	**0**	**0**	**0**	**0**
Myocardial necrosis	**2**	**0**	**1**	**1**	**0**
Lesions	**1**	**0**	**0**	**0**	**0**
Gr1-Deltamethrin exposed group, Gr2-DM for 10 days + NS extract for 10 days, Gr3-DM for 10 days + SC extract for 10 days, Gr4-DM for 10 days + NS & SC extract for 10 days, and Gr5-Control group treated with technical grade corn oil					
0 = No change, 1 = Mild change, 2 = Moderate change, 3 = Severe change					

The deltamethrin exposure in mice causes the cardiotoxicity, during histopathological examination there was several changes in cardiac tissues were observed. Heterogenous cell size, vacuole formation, architecture of cardiomyocytes was not normal in deltamethrin treated groups. In control group 5 there was no such changes were observed. In Gr1, lesions were also observed due to deltamethrin exposure.

Similar alterations were observed by Ibrahim and Abdel-Daim in 2015 due to tilmicosin-induced cardiotoxicity in mice and cardioprotective effects due to *Spirulina platensis*. Abdel-Daim

et al 2016, also found the cardiac tissues alterations due to tilmicosin-induced cardiotoxicity in mice and cardiac protective effects due to *Commiphora molmol*. In one of the research studies by Papaefthimiou and Theophilidis in 2001 also reported the cardiotoxicity and cardiac tissues alterations due to deltamethrin exposure in the heart of bees. Santos *et al*, in 2011 reported that deltamethrin causes damage to cardiac tissues and liver tissues, they also observed histopathological alterations in cardiac tissues of wistar rats. So, with above all findings and reports it can be concluded that deltamethrin in high dose i.e. 18 mg/kg is causing damage to heart tissues and which can be recovered by seed extracts of *Nigella sativa* and *Syzygium cumini* alone as well as in combination of these two extracts.

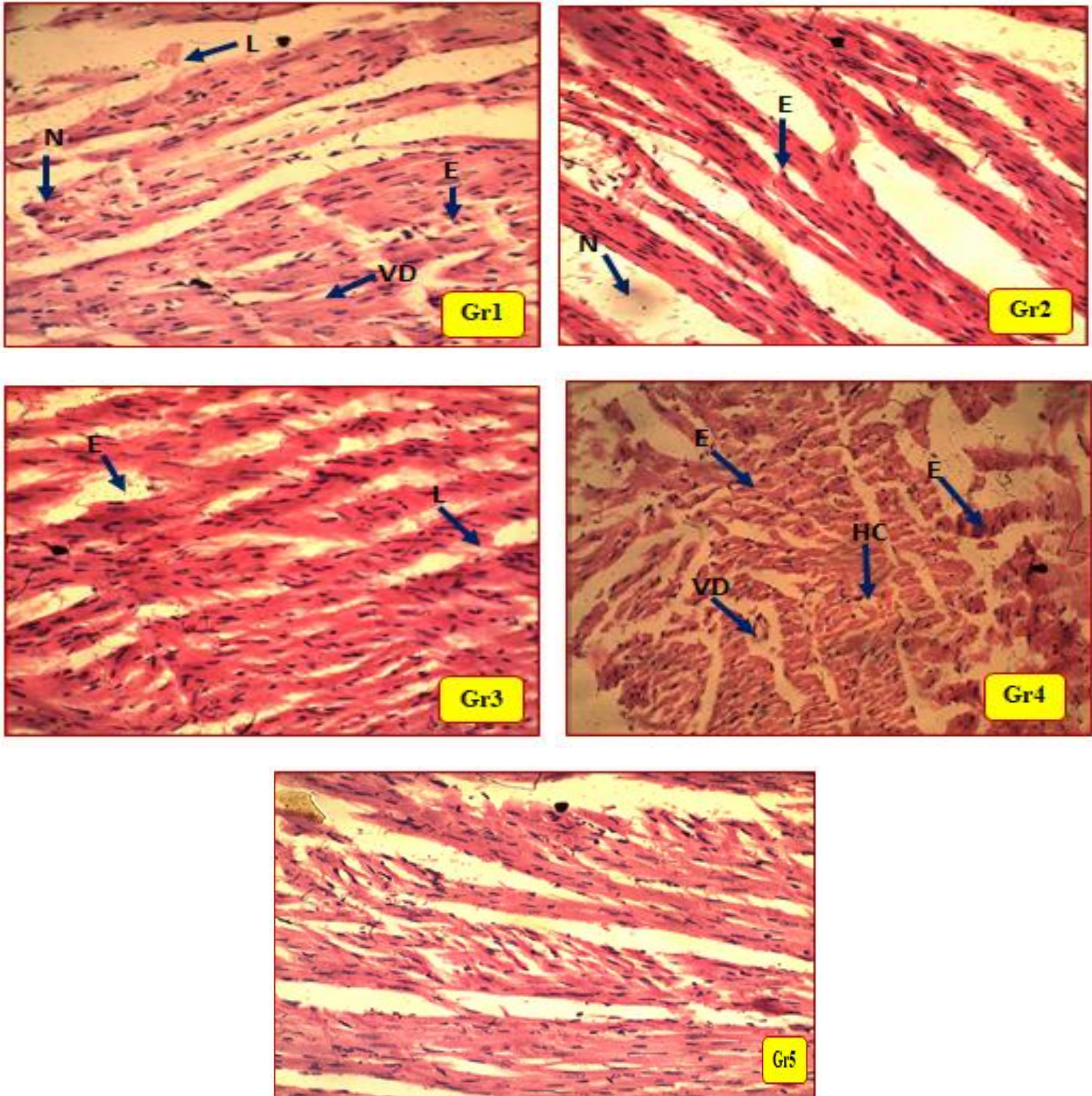


Fig 5.86: Histological observation under microscope at 40X magnification of heart tissues of five groups of mice. Gr1- Deltamethrin exposed mice, Gr2- DM for 10 days + NS extracts for 10 days, Gr3- DM for 10 days + SC extracts for 10 days, Gr4- DM for 10 days + NS and SC extracts for 10 days, and Gr5-Control group treated with corn oil. L- lesions, N-Necrosis, E-Edema, VD- Vacuolar degeneration, HC- Heterogenous cells.

LIVER:

The following parameters were considered for histopathological examination of liver tissues under microscope-

Table 5.25: Parameters for Histological examination of hepatic tissues due to DM exposure and obtained results

Parameters	Gr1	Gr2	Gr3	Gr4	Gr5
Central Vein	**1**	**2**	**2**	**1**	**4**
Normal hepatocytes (NH)	1	2	3	3	**4**
Degenerated hepatocytes (DH)	**4**	**2**	**2**	**1**	**0**
Infiltration of PMN cells	**4**	**2**	**1**	**1**	**0**
Normal sinusoids (NS)	**1**	**2**	**2**	**3**	**4**
Fat vacuoles (FV)	**3**	**1**	**1**	**1**	**2**
Cellular debris (CD)	**4**	**1**	**2**	**1**	**0**
Mildly dilated central vein (MDCV)	**2**	**0**	**0**	**1**	**0**
Gr1-Deltamethrin exposed group, Gr2-DM for 10 days + NS extract for 10 days, Gr3-DM for 10 days + SC extract for 10 days, Gr4-DM for 10 days + NS & SC extract for 10 days, and Gr5-Control group treated with technical grade corn oil					
0 = No change, 1 = Mild change, 2 = Moderate change, 3 = Severe change, 4 = Very severe changes					

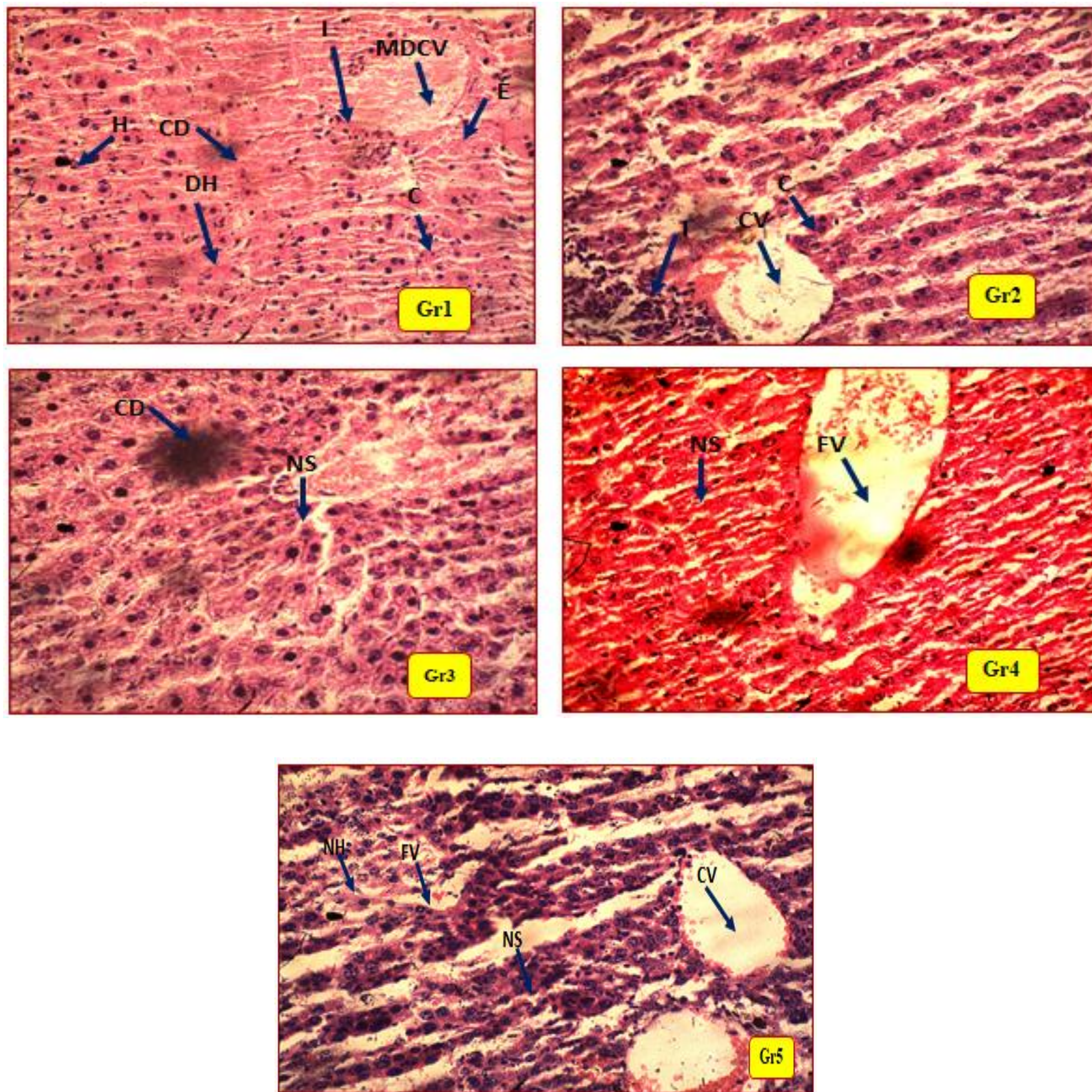


Fig 5.87: Histological observation under microscope at 40X magnification of heart tissues of five groups of mice. Gr1- Deltamethrin exposed mice, Gr2- DM for 10 days + NS extracts for 10 days, Gr3- DM for 10 days + SC extracts for 10 days, Gr4- DM for 10 days + NS and SC extracts for 10 days, and Gr5-Control group treated with corn oil. H- Hemorrhage, I- Infiltration of MN cells, E- Emptying of hepatocytes, C- Congestion, CV- Central vein, FV- Fat vacuoles, NS- Normal sinusoid, MDCV- Mildly dilated central vein, NH- normal hepatocytes, DH- Degenerated hepatocytes, CD- Cellular debris.

KIDNEY:

The following parameters were considered for histopathological examination of renal tissues under microscope-

Table 5.26: Parameters for Histological examination of renal tissues of mice due to DM acquaintance and observed results under microscopic investigation

Parameters	Gr1	Gr2	Gr3	Gr4	Gr5
Enlarged Renal vein (ERV)	**4**	**2**	**3**	**1**	**1**
Degenerated tubules (DT)	4	2	2	2	**0**
Cellular debris (CD)	**4**	**2**	**2**	**1**	**0**
Renal Tubule (RT)	**4**	**2**	**1**	**1**	**0**
Glomerulus (G)	**1**	**2**	**2**	**3**	**4**
Atrophied glomeruli (AG)	**3**	**1**	**1**	**1**	**2**
Leukocyte infiltration (LI)	**4**	**2**	**2**	**1**	**0**
Enlarged sinusoid (ES)	**4**	**1**	**2**	**1**	**0**
Tissue congestion (TC)	**3**	**1**	**1**	**1**	**0**
Wide cellular spaces (WCS)	**3**	**2**	**1**	**1**	**1**
Gr1-Deltamethrin exposed group, Gr2-DM for 10 days + NS extract for 10 days, Gr3-DM for 10 days + SC extract for 10 days, Gr4-DM for 10 days + NS & SC extract for 10 days, and Gr5-Control group treated with technical grade corn oil					
0 = No change, 1 = Mild change, 2 = Moderate change, 3 = Severe change, 4 = Very severe changes					

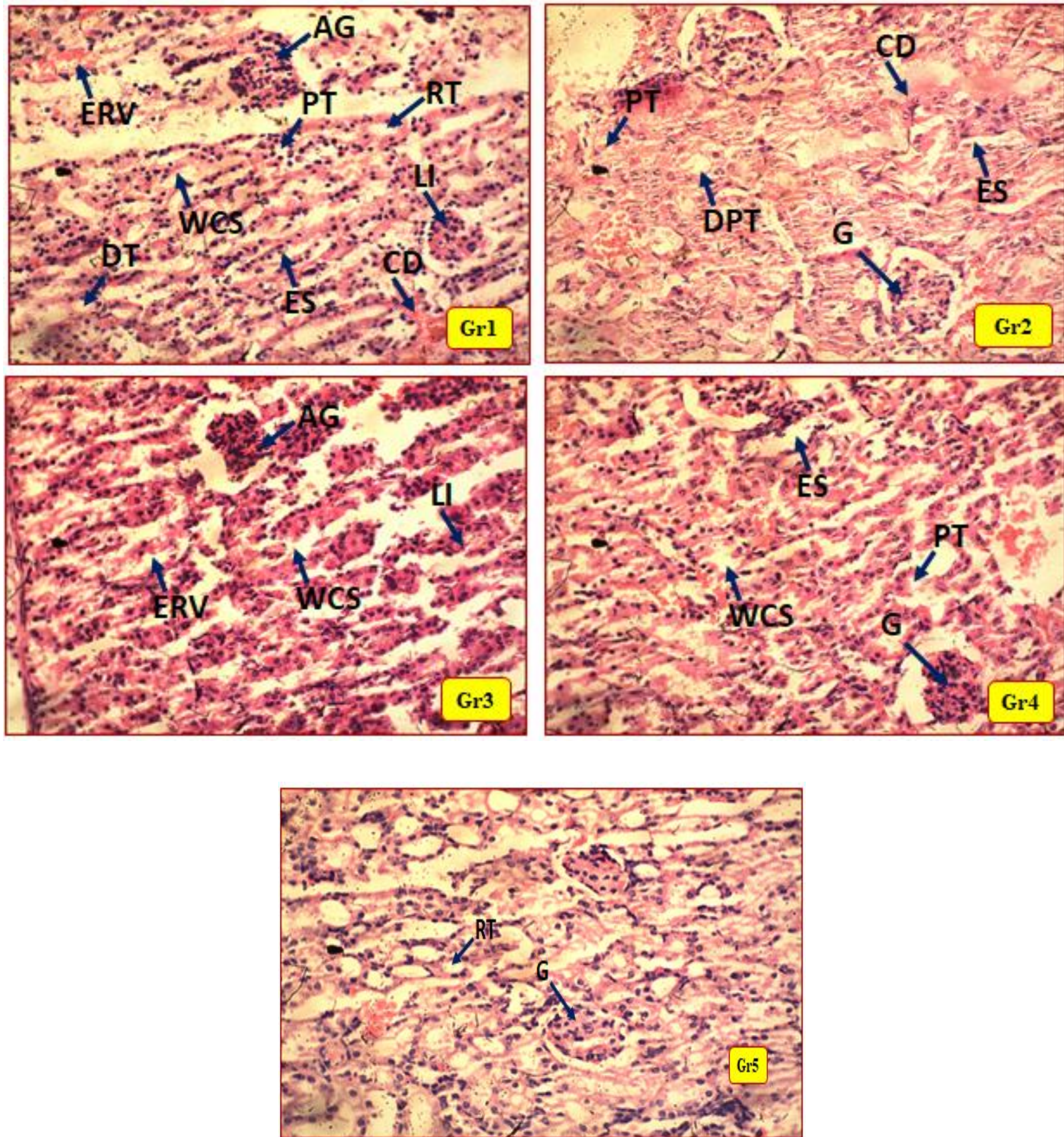


Fig 5.88: Histological observation under microscope at 40X magnification of heart tissues of five groups of mice. Gr1- Deltamethrin exposed mice, Gr2- DM for 10 days + NS extracts for 10 days, Gr3- DM for 10 days + SC extracts for 10 days, Gr4- DM for 10 days + NS and SC extracts for 10 days, and Gr5-Control group treated with corn oil. RT- Renal tubule, DT- Degenerated tubule, PT- Proximal tubule, G- Glomerulus, AG- Atrophied glomerulus, ES- Enlarged sinusoid, WCS- Wide cellular space, ERV- Enlarged renal vein, DPT- Dilated proximal tubule, CD- Cellular debris.

SPLEEN:

The following parameters were considered for histopathological examination of spleen tissues under microscope-

Table 5.27: Parameters for histological examination of splenic tissues in DM treated mice and recorded observation values under microscope

Parameters	Gr1	Gr2	Gr3	Gr4	Gr5
White pulp (WP)	**4**	**2**	**3**	**1**	**1**
Red pulp (RP)	4	2	2	2	**0**
Marginal zone (MZ)	**4**	**2**	**2**	**1**	**0**
Venous sinuses (VS)	**4**	**2**	**1**	**1**	**1**
Megakaryocytes (M)	**4**	**2**	**2**	**3**	**1**
Hemosiderin deposits (HD)	**3**	**1**	**1**	**1**	**0**
Fibrosis (F)	**3**	**1**	**2**	**2**	**1**
Cellular debris (CD)	**3**	**1**	**1**	**1**	**0**
Gr1-Deltamethrin exposed group, Gr2-DM for 10 days + NS extract for 10 days, Gr3-DM for 10 days + SC extract for 10 days, Gr4-DM for 10 days + NS & SC extract for 10 days, and Gr5-Control group treated with technical grade corn oil					
0 = No change, 1 = Mild change, 2 = Moderate change, 3 = Severe change, 4 = Very severe changes					

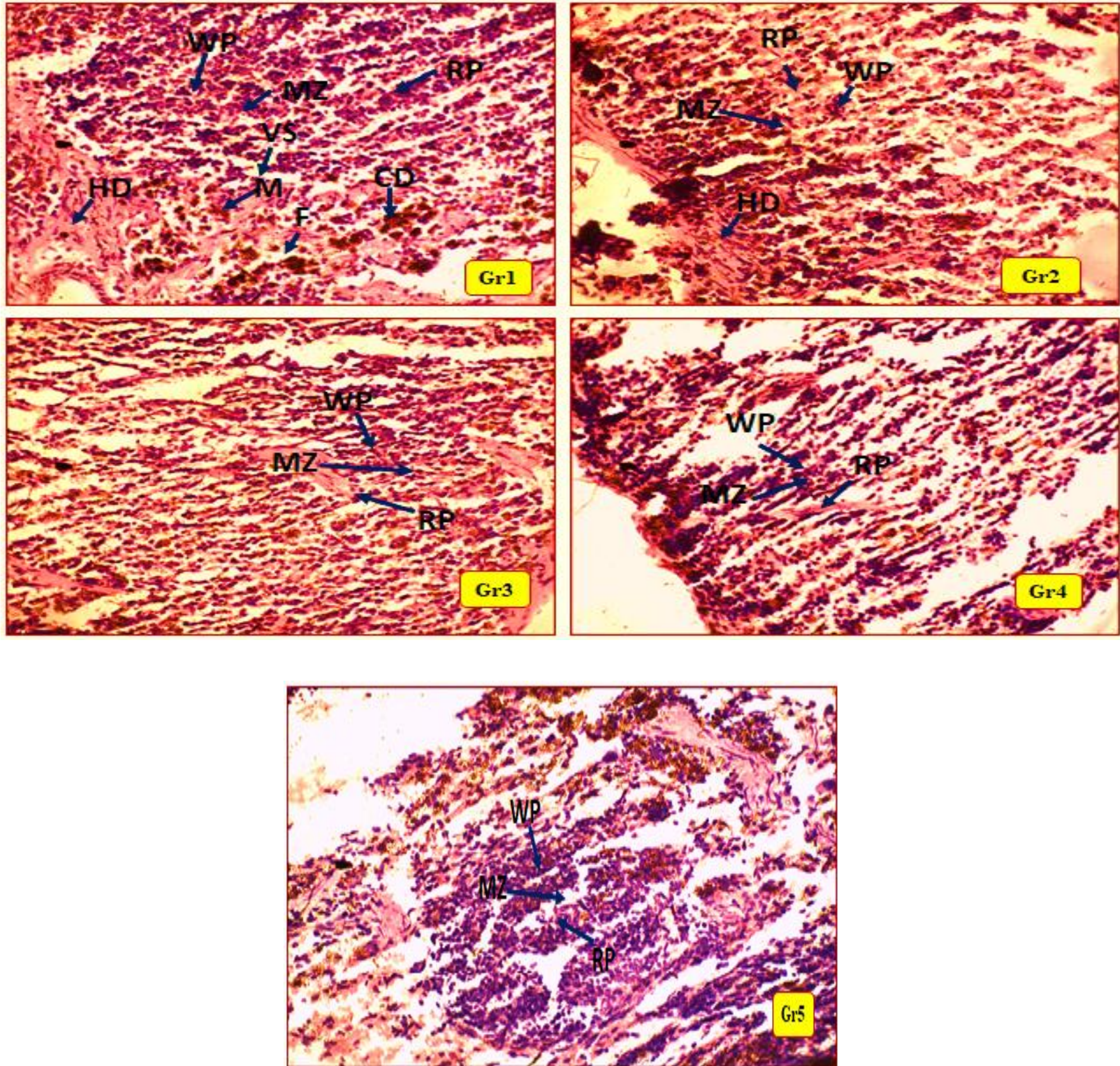


Fig 5.89: Histological observation under microscope at 40X magnification of splenic tissues of five groups of mice. Gr1- Deltamethrin exposed mice, Gr2- DM for 10 days + NS extracts for 10 days, Gr3- DM for 10 days + SC extracts for 10 days, Gr4- DM for 10 days + NS and SC extracts for 10 days, and Gr5-Control group treated with corn oil. RP- Red pulp, WP- White pulp, MZ- Marginal zone, F- fibrosis, HD- Hemosiderin deposits, CD- Cellular debris, M- Megakaryocytes, VS- Venous sinuses.

OBJECTIVE 5: To study the immunomodulatory effects of deltamethrin and bioactive compounds of *Nigella sativa* and *Syzygium cumini* with MHC I and MHCII molecule of human and mice origin using bioinformatics tools.

5.14: PatchDock Results:

Table 5.28: The IUPAC name of the ligand molecules (L1-L7)

Ligand	Chemical Formula	Canonical SMILES	IUPAC Name
L1	$C_{22}H_{19}Br_2NO_3$	N#CC(C1CCCC(C1) OC1CCCCC1) OC(=O) C1C(C1(C) C) C=C(Br)Br	[(S)-cyano-(3-phenoxyphenyl)methyl] (1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane-1-carboxylate
L2	$C_{10}H_{12}O_2$	CC(C1=CC(=O) C(=CC1=O) C) C	2-methyl-5-propan-2-ylcyclohexa-2,5-diene-1,4-dione
L3	$C_{20}H_{24}O_4$	CC(C1=CC(=O) C2(C(C1=O) C1(C2C(=O) C(=CC1=O) C(C) C) C) C) C	4b,8b-dimethyl-3,7-di(propan-2-yl)-4a,8a-dihydrobiphenylene-1,4,5,8-tetrone
L4	$C_7H_6O_5$	OC(=O) C1CC(O)C(C(C1) O) O	3,4,5-trihydroxybenzoic acid
L5	$C_{15}H_{10}O_8$	OC1CC(O)C2C(C1) OC(C(C2=O) O) C1CC(O)C(C(C1) O) O	3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-4H-1-benzopyran-4-one
L6	$C_{15}H_{10}O_7$	OC1CC(O)C2C(C1) OC(C(C2=O) O) C1CCC(C(C1) O) O	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one
L7	$C_{10}H_{10}O_4$	COC1CC(C=CC(=O) O) CCC1O	(E)-3-(4-hydroxy-3-methoxyphenyl) prop-2-enoic acid

In the above table the ligand molecules are L1- Deltamethrin, L2- Thymoquinone, L3-Dithymoquinone, L4- Gallic acid, L5-Antimellin, L6-Quercetin, L7-ferulic acid. These seven molecules were analysed for interaction with MHC I and MHC II with human and mice origin.

The evaluation of immunomodulation effects have been calculated for all these molecules on the basis of binding energy. These ligands are going to bind with MHC I and MHC II to show the immunomodulation effects, L1-Deltamethrin is a pesticide molecule and remaining L2-L6 are bioactive molecules present in seed extracts of *Nigella sativa* and *Syzygium cumini*. These bioactive molecules are already established for antioxidant potential, antimicrobial effects and cytotoxic effects.

Table 5.29: Prediction of physicochemical properties of the title compounds L1-L7

Ligand	**MW**	**#Heavy atoms**	**#Aromatic heavy atoms**	**#Rotatable bonds**	**#H-bond acceptors**	**#H-bond donors**	**MR**	**TPSA**	**Log P**
L1	505.2	28	12	7	4	0	115.11	59.32	5.16
L2	164.2	12	0	1	2	0	47.52	34.14	1.85
L3	328.4	24	0	2	4	0	91.24	68.28	2.62
L4	170.12	12	6	1	5	4	39.47	97.99	0.21
L5	318.24	23	16	1	8	6	80.06	151.59	0.79
L6	302.24	22	16	1	7	5	78.03	131.36	1.23
L7	194.18	14	6	3	4	2	51.63	66.76	1.36

(Where, LogP- logarithm of compound partition coefficient between n-octanol and water; TPSA- topological polar surface area; natom, number of atoms; MW- molecular weight; nON- number of hydrogen bond acceptors; nOHN- number of hydrogen bond donors; nVio- number of violations; Nrotb- number of rotatable bonds).

In the above table the physicochemical properties of title compounds L1-L7 have been shown which reveals the one of the most important parameter of H-donor and H-acceptor for reactivity of these molecules. While topological polar surface area defines the surface sum over all polar atoms or molecules, primarily oxygen and nitrogen, also including their attached hydrogen atoms.

Table 5.30: Prediction of molecular properties descriptors of the title compounds L1-L7

Ligand	**ESOL LogS**	**ESOL Class**	**Ali LogS**	**Ali Class**	**Silicos-IT LogSw**	**Silicos-IT class**	**GI absorption**	**BBB permeant**	**Pgp substrate**
L1	-6.73	Poorly soluble	-7.23	Poorly soluble	-7.22	Poorly soluble	High	No	No
L2	-2.18	Soluble	-2.55	Soluble	-2.03	Soluble	High	Yes	No
L3	-3.05	Soluble	-3.13	Soluble	-4.18	Moderately soluble	High	Yes	No
L4	-1.64	Very soluble	-2.34	Soluble	-0.04	Soluble	High	No	No
L5	-3.01	Soluble	-3.96	Soluble	-2.66	Soluble	Low	No	No
L6	-3.16	Soluble	-3.91	Soluble	-3.24	Soluble	High	No	No
L7	-2.11	Soluble	-2.52	Soluble	-1.42	Soluble	High	Yes	No

Table 5.31: Prediction of bioactivity score of the title compounds L1-L7

Ligand	GPCR	ICM	KI	NRL	PI	EI
L1	-0.14	-0.32	-0.33	-0.11	-0.04	0.21
L2	-1.401	-0.311	-1.271	-1.472	-1.445	-0.4
L3	-0.18	-0.09	-0.49	-0.137	-0.1	0.1
L4	-0.77	-0.26	-0.88	-0.52	-0.94	-0.17
L5	-0.06	-0.182	0.284	0.315	-0.205	0.299
L6	-0.06	-0.19	0.28	0.36	-0.25	0.28
L7	-0.47	-0.3	-0.72	-0.14	-0.81	-0.12

(In above mentioned table, GPCR-GPCR ligand; ICM - Ion channel modulator; KI - Kinase inhibitor; NRL - Nuclear receptor ligand; PI - Protease inhibitor; EI - Enzyme inhibitor)

Table 5.32: Prediction of Drug-likeness model score of the ligands L1- L7

Ligand	Lipinski #violations	Ghose #violations	Veber #violations	Egan #violations	Muegge #violations	Bioavailability Score	PAINS #alerts
L1	1	2	0	1	1	0.55	0
L2	0	0	0	0	1	0.55	1
L3	0	0	0	0	0	0.55	0
L4	0	2	0	0	1	0.56	1
L5	1	0	1	1	2	0.55	1
L6	0	0	0	0	0	0.55	1
L7	0	0	0	0	1	0.56	0

Table 5.33: Prediction of solubility and toxicity of the title compounds L1-L7

Ligand	ClogS	Mutagenic	Tumorigenic	Reproductive	Irritant
L1	-6.97	None	None	None	None
L2	-1.68	None	Yes	Yes	Yes
L3	-3.12	Yes	Yes	Yes	Yes
L4	-0.74	None	Yes	Yes	None
L5	-2.2	None	Yes	Yes	Yes
L6	-2.49	None	None	Yes	Yes
L7	-1.72	None	None	None	Yes

Table 5.34: Pharmacokinetics profile of the title compounds L1-L7

Ligand	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s) skin permeation	Synthetic Accessibility	Conformational Energy (Kcal/Mol)
L1	Yes	Yes	Yes	No	Yes	-4.98	3.98	80.62
L2	No	No	No	No	No	-5.74	2.83	4.03
L3	No	No	No	No	No	-6.83	4.65	6.85
L4	No	No	No	No	Yes	-6.84	1.22	-5.83
L5	Yes	No	No	No	Yes	-7.4	3.27	60.85
L6	Yes	No	No	Yes	Yes	-7.05	3.23	62.37
L7	No	No	No	No	No	-6.41	1.93	-8.22

Table 5.35: Prediction of ADMET profile of the title compounds L1-L7

Ligand	Blood-Brain Barrier (BBB)	Human Intestinal Absorption (HIA)	Caco-2 Permeability	CYP Inhibitory Promiscuity	AMES toxicity	Carcinogenicity	Rat Acute ToxicityLD50 mol/Kg
L1	BBB+	HIA+	Caco2+	High	Non toxic	Non Carcinogenic	3.79
L2	BBB+	HIA+	Caco2+	Low	Non toxic	Non Carcinogenic	2.61
L3	BBB+	HIA+	Caco2+	High	Non toxic	Non Carcinogenic	2.75
L4	BBB-	HIA+	Caco2-	Low	Non toxic	Non Carcinogenic	1.86
L5	BBB-	HIA+	Caco2-	High	Non toxic	Non Carcinogenic	3.02
L6	BBB-	HIA+	Caco2-	High	Non toxic	Non Carcinogenic	3.02
L7	BBB-	HIA+	Caco2+	Low	Non toxic	Non Carcinogenic	1.43

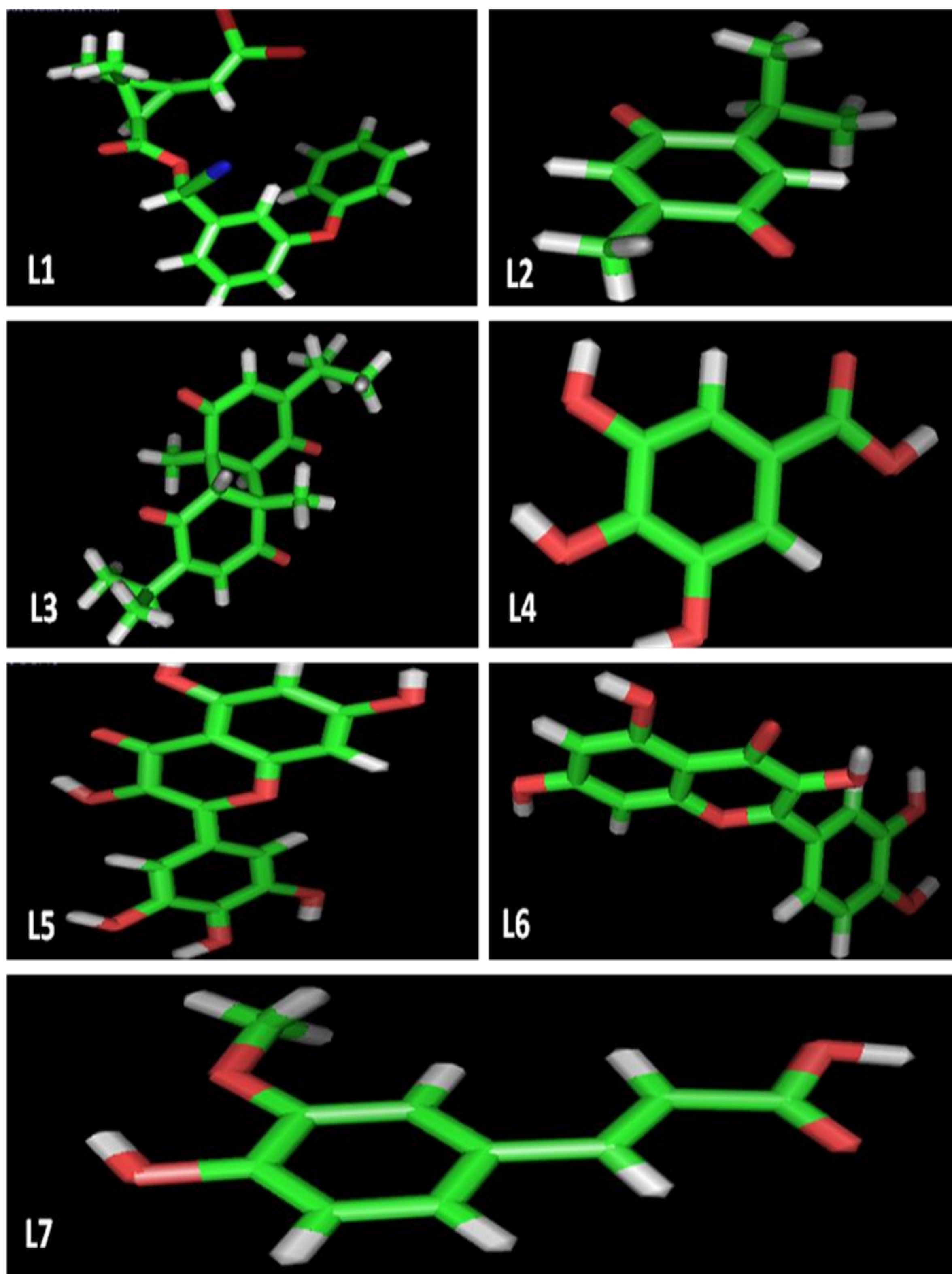


Fig 5.90: Structure of ligand molecules (L1- Deltamethrin, L2- Thymoquinone, L3-Dithymoquinone, L4- Gallic acid, L5-Antimellin, L6-Quercetin, L7-ferulic acid)

Table 5.36: Prediction of Binding energy and Inhibition constant of ligands L1-L7

Binding Energy (Kcal/mol)				
Ligand	HUMAN		MICE	
	MHC-I	MHC-II	MHC-I	MHC-II
L1	5700	5704	5204	5332
L2	3506	3172	3230	3338
L3	4894	4748	4498	4586
L4	3004	2740	2554	3014
L5	4342	4166	3886	3924
L6	4292	4150	3806	3886
L7	3434	3510	3270	3090

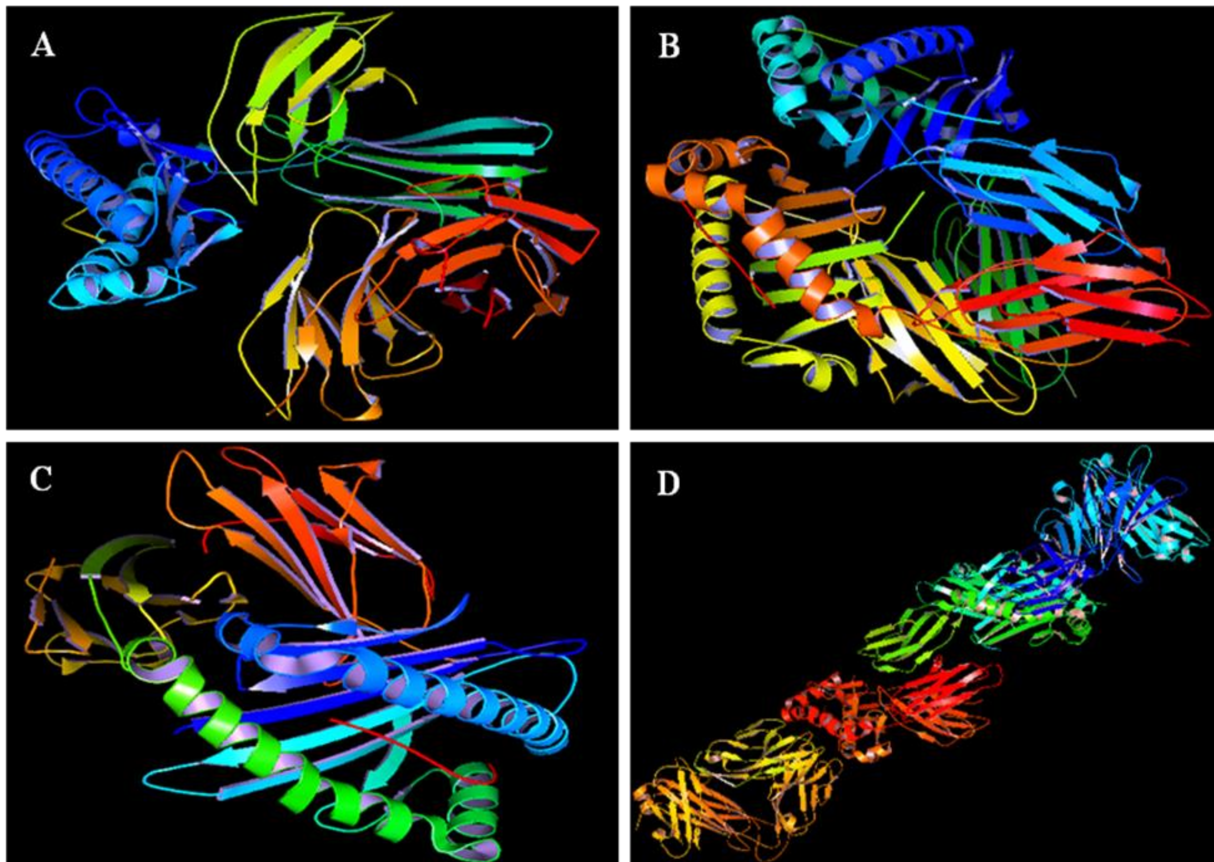


Fig 5.91: A- Human MHC Class I, B- Human MHC Class II, C- Mouse MHC Class I, D-Mouse MHC Class II

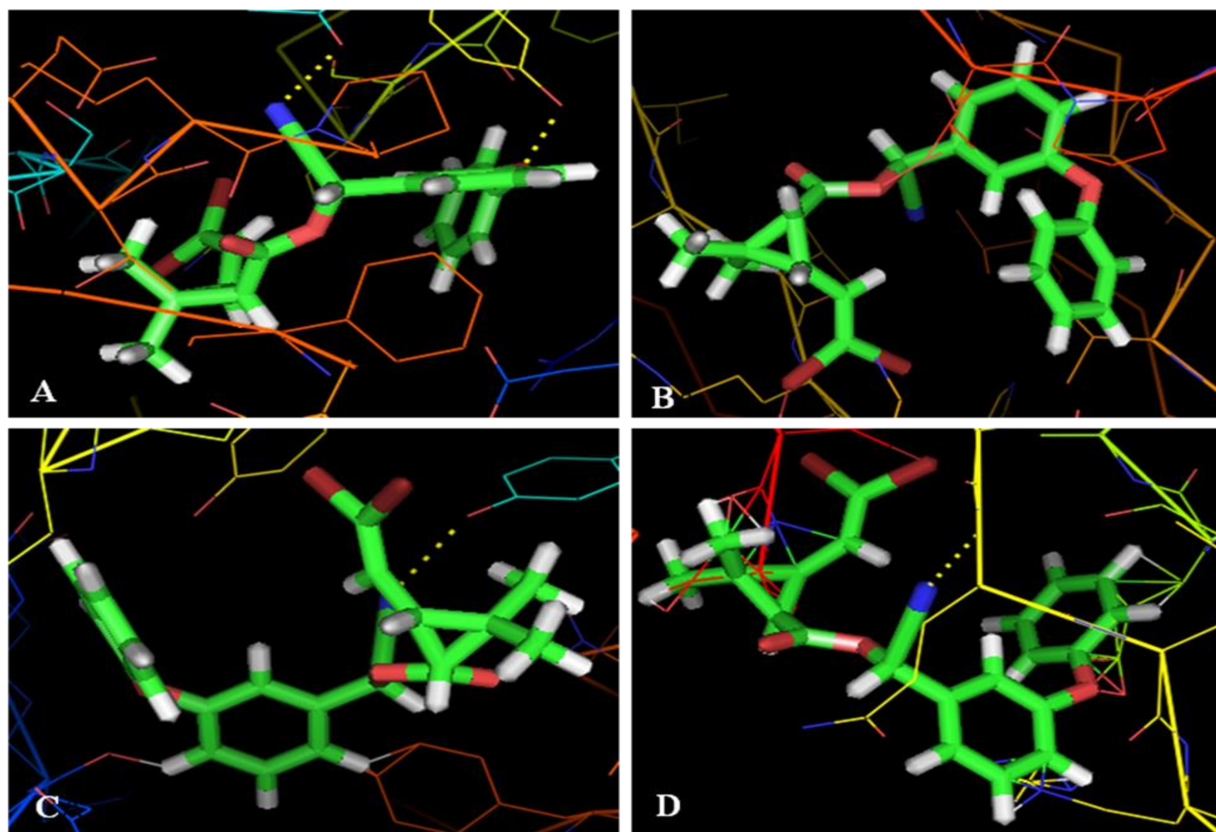


Fig 5.92: Deltamethrin interaction with MHC molecule (A- Human MHC Class I-L1, B- Human MHC Class II-L1, C- Mouse MHC Class I-L1, D-Mouse MHC Class II-L1)

5.15: AutoDock Results:

Docking of "MHC-1" with "deltamethrin" with the help of AutoDock

After performing the docking of the MHC_1 with ligand deltamethrin by the help of AutoDock, in return on accessing the option in AutoDock "Play ranked by energy" we visualized all the conformation of deltamethrin docked with MHC_1 in accordance with their free binding energies. Total 10 docked conformations were returned by the AutoDock after the successful completion of Autodock. All the conformations returned by AutoDock are shown below. In the following conformations MHC-1 is shown in ribbon view and deltamethrin is shown in lines with atomic spheres.

Docked conformations Vs binding energy calculation:

Table 5.37: Docked confirmation with binding energy value obtained through AutoDock tool

Conformation Number	Binding energy (Kcal/mol)
Docked Conformation #1	-5.79
Docked Conformation #2	-5.47
Docked Conformation #3	-4.98
Docked Conformation #4	-4.51
Docked Conformation #5	-4.49
Docked Conformation #6	-4.05
Docked Conformation #7	-3.96
Docked Conformation #8	-3.95
Docked Conformation #9	-3.74
Docked Conformation #10	-2.84

Least binding energy value most probable binding confirmation between ligand molecules, deltamethrin and MHC I molecule of human origin.

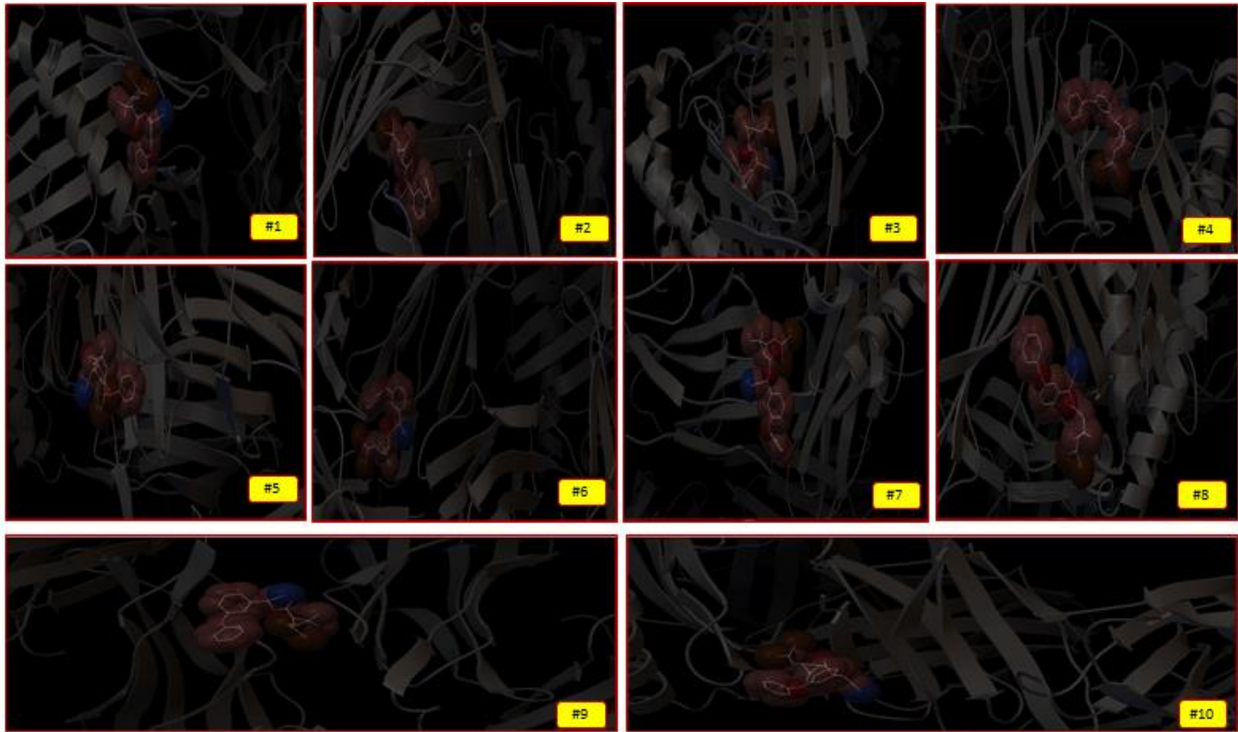


Fig 5.93: Docked confirmation 1-10, showing interaction of deltamethrin with MHC I

From the energy table and docked conformations it is clear that conformation 1 is the most preferable configuration of MHC1 docked with Deltamethrin whose distance image is shown below calculated using PyMOL (Sanner, 1999; Schrödinger, 2010).

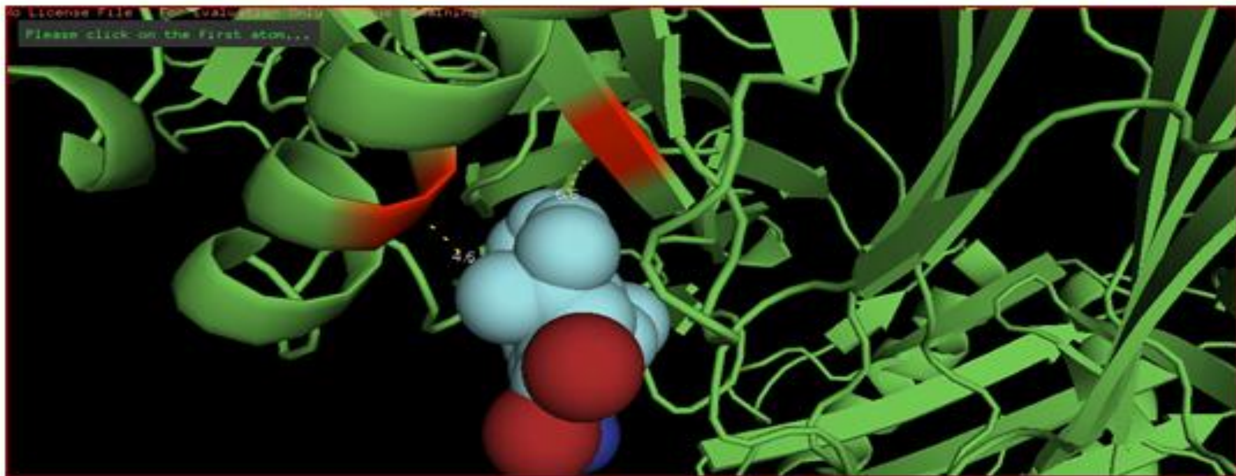


Fig 5.94: Docked conformation #1 of deltamethrin, shows potential binding with MHC I at amino acid no. 3(H) and 169(R) respectively with measured distance 4.6Å and 6.6Å respectively

Discussion

As we know that lower binding energy lead to better binding and if binding energy value is negative that means binding is spontaneous and does not require any energy for the binding to take place. So, we can say that the conformation with most negative binding energy/affinity will be the most preferable conformation when we opt to choose the best conformation on the basis of binding energy/affinity. As the above conformations were ranked by energy in ascending order that is docked conformation #1 is having least binding energy and possibly the most preferable binding site for the deltamethrin. Therefore, docked conformation #1 is the most preferable conformation out of all conformation of MHC_1 docked with deltamethrin. MHC I molecules enables natural killer cells to do necessary action against pathogens or antigens (Kim *et al*., 2005), but due to deltamethrin interaction with MHC I molecules the necessary action of natural killer cells, macrophages and other immune cells will be affected and lead to develop immune compromise state of an individual (Kindt *et al*., 2007).

Docking of "MHC-II" with "deltamethrin" with the help of AutoDock

After performing the docking of the MHC_II with ligand deltamethrin by the help of AutoDock, in return on accessing the option in AutoDock "Play ranked by energy" we will be able to visualize all the conformation of deltamethrin docked with MHC_II in accordance with their free binding energies. Total 10 docked conformations were returned by the AutoDock after the successful completion of Autodock. All the conformations returned by AutoDock are shown below. In the following conformations MHC-II is shown in ribbon view and deltamethrin is shown in lines with atomic spheres (Kindt *et al*., 2007).

Docked conformations binding energy table:

Table 5.38: Docked confirmation with binding energy value of deltamethrin with MHCII

Conformation Number	Binding energy (Kcal/mol)
Docked Conformation #1	-5.74
Docked Conformation #2	-5.13
Docked Conformation #3	-4.52
Docked Conformation #4	-4.31
Docked Conformation #5	-4.02
Docked Conformation #6	-3.88
Docked Conformation #7	-3.82
Docked Conformation #8	-3.44
Docked Conformation #9	-3.30
Docked Conformation #10	-2.61

Fig 5.95: Docked confirmation 1-10, showing interaction of deltamethrin with MHC II

From the energy table and docked conformations it is clear that conformation 1 is the most preferable configuration of MHC2 docked with Deltamethrin whose distance image is shown below calculated using PyMOL (Sanner, 1999; Schrödinger, 2010).

Fig 5.96: Docked conformation #1 of deltamethrin, shows potential binding with MHC II at amino acid no. 92(L) and 177(H) respectively with measured distance 6.0Å and 4.8 Å respectively.

Discussion

As we know that lower binding energy lead to better binding and if binding energy value is negative that means binding is spontaneous and does not require any energy for the binding to take place. So, we can say that the conformation with most negative binding energy/affinity will be the most preferable conformation when we opt to choose the best conformation on the basis of binding energy/affinity. As the above conformations were ranked by energy in ascending order that is docked conformation #1 is having least binding energy and possibly the most preferable binding site for the deltamethrin. Therefore, docked conformation #1 is the most preferable conformation out of all conformation of MHC_II docked with deltamethrin. When there is mutation in MHC II molecules or some other molecules besides immune functioning cells binds or interact with MHC II molecules then it affects the normal antigen processing mechanism mediated by MHC II molecules (Kreiter *et al*., 2005; Kindt *et al*., 2007)).

SUMMARY AND CONCLUSIONS

6.1: SUMMARY

In the present study, efforts have been made to delineate the immunotoxicity, oxidative stress conditions, hematological changes, cellular toxicity, and in-silico immunomodulation exaggerated due to deltamethrin pyrethroid pesticide and to encounter protective effects on various parameters were established with seed extracts of *N. Sativa* and *S. cumini* alone and synergistic approach. There were various biochemical and immunological parameters elevated or reduced due to deltamethrin exposure in DM group leading to various metabolic dysfunction and deteriorated immunological status. Deltamethrin causing the oxidative stress lead to production of free radicals such as reactive oxygen species (ROS) which exceeds endogenous antioxidant grade of body. Human beings exposed to deltamethrin are on high risk of immunotoxicity due to weakened immune system function and higher chance of infections caused by bacteria, fungi, protozoa etc. From the earlier discussion, it is obvious that a adjacent association exist between deltamethrin induced oxidative stress which provokes biochemical, pathological, histological leading to changes in hematological parameters, immunological parameters causing structural, metabolic and functional disintegrity. It is recorded deltamethrin induces hepatotoxicity, cardiotoxicity, lung toxicity, nephritic toxicity and splenic toxicity. Immune system is the utmost vulnerable region due to deltamethrin and generally lead to immune compromised conditions of an individual. It is the submission of author that the result obtained from the present study have given insight into mechanism of deltamethrin induced toxicity and protective effects of botanical extracts derived from *N. sativa* and *S. cumini*.

6.2: CONCLUSIONS

Verdicts in the current investigation are evocative of the following inferences-

- The anomalous biochemical profiles of mice blood parameters reproduced the transformed cellular metabolic expression mechanism caused by deltamethrin pesticide persuaded oxidative stress conditions. This is directly corelate with the blood biochemical parameters. It advocates that the deltamethrin downregulates the expression of the antioxidant enzymes of the cells of different organs and causes the upregulation of anti-antioxidants enzymatic expression. These metabolic alarms can be subjugated as the indications of oxidative stress facilitated toxicity and eventually distress the immune system function and leading to defects in the defense mechanism.
- Herbal extracts derived from seeds of *N. sativa* and *S. cumini* treatment given to deltamethrin induced Swiss albino mice have evidently elevated the antioxidant enzymes expression of GPx, GST, SOD, CAT, GSH, ATPase and however reduced the expression of ALKP, AST, ALT, lipid peroxidation, MDA, NO, Cholesterol, ALPase and ACPase were observed in blood serum, heart , lungs, spleen, kidney and liver. The immunomodulatory effects of seed extracts of have been established in the current research findings.
- The seed extract of *N. sativa* and *S. cumini* has intensely shown the antioxidant properties, antibacterial effects, antifungal effects, and protective effects in in-vitro as well as in-vivo condition in deltamethrin induced toxicity significantly.
- The bioactive compounds derived from *N. sativa* and *S. cumini* have shown significant protective effects with MHC I and MHC II molecules in in-silico studies of immunomodulatory effects of bioactive compounds.
- In presence of phytochemical extracts of *N. sativa* and *S. cumini* overcome the deltamethrin induced body weight and organ weight (liver, kidney, heart, lungs and spleen weight) loss in mice models significantly.
- Oxidative stress persuaded swelling due to deltamethrin exposure was noticeable in DM group of mice with noticeable upsurge in TNF-α and decrease in IL-6. Deltamethrin affected both cellular immunity as well as humoral immunity, and botanical extracts *of N. sativa* and *S. cumini* overcome the effects of deltamethrin pesticides in treatment groups.

- The main significances of reactive oxygen species impairment to a hematological profile is deprivation of red blood corpuscles, white blood corpuscles count, and blood protein like hemoglobin, albumin, and total protein content of blood while an obvious enhancement in hematological profile has been made by *N. sativa* and *S. cumini* extract.
- In-silico studies also revealed the toxic effects on the immune functions because deltamethrin binding affinity with MHC I and MHC II molecules can lead to significant effects on cellular immunity as well as humoral immunity. The bioactive compounds derived from *N. sativa* and *S. cumini* shown the protective effects due to deltamethrin and act as potential positive immunomodulator.

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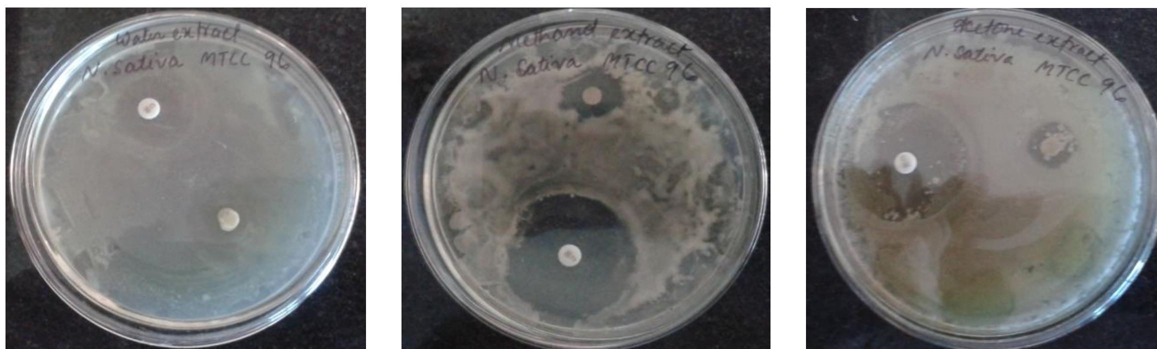
APPENDICES

Antibacterial Results of *Nigella Sativa*

Antibacterial test of water, methanol and acetone extract of *N. sativa* against *Bacillus subtilis*

Antibacterial test of water, methanol and acetone extract of *N. sativa* against *S. typhimurium*



Antibacterial test of water, methanol and acetone extract of *N. sativa* against S. *aureus*

Antibacterial Results of *Syzygium cumini*

Antibacterial test of water, methanol and acetone extract of *S. cumini* against *Bacillus subtilis*

Antibacterial test of water, methanol and acetone extract of *S. cumini* against *S. typhimurium*

Antibacterial test of water, methanol and acetone extract of *S. cumini* against *Salmonella enteric*

Antibacterial test of water, methanol and acetone extract of *S. cumini* against S. *aureus*