ALTERATIONS IN BIOCHEMICAL AND OXIDATIVE BIOMARKERS IN SUBACUTE ORAL TOXICITY OF IMIDACLOPRID ALONE AND IN CONJUNCTION WITH ARSENIC IN WISTAR RATS

By

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Thesis submitted to Faculty of Postgraduate Studies
in partial fulfillment of requirements
for the degree of

MASTERS OF VETERINARY SCIENCE
IN
VETERINARY PHARMACOLOGY AND TOXICOLOGY

Division of Veterinary Pharmacology and Toxicology

Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu

Main Campus, Chatha, Jammu-180009

2017

Certificate – I

This is to certify that the thesis entitled **"Alterations in biochemical and oxidative biomarkers in subacute oral toxicity of imidacloprid alone and in conjunction with arsenic in wistar rats"** submitted in partial fulfillment of the requirements for the degree of **Masters of Veterinary Science** in subject of **Veterinary Pharmacology and Toxicology** is a record of bonafide research, carried out by **Lakshay Mahajan**, Registration No **J-15-MV-433** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that help and assistance received during the course of thesis investigation have been duly acknowledged.

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Certificate – II

We, the members of Advisory Committee of **Mr. Lakshay Mahajan**, Registration No. **J-15-MV-433,** a candidate for the degree of **Masters of Veterinary Science** in **Veterinary Pharmacology and Toxicology** have gone through the manuscript of the thesis entitled **"Alterations in biochemical and oxidative biomarkers in subacute oral toxicity of imidacloprid alone and in conjunction with arsenic in wistar rats"** and recommend that it may be submitted by the student in partial fulfillment of the requirements for the degree.

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Acknowledgements

I bow in reverence to God Almighty the merciful, the compassionate who bestowed upon me gracious blessings enabling me to achieve the target.

I wish to acknowledge and express my feeling of deep gratitude indebtedness and reverence to my esteemed guide and chairman of my advisory committee, ***Dr. Pawan Kumar Verma***, Assistant Professor, Division of Veterinary Pharmacology and Toxicology, SKUAST- Jammu, whose austere guidance, valuable and timely suggestions, stimulating discussions and constructive criticisms enabled me to complete this work. His affectionate instinct and incessant encouragement were always boon to me from the very inception of the work to its completion. Words would not suffice my deep sense of gratitude for his altruistic approach in monitoring my smooth progress and providing unremitting help in each episode of my work.

I also acknowledge with great pleasure and appreciation to members of my advisory committee ***Dr. Mudasir Sultana***, Professor and Head, Division of Veterinary Pharmacology and Toxicology, ***Dr. S.R. Upadhyay***, Assistant Professor, Division of Veterinary Medicine and ***Dr. P.S. Mahapatra***, Associate Professor and Head, Division of Veterinary Physiology and Biochemistry for their generous help, innovative guidance and invaluable suggestions at various stages which were of great value and cooperation throughout the study.

It is a pleasant opportunity for me to express my gratitude towards ***Prof. Mudasir Sultana***, Head, Division of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Sciences and Animal Husbandry, R.S.Pura, Jammu for providing necessary facilities, help and support for conducting my masters research activities.

I acknowledge my genuine and heartfelt respect and gratitude to ***Dr. Rajinder Raina***, Professor, Division of Veterinary Pharmacology and Toxicology and ***Dr. Nrip Kishore Pankaj***, Assistant Professor, Division of Veterinary Pharmacology for their kind cooperation and impeccable guidance during the course of the study.

With deep sense of gratitude, I respectfully acknowledge ***Dr. Pradeep Kumar Sharma***, Honourable Vice Chancellor of SKUAST-Jammu for allowing me to undertake the study and for providing necessary infrastructural facilities to carry out my research work.

I extend my sincere gratitude towards ***Dr. Tasleem Ahmad Shamas Ganai***, Director Education for being kind enough to provide all the necessary facilities and guidance. Thanks also goes to ***Dr. M.M.S. Zama***, Dean, F.V.Sc. & A.H. and ***Dr. M.S. Bhadwal***, Associate Dean, F.V.Sc. & A.H. for their courteous and indulgent moral, extraordinary help, technical and timely academic support throughout the course of the study.

I also express my thanks to the staff members of the Division of Veterinary Pharmacology and Toxicology especially ***Mr. Pavan***, ***Mr. Bishan*** , ***Mr. Ashwani***, ***Mr. Mohan*** and ***Mr. Ajay*** for their co-operation and help, thereby making my work a smooth going process.

Friendship is beyond the bounds of acknowledgement. But I will still not like to miss the opportunity to place on record the affection, cooperation and emotional support provided by my friends and colleagues, ***Dr. Makhmoor Ahmed***, ***Dr. Mahrukh Ahmed***, ***Gurpreet Singh*** and ***Aabid Hussain Thoker***.

No words could adequately express all my ***parents*** have done for me throughout my life but I will be failing in my duty if I do not place on record the deepest sense of gratitude to them and my brother whose love, patience and sacrifice made it possible for me to complete my thesis. They have always been a constant inspiration and whose constant admonitions have helped me in my academic pursuits. Their sacrifices, prayers and good wishes made me to reach this stage.

Last but not the least I am grateful to almighty God for giving me the strength, courage, perseverance and patience to achieve this memorable milestone of my life.

Any omission in this brief acknowledgement does not mean lack of gratitude.

Needless to say, all errors and omissions are mine.

Lakshay.

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Place: R S Pura

Date: 18.09.2017

ABSTRACT

Title of the Thesis	:	**Alterations in biochemical and oxidative biomarkers in subacute oral toxicity of imidacloprid alone and in conjunction with arsenic in wistar rats**
Name of the Student & Registration No.	:	Lakshay Mahajan (J-15-MV-433)
Major Subject	:	Veterinary Pharmacology and Toxicology
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Degree to be awarded	:	M.V.Sc (Veterinary Pharmacology and Toxicology)
Year of award of Degree	:	2017
Name of the University	:	Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu (J&K)

The present study was aimed to evaluate the alterations in biochemical and oxidative biomarkers indicating hepatic, renal, testicular damage induced by the subacute exposure of imidacloprid and arsenic in male wistar rats. Rats (180-200 g) were divided into eight groups of six rats each and were subjected to various daily oral administrations for 28 days. Group I served as control, group II received imidacloprid @ 16.9 mg/kg b.wt by oral gavage, group III, IV and V received arsenic @ 50, 100 and 150 ppb orally in drinking water whereas group VI, VII and VIII received both imidacloprid and arsenic at different dose levels respectively. Total plasma proteins and albumin levels revealed a significant ($P<0.05$) fall in higher doses of arsenic as well as in combination groups while SGOT, SGPT, ACP, ALP, BUN and CR levels were increased significantly ($P<0.05$) in all treated groups as compared to the control. IMI and arsenic caused significant ($P<0.05$) elevation in MDA and AOPP whereas significant ($P<0.05$) decrease in TTH, GST, GR, GPx, SOD and CAT activities were observed in the blood, liver, kidney and testicular tissues. These finding were further confirmed by histological alterations in these tissues. In liver, mild to moderate degenerative changes were observed in IMI and arsenic administered rats. Arsenic administrations produce mild degenerative changes of renal tubular epithelium in a dose dependent manner. Microscopic examination of testes presented mild edematous fluid accumulation in interstitial spaces in the IMI administered group and arsenic exposure demonstrated mild degenerative changes in seminiferous tubules with the increasing doses of arsenic. Co-administration of IMI and arsenic produced more severe biochemical, antioxidant alterations in blood, liver, kidney and testes as compared to the individual administration of either toxicant in the wistar rats.

Keywords: Imidacloprid, Arsenic, Biochemical, Oxidative stress, Hepatoxicity

Signature of Major Advisor Lakshay **Signature of Student**

CHAPTER – I

INTRODUCTION

Agriculture and its allied sectors contribute nearly 22% of Gross Domestic Product (GDP) of India while about 65-70% of the population depends on agriculture for livelihood (Sachdeva, 2007). It is estimated that about 25% of crop production in the country is lost on account of pests and diseases. The biocidal agricultural chemicals collectively known as Pesticides are, without any question, the largest group of widely broadcast poisonous substances and constitute an important class of compounds that are being used extensively throughout the world to control agricultural pests in order to increase the crop production and public health. Therefore, these chemicals will continue to remain a key input in modern agriculture (Helferich and Winter, 2001). The production of pesticides started in India in 1952 and presently, India is the second largest producer of pesticides in Asia after China and ranks twelfth in the world for the use of pesticides (Mathur, 1999). Pesticides, though present in the environment in small quantities as compared to other contaminants, account for public and safety concern due to their biological activity (Kamrin, 1997). The pesticides are classified as insecticides, fungicides, weedicides, herbicides, nematodicides and rodenticides, of which insecticides constitutes 77% of the total pesticides used in different agricultural and animal husbandry practices and in public health operations (Chauhan and Lokesh, 2006).

There are more than 1500 pesticides listed in the compendium of pesticides common names. Roughly 55,000 tons of pesticides are annually applied in field crops. Chemicals have been used to control insects for centuries but have come into widespread use only within the past century with the development of variety of synthetic insecticides. Although Indian average consumption of pesticide is far lower than many other developed economies, the problem of pesticide residue is very high in our country. Improper handling and unsafe spraying of these agrochemicals cause risk of health hazards (Bag, 2000; Gupta, 2004). Centre for Science and Environment (CSE) reported that pesticide exposure causes acute poisonings, cancer, neurological impairment, reproductive and developmental problems (Takagi *et al*., 1997; Arora, 2007). In this

context, pesticide safety, regulation of pesticide use, proper application technologies and integrated pest management are some of the key strategies for minimizing human and animal exposure to pesticides.

Pesticides are contemplated to be worldwide contaminants and known to exert various toxicological effects in humans and animals (Garry *et al*., 2002). There are a number of possible ways in which humans can be exposed and toxic effects of these pesticides may have consequences on food consumers, production workers, formulators, farmers and other applicators. Jammu and Kashmir being a horticulture state is using these crop protection chemicals at an alarming rate. Indiscriminate and unhindered use of synthetic chemicals in various fields poses severe and serious health risk to mankind. Rapid industralization, demand for increased agricultural productivity and newer formulae in the development of medicines have all contributed to increased use of synthetic chemicals in one or other way.

Among the insecticides, neonicotinoids are the only major new class of insecticides developed in the past three decades. Worldwide annual sales of neonicotinoids are approximately one billion dollars accounting for 11 to 15% of the total insecticide market (Tomizawa and Casida, 2005). Imidacloprid was introduced to the market in 1991 as the first CNI (chloronicotinyl insecticide, syn. neonicotinoid) and has been highlighted because of its extremely high intrinsic insecticidal potency, low mammalian toxicity, broad insecticidal spectra, excellent systemic properties and plant compatibility, so it has been the largest selling insecticide worldwide for agriculture and as a veterinary medicinal remedy to control ectoparasitic insects in the last decade (Moria *et al*., 1992). For agriculture use, Imidacloprid is registered worldwide in more than 120 countries and for more than 140 crops (Mencke and Jeschke, 2002). In India, it is available under various brand names e.g. Tatamida 17.8% (Rallis India Ltd.), Admire 70%, Confidor 17.8% (Bayer India Ltd.) and Conidor 17.8% (BASF India Ltd.)

The selective toxicity of imidacloprid to insects and not to mammals is attributed to differences in binding affinity or potency at the nicotinic acetylcholine receptor (Tomizawa and Casida, 2003). The specificity of nicotinoid insecticides appears to be related to receptor subtype, function, neuronal region, development stage and their metabolic lability. The selectivity also depends on major structural differences in the

neuronal nicotinic acetylcholine receptor binding sites of mammals and insects. The selectivity of chloronicotinyl compounds for insects can be partly explained by differences in the ionization of the pyrolidine nitrogen. Imidacloprid is poorly ionized in neutral media, in contrast to nicotine and thus, passes easily through the insect lipophilic barriers (Solecki, 2001).

Imidacloprid is a systemic, chloro-nicotinyl insecticide with soil, feed and foliar uses for the control of sucking insects including rice hoppers, aphids, thrips, termites, soil insects and some beetles. It is mostly commonly used on rice, cereal, maize, potatoes, sugar beets, fruits and cotton, and is especially systemic when used as seed or soil treatment. It works by interfering with the transmission of stimuli in the insect nervous system. Specifically, it mimics the action of Ach and causes a blockade in a type of neuronal pathway (nicotinergic) that is more abundant in insects than in warm- blooded animals (making the chemical selectivity more toxic to insects than warm-blooded animals). This blockage results in the insect's paralysis and eventual death. It is effective on contact and via stomach action (Kidd and James, 1994).

Due to the favourable mammalian safety characteristics, imidacloprid has been developed for veterinary use and is used as flea control agents on cats and dogs. As endoparasiticide, it exerts activity against the gastrointestinal nematodes (*Haemonchus contortus* in sheep) only at higher concentrations (Mencke and Jeschke, 2002). The persistence and extreme stability of pesticides in environment are the ultimate source of contamination at the dietary level. The situation in practice is not toxicity resulting from a single or few large doses of a given pesticide but due to oral intake of very small quantities over a reasonable period of time. Agrochemicals, automobile waste, metals and metalloids are the major contaminants of environmental contamination are recognized as a worldwide problem. Agrochemicals along with heavy metals from emissions and groundwater sources are the dominant compounds of the chemical load of environment of man and animals (Kacmar *et al.*, 1999). Among heavy metals, Arsenic (As) is categorized as a ubiquitous trace element and is the 52nd most abundant element in the earth's crust (Hanston *et al*., 2003). Arsenic is the number one substance in the most recent Comprehensive, Environmental, Response, Compensation and Liability Act

(CERLA) priority list of hazardous substances published by the agency for Toxic Substances and Disease Registry (ATSDR, 2007).

Although arsenicals have been used throughout history but more detailed documentation of its use began in the late 19th century. Fowler's solution, first used in 1786, is a 1% solution of potassium arsenite used for of various diseases including malaria, syphilis, asthma, chorea, eczema and psoriasis (Scheindlin, 2005). In 1910, Paul Ehrlich introduced a new arsenic based drug namely Salvarsan, which came to be known as the "Magic bullet" for treating syphilis and was used until the use of penicillin became more prevalent in the 1940's (Riethmiller, 2005; Yarnell, 2005). Arsenic may be present in the environment either in the organic or inorganic form. The most common pathway for an elevated environmental exposure to inorganic arsenic worldwide is through drinking water. Usual route of arsenic assimilation could be through ingestion and inhalation while some degree of skin absorption also occurs (Monies, 1999), although some of the inhaled arsenic may be absorbed in the intestine after clearance from the upper respiratory tract (Vahter and Norin, 1980). Following ingestion, arsenic appears rapidly in the circulation where it binds primarily to haemoglobin (Axelson, 1980). Skin, bone and muscles represent the major storage organs (Osborne, 1925). Inorganic arsenic does not appear to cross the blood brain barrier (BBB), however transplacental transfer of arsenic in humans (Gibson and Gage, 1982) and mice (Hood *et al.*, 1987) has been established.

Biotransformation of absorbed arsenic mostly takes place in liver and kidneys (Ford, 2002). Methylated metabolites formed during the process of biotransformation are distributed throughout the body (Dopp *et al*., 2004). Urine is the major gateway to excrete arsenic as almost 60% of the amount is excreted in urine and only about 6% in faeces (Goyer and Clarkson, 2002). Biotransformation of arsenic is considered the primary detoxification mechanism since the inorganic arsenicals are most toxic to living organisms (Yamauchi and Fowler, 1994). These conversions of arsenic species are important in the methylation of arsenate to dimethylarsenic acid (DMA) which is believed to be the principal detoxification mechanism (Aposhian, 1989).

Arsenic toxicity differs from that of other protoplasmic poison which acts by denaturing and precipitating the cellular proteins. It has been assumed that the arsenic

affects the functional activity rather than structural integrity (Stocken and Thompson, 1946). Arsenic toxicity is mainly attributed to low methylating ability. Chimpanzee, monkeys, humans and guinea pigs are prone to its toxicity as they seem to be poor methylators. Disturbance in the methylating ability in rats, chicken and rabbit is leading to its toxic effects in these species (Vahter, 1994). Arsenic causes many hazards in animals and humans such as oxidative stress, DNA hypo/ hypermethylation or alteration of several gene expressions. Arsenic causes damage in biological system because of its ability to generate oxidative stress in the cells. It generates free radicals/ reactive oxygen species (ROS) viz. hydrogen peroxide (H_2O_2), nitric oxide (NO), superoxide anions (O_2^-) and hydroxy radicals (OH^-) that leads to oxidative damage in visceral organs due to imbalance in antioxidant system (Yamanaka *et al*., 1991). The excessive generation of ROS is very damaging as they can attack lipids, proteins and DNA resulting in lipid peroxidation and DNA mutation leading to several diseases (Singh *et al*., 2009). Based on the substantial evidence of cancer risk associated with chronic exposure to relatively low concentration of arsenic in drinking water, the international agencies suggested 50 µg/L as the maximum contaminant level (MCL) with an increase in risk for cancer (NRC, 1999). The United States Environment Protection Agency had revised the MCL for arsenic in drinking water to 10 µg/L (USEPA, 2001). The WHO has suggested a provisional guideline value of 10 µg/L of arsenic in drinking water and the European Union has set 50 µg/L and 10 µg/L as the limit and guide values of arsenic in drinking water respectively (WHO, 1981).

OBJECTIVES OF THE STUDY:

In view of the weight of evidence of binary mixture toxicity, the present study was undertaken with the following objectives:

1. To evaluate the alterations in blood biochemical and oxidative biomarkers in subacute toxicity of imidacloprid in wistar rats.
2. To evaluate the alterations in blood biochemical and oxidative biomarkers in subacute toxicity of arsenic in wistar rats.
3. To evaluate the alterations in blood biochemical and oxidative biomarkers after concurrent exposure of imidacloprid and arsenic in wistar rats.

CHAPTER II

REVIEW OF LITERATURE

Pesticides are considered to be essential for agricultural development, but poor application procedures are adopted by farmers that can cause serious ambient contamination (Saitta *et al*., 2000; Gonzalez *et al*., 2003). The term pesticide includes insecticides, herbicides, rodenticides as well as fumigants and wood preservatives. The extensive use of chemicals in agriculture is also compromising soil and water quality (Arnaud *et al*., 2005) and residues may remain in foodstuff in amounts above maximum residual level (MRL) that results in serious consequences or potential adverse health effects (Yassi *et al*., 2001) like respiratory diseases (Salameh *et al*., 2003), neurological dysfunctions (Paolini *et al*., 2004), cancers (Flower *et al*., 2004) and reproductive disorders (Kumar, 2004). It also has side effects on the environment (Forget, 1993) other than the life forms. This problem is being viewed seriously by international organizations, such as United States Environmental Protection Agency (USEPA), Codex Alimentarious Commission, WHO and FAO of the United Nations (Cao *et al*., 2005). It has been estimated that 85-90% of the pesticides applied in agriculture never reach their target organisms, but instead are dispersed in the air, water and soil.

2.1 Neonicotinoids

Neonicotinoids, the most important new class of synthetic neuro-active insecticides are used to control sucking insects both on plants and on companion animals. The neonicotinoids are the only major new class of insecticides developed in the last three decades. Imidacloprid, nitenpyram, acetamiprid, thiacloprid, thiamethoxam and other members of this class act as agonist at the insect nicotinic acetylcholine receptor (nAchR). Worldwide annual sales of these insecticides are approximately one billion dollars, accounting for 11-15% of the total insecticide market (Tomizawa and Casida, 2005).

2.1.1 Imidacloprid

Imidacloprid was the first representative and an important neonicotinoid insecticide and was the first neonicotinoid registered by the USEPA for use as a pesticide. Since being introduced in the market as a insecticide in 1992 and ranked as one the top selling pesticide in the world in 2001-2002, IMI is registered and marketed worldwide for use in more than 120 countries and on over 16 crops. IMI is classified as a "Group E" carcinogen, indicating no evidence of carcinogenicity in humans (USEPA, 2003).

Imidacloprid with unique physical and toxicological properties as compared to other classes of insecticides, was synthesized for the first time by Bayer chemists in 1985 for the purpose of controlling many important pests already resistant to other insecticides (Cox, 2001). It has high selectivity factors for insects than organophosphates, methylcarbamates and organochlorines. This is attributable to both target site specificity (Casida and Quistad, 1998) and detoxification. The selectivity of chloronicotinyl insecticide for insects as opposed to mammals can be partly explained by differences in the ionization of the pyrolidine nitrogen. Imidacloprid is poorly ionized in neutral media in contrast to nicotine and thus, passes easily through insect lipophilic barriers (Solecki, 2001).

2.1.1.1 Physiochemical Properties of Imidacloprid:

Common Name : Imidacloprid
Chemical Name: 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine
Molecular formula : $C_9H_{10}ClN_5O_2$
Molecular mass : 255.66 g/mol. ($20^{O}C$ at pH 7)
Melting point : 143-145^{O}C
Appearance : White crystalline powder
Stability : Stable to hydrolysis at pH 5-11
Solubility in water : 0.51 g/L ($20^{O}C$)
Vapour pressure : 3×10^{-12} mm Hg (at $20^{O}C$)
Toxicity class : II and III
CAS Registry number : 138261-41-3
Oral LD_{50} : 450 mg/kg b.wt (in rats) and 131 mg/kg b.wt (in mice)
Dermal LD_{50} : greater than 5000 mg/kg b.wt (in rats)

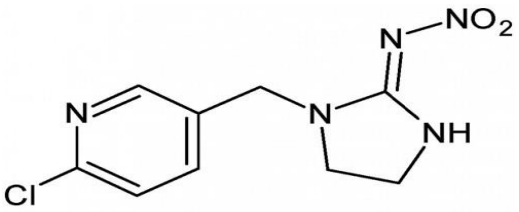

Figure 1: Chemical structure of Imidacloprid

2.1.1.2 Pharmacokinetics of Imidacloprid:

The gastrointestinal tract of rats absorbed 92% of the imidacloprid dose. Plasma concentration peaked 2.5 hours after administration (WHO, 2004). Little systemic absorption through the skin occurs following dermal exposure in pets (Wismer, 2004). It was readily absorbed and rapidly distributed to the tissues and organs within 5 min after intravenous injection and 1 hour after oral dosage, with the exception of fatty tissues, the central nervous system and the mineral part of the bone (Klein, 1987b). Metabolism occurs primarily in the liver (Thyssen and Machemer, 1999). Imidacloprid metabolize in two major pathways: the first pathway, imidacloprid may be broken by oxidative cleavage to 6-chloronicotinic acid and imidazolidine. Imidazolidine is excreted in the urine and 6-chloronicotinic acid undergoes further metabolism via, glutathione conjugation to form mercaptonicotinic acid and a hippuric acid (Klein and Karl, 1990). It may also be metabolized by hydroxylation of the imidazolidine ring in the second major pathway (Klein, 1987a). Metabolic products from the second pathway include 5-hydroxy and olefin derivatives (Klein, 1987a).

The metabolic products 5-hydroxy and olefin derivatives resulting from hydroxylation of the imidazolidine ring are excreted in both the faeces and urine (Klein and Karl, 1990; Schulz-Jander and Casida, 2002). Metabolites found in urine include 6-chloronicotinic acid and its glycine conjugate. Metabolites in the faeces accounted for roughly 80% of the administered dose in rats and included monohydroxylated derivatives in addition to unmetabolized imidacloprid. Olefin, guanidine and the glycine conjugate of the methylnicotinic acid were identified as minor metabolites (Roberts and Hutson, 1999). Rats excreted 96% of the imidacloprid within 48 hours following an oral dosing, with 90% excreted in the first 24 hours (Klein, 1987a).

2.1.1.3 Toxicological Profile

Imidacloprid is a general use insecticide and has unique physical and toxicological properties as compared with earlier class of insecticides and is comparatively more selective for insects as compared to mammals. Selective toxicity involving low hazards for mammals and high potency to pests, are essential for safe and effective pesticides. Nicotine was used for centuries to control sucking insects despite relatively low efficacy and high toxicity to mammals. The neonicotinoids are more toxic to insects and less toxic to mammals, providing an excellent example of selective toxicity (Kagabu, 1997; Yamamoto *et al.*, 1998).

Berney *et al.* (1999) reported that patridges have been poisoned and killed by agricultural use of imidacloprid. IMI residues were found in crop in crop, gizzard and liver of patridges. Godfrey (1999) observed that a cat developed a severe skin rash following treatment with an imidacloprid containing product. The rash centered at the spot where the IMI was applied.

Cox (2001) reported that IMI affects reproduction and on exposure of pregnant animals results in more frequent miscarriages and smaller off-springs. IMI is acutely toxic to some birds species including sparrows, quail, canaries and pigeons with LD_{50} of less than 50 mg/kg b.wt. Premlata *et al.* (2004) studied subacute toxicity of imidacloprid in wistar rats for 28 days. She found difficulty in breathing, respiratory distress, open mouth breathing, cyanosis of paws and legs, exophthalmus and decreased spontaneous motor activity after 15-20 minutes following intra-peritoneal administration of imidacloprid in rats.

Tomizawa and Casida (2005) reported that no specific antidotes for neonicotinoid poisoning in mammals. Treatment with an acetylcholinesterase (AChE) - reactivating oxime (e.g. pralidoxime-important in organophosphate poisoning) or a nicotinic antagonist might be either ineffective or contraindicated. Symptomatic treatment is recommended for any possible acute poisoning case.

2.1.1.4. Alterations in Biochemical Parameters

In a chronic toxicity study in wistar rats were administered with imidacloprid at different concentrations (100, 300, 900 and 1800 ppm) for 2 years. There was no inhibition of cholinesterase in brain, plasma or erythrocytes at any level. At 1800 ppm,

serum alkaline phosphatase, creatinine kinase, aspartate aminotransferase levels were elevated with a reduction in plasma cholesterol (USEPA, 1998). Kaur *et al.* (2006) reported that repeated oral administration of IMI @ 1 mg/kg for 21 days in cow calves significantly elevated alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and had no significant effects on total serum proteins, aspartate aminotransferase, acid phosphatase, BUN, plasma creatinine, blood sugar, plasma cholesterol and cholinesterase enzymes.

Premlata *et al.* (2006) studied subacute toxicity of imidacloprid in adult male rats following intra-peritoneal administration at the rate of 20 and 40 mg/kg daily for 28 days. There was decrease in blood urea level on day 14 and creatinine level on day 28 but there was no effect on Hb, PCV and TEC counts at both the dose levels. Imidacloprid caused an inconsistent effect on TLC showing an increase in TLC on seventh day at lower dose and a gradual decrease in TLC upto 28 days at higher dose. Kammon *et al.* (2010) reported that oral administration of imidacloprid at the rate of 139 mg/kg body weight through oral gavage in chicken resulted in significant increased levels of AKP, ALT, AST and plasma glucose. Balani *et al.* (2011) evaluated the toxic effects of oral administration of imidacloprid in male white leghorn chicken at 1.25, 1.67 and 2.5 mg/kg b.wt for 28 days and noticed an increase in ALT, no change in AST, serum total protein, total globulins, albumin and serum creatinine levels. Non-significant decline in total protein and a significant reduction in albumin levels was reported in birds fed with 1-2 mg/kg b. wt of Imidacloprid for 28 days which is indicative of immunosuppression (Siddiqui *et al*., 2007). Sridhar (2010) recorded a natural toxicity of imidacloprid in buffaloes and observed slight increase in AST and ALT whereas there was no change in serum BUN and creatinine. Bhardwaj *et al*. (2010) observed that oral administration of imidacloprid in female rats at the rate of 5, 10 and 20 mg/kg b. wt for 90 days resulted in elevation of ALT, AST, glucose, BUN and decreased acetylcholinesterase in serum and brain. Similarly, Soujanya *et al*. (2013) reported that oral administration of imidacloprid at the rate of 80 mg/kg b. wt for 28 days in male rats resulted in nephrotoxicity which was evident from significantly increased hepatic biomarkers, serum creatinine levels and plasma proteins in imidacloprid treated rats whereas co-treatment with vitamin C brought mild to moderate improvement in creatinine levels.

Mohany *et al.* (2012) reported that oral administration of imidacloprid at 0.21 mg/kg b. wt for 28 days in male albino rats resulted in elevation of AST, ALT, ALP and MDA levels. Abbassy *et al.* (2014b) investigated the toxicity of imidacloprid (0.943, 0.365, 45 and 56 mg/kg) and tetraconazole (0.174, 0.104, 124.8 and 156 mg/kg) in male rats given daily oral doses for 30 days. The results indicated IMI and tetraconazole did not cause any significant effects on the activities of AST, ALT, GGT, LDH and ALP enzymes. There was non-significant difference in creatinine, uric acid, total protein, albumin and glucose in serum of treated rats. There was also no effect on PCV, Hb, RBC count and WBC in the blood of treated rats.

2.1.1.5. Alterations in Antioxidant Parameters

Imidacloprid at the rate of 10 µM through intravenous administration in rats resulted in increased nitric oxide levels and upregulated inflammatory cytokines tumor necrosis factor-alpha (TNF-α), Interleukin-6 (IL-6), Interleukin-1 beta (IL-1β) in liver. Elevated levels of xanthine oxidase, myeloperoxidase activity, depleted intracellular glutathione in liver and brain also observed (Duzguner and Erdogan, 2010)**.** Oral administration of imidacloprid at the rate of 14.9 mg/kg b. wt. In male mice produced oxidative stress which was evident from increased levels of lipid peroxidation (LPO), activities of antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione peroxidise (GP_X), glucose-6-phosphate dehydrogenase (G6PD), glutathione-s-transferase (GST) and decreased levels of reduced glutathione (GSH) whereas Vitamin C (200 mg/kg) treated mice showed ameliorating effect documented by decreased levels of LPO (El-Gendy *et al*., 2010).

Kapoor *et al.* (2010) evaluated the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidise (GP_X) and level of glutathione (GSH) and lipid peroxidation (LPO) in liver, kidney and brain of rats after oral administration of imidacloprid (5, 10, 20 mg/kg/day) for 90 days. Further Kapoor *et al.* (2011) evaluated the technical grade imidacloprid for its effect on ovarian morphology, hormones and antioxidant enzymes in female rats after 90 days oral exposure. Imidacloprid at 5 and 10 mg/kg b. wt/day has not produced any significant changes but 20 mg/kg b. wt/day dose produced significant alterations in levels of LH, FSH and Progesterone along with significant changes in SOD, CAT, GP_X, GSH and LPO. He concluded that imidacloprid

at 20 mg/kg b. wt/day dose level has produced significant toxicological impact on ovary of female rats as evident by patho-morphological changes, hormonal imbalance and generating oxidative stress and can be considered primarily as Lowest Observed Effect Level (LOEL) for chronic study.

Zhang *et al.* (2011) studied the effect of acetamiprid (30 mg/kg b.wt) in mice. He concluded that acetamiprid had damaged seminiferous tubules and Leydig cells, there was degeneration of mitochondria and endoplasmic reticulum of Leydig cells. These effects were mediated by rise in oxidative stress, as acetamiprid increased malondialdehyde and nitric oxide in the testes and reduced the activity of CAT, GPx, SOD and activated p38. The concentration of acetamiprid in the testes as lower than that in the liver, along with low levels of liver function tests (AST, ALT and ALP), which suggest that male reproductive system may be affected through indirect action of its metabolites. Vitamin E (@ 20 mg/kg b.wt) significantly ameliorated the effects of acetamiprid.

Ince *et al.* (2013) concluded that IMI produced significant increase in blood, liver, kidney and heart malondialdehyde (MDA) levels and decrease in blood and liver glutathione levels (GSH) levels. In addition, IMI treatment decreased erythrocyte, liver and kidney SOD and CAT activities. However, Treatment with TQ reversed IMI-induced oxidative stress, lipid peroxidation and activities of antioxidant enzymes. Lonare *et al.* (2014) evaluated the neurotoxic effect and biochemical alteration as a result of imidacloprid and potential protective role of Curcumin (100 mg/kg; orally) against it in rats. Rats were administered with IMI (45 and 90 mg/kg body weight; orally) alone and in combinations for a period of 28 days. Significant decreases in SLA and pain threshold were observed in animals treated with IMI while the effects were attenuated with Cur co-treatment. Acetylcholinesterase, ATPase and serum biochemical (creatine kinase, LDH, SDH and ALP) were significantly decreased as result of IMI exposure and these enzyme levels were reversed in groups treated with Cur in IMI treatments. Also, IMI caused significant decrease in antioxidant enzymes activity and non-enzymes level with increase in LPO while Cur administration restored the altered activity of antioxidant system with decrease in LPO. In conclusion, Cur restores the altered functions of biochemical markers and neurotoxicity in IMI exposed rats.

Babu *et al.* (2014) studied the effect of imidacloprid (200 ppm) induced oxidative stress in layer birds of above 2 months age for 90 days. The hepatic tissue antioxidant profile revealed a significant reduction in GSH concentration and rise in TBARS levels. The alterations in oxidative stress profiles indicate hepatotoxicity and moderate protection was provided by *Withania somnifera* (@ 500 ppm) and Vitamin C (@ 200 ppm) to ameliorate the toxic effects of imidacloprid. Ahmed and Nasr (2015) investigated the neuro-protective effect of broccoli water extract and ferulic acid on imidacloprid induced oxidative stress and DNA damage in male albino rats. Rats were co-treated with broccoli water extract (200 mg/kg) with IMI (80 mg/kg) orally for 28 days. The results revealed that IMI induced low serum levels of total antioxidant capacity (TAC), whereas lipid peroxidation (LPO) content was increased. Broccoli and ferulic acid significantly attenuated the IMI-induced increase in LPO, TNF-α, Nitric oxide, MPO, GST and CAT activities. IMI decreased glutathione (GSH) while co-treatment with broccoli and ferulic acid significantly improved the GSH levels.

Low doses (one-fourth of LD_{50}) of dichlorovos (DDVP), lambda-cyhalothrin (LMB), cypermethrin (CPM) and Imidacloprid (IMI) were administered to adult male rats for 3 weeks. Erythrocyte antioxidant enzymes, biomarkers of tissue toxicity were assessed. G6PD, GST, Ach and body weight reduced significantly in DDVP and LMB-treated rats only, GSH levels reduced significantly in rats treated with DDVP and IMI while LPO levels increased in plasma of DDVP and erythrocytes of DDVP, CPM and IMI-exposed rats (Nwozo *et al*., 2015; Lonare *et al.* 2015).

Keshta *et al.* (2016) studied thiamethoxam (THIA @ 156 mg/kg b.wt) and acetamiprid (AC @ 100 mg/kg b. wt) induced antioxidant, biochemical and histopathological alterations at durations of 10, 20 and 30 days in rats. They significantly increased the levels of MDA and nitric oxide (NO), but there was significant reduction in activities of CAT and SOD at 10, 20 and 30 days. Also, they significantly elevated the concentrations of total cholesterol, triglycerides, LDH and creatine kinase whereas there was significant fall in AChE activity and testosterone levels.

Wang *et al.* (2016) investigated the soil ecological effect of Imidacloprid on *Eisenia fetida* (earthworm) at various concentrations of 0.10. 0.50 and 1.0 mg/kg soil respectively after 7, 14, 21 and 28 days to evaluate its effect on ROS generation,

antioxidant enzyme activity (SOD, CAT, GST), MDA content and DNA damage of *E. fetida*. Significant rise of ROS level, SOD and GST activity was observed. CAT activity was inhibited and reflected a dose-dependent relationship on days 7, 14 and 21. High MDA levels were observed and the olive tail moment (OTM) as well as percentage of DNA in the comet tail declined with increasing concentrations and exposure time after 7 days. Therefore, he concluded that the sub-chronic exposure of IMI caused DNA damage and LPO leading to antioxidant responses in earthworm.

Imidaclopid was evaluated for its effect on oxidative stress and histopathological changes in liver of Wistar albino rats at two dose levels (19 and 38 mg/kg/day) administered orally for 10, 20 and 30 days. Imidacloprid at 38 mg/kg dose significantly increased organ weight and MDA levels in 20 and 30 days group. There was significant fall in levels of cytoplasmic and membrane proteins along with activities of enzymes SOD and GPx at 38 mg/kg dose administered for 20 and 30 days. GSH levels were reduced significantly at 38 mg/kg dose administered for 30 days (Lohiya *et al.,* 2017).

2.2. Arsenic

Arsenic, one of the most toxic metalloids, is ubiquitous in the environment. Globally, millions of people are being exposed to arsenic through consumption of contaminated drinking water and food. It is solid, tin-white to steel gray coloured element, has a molecular weight of 74.9 and is classified as a metalloid. It has both metallic and non-metallic properties. Arsenic is usually not mined as such, but is recovered as by-product from the smelting of copper, lead, zinc and other ores. All this can result in the release of arsenic into the environment. Arsenic exists in three states (elemental, trivalent and pentavalent), the trivalent form of arsenic is considered to be more toxic due to its ability to bind with the sulfhydryl group of proteins and disrupt the enzyme activity (Shila *et al*., 2005; Yu *et al*., 2006).

In-spite of the fact that skin lesions are characteristic features of arsenic toxicity (Smith, 2000), chronic arsenic exposure may affect a number of organs (Spallholz *et al*., 2004; Khan *et al*., 2007). Arsenic exposure has been associated with health problems including hypertension (Chen *et al*., 1995), cardiovascular diseases, developmental abnormalities diabetes, hearing loss, fibrosis of liver and lungs, haematological disorders,

neurological and reproductive problems, black foot disease and cancer (Kapaj *et al.*, 2006; Khan *et al.*, 2006; Bhattacharya and Haldar, 2012). The adverse effects of arsenic in humans have been documented in Bangladesh, India, China, Taiwan, Chile and the United States of America (Walvekar *et al*., 2007; Wang *et al*., 2007).

2.2.1. Toxicodynamics

Arsenic affects mitochondrial enzymes and impairs cell respiration, which may lead to cellular death (Brown *et al.*, 1976). Pentavalent arsenic compounds are un-coupler of mitochondrial oxidative phosphorylation. They compete with inorganic phosphate in the formation of adenosine triphosphate (ATP), with subsequent formation of unstable arsenate ester that is rapidly hydrolyzed in a process called arsenolysis. Trivalent arsenicals inhibit many enzymes by reacting with biological ligand containing –SH group, especially the pyruvate dehydrogenase system is most sensitive to trivalent arsenicals because of presence of two –SH groups in lipoic acid (Klassen *et al.*, 2001). Interruption of the electron transport chain (ETC) and oxidative phosphorylation can have serious consequences including the generation of reactive oxygen species (ROS). The ROS generation may be associated with the cell death via apoptosis (Buttke and Sandstrom, 1994).

Methylation has long been considered the primary mechanism of detoxification of inorganic arsenic in mammals (Buchet *et al*., 1981). Arsenite acts as the initial substrate for arsenic methyltransferases that subsequently produce monomethylarsonic and dimethylarsonic acid species (Zakharyan *et al*., 1995; Aposhian, 1997). These pentavalent methylated species are more rapidly eliminated from the body than the inorganic precursors and are comparatively less toxic based on the lethal doses (Tatken and Lewis, 1983).

2.2.2. General Toxicity

Arsenic exists in the elemental form and in trivalent and pentavalent oxidation states. The toxicity of a given arsenical is related to the rate of its clearance from the body and its degree of accumulation in tissues. In general, toxicity increases in the sequence: organic arsenicals < As^{5+} < As^{3+} < arsine (AsH_3). The organic arsenicals are usually excreted more rapidly than the inorganic forms and are less toxic. Trivalent compounds like sodium arsenite are more toxic than pentavalent compounds such as lead arsenite

(Klassen *et al.*, 2001). The hepatotoxic effects of arsenic in man and animals have been well documented. Industrial or occupational exposure may cause various disorders in liver, e.g. cirrhosis, fibrosis, non-cirrhotic portal hypertension, cholestasis, moderate metabolic disorders, hepatic cancers and necrosis. Intake of some Ayurvedic and Unani arsenic preparations has been reported to cause Indian Childhood Cirrhosis (ICC) in the off-springs (Athar and Vohora, 1995).

Arsenic acid and some other arsenicals are known to cause tubular damage including necrosis in kidney. Haemoglobinuria occurred in cases of toxicity manifested following the exposure to arsine gas and As (III) halogenides. Urinary arsenic concentration has been regarded as a good index for assessing the degree of arsenic intoxication (Athar and Vohora, 1995). Arsenic is a capillary poison and affects tissues rich in oxidative enzyme systems resulting in hypovolemia, shock and circulatory failure. Myocarditis, peripheral neuropathy, ECG changes and perivascular disturbances (e.g. Raynoud's syndrome, mesenteric thrombosis and Black-foot disease) may result from exposure to arsenic compounds. Arsine and As (III) halogenoids are particularly toxic. They are taken up by erythrocytes and cause hemolysis and hemoglobinuria. Its chronic exposure may lead to bone marrow depression, anaemia, leucopenia and basophilic spelling (Athar and Vohora, 1995).

2.2.3. Alterations in Biochemical Parameters

Vutukuru *et al*. (2007) evaluated the acute effects of arsenic and hexavalent chromium on the activity of ALT and AST in the Indian major carp, Labeo rohita for 24 hr and 96 hr. They concluded that ALT activity rises significantly in arsenic exposed fish indicating serious hepatic damage and distress condition in fish. However, no such significant change was observed in chromium-exposed fish suggesting that arsenic is more toxic to the fish. Patel and Kalia (2010) suggested that sub-chronic arsenic exposure further accelerated the nephrotoxicity in diabetic group, which seemed to be dependent on compromised oxidative stress. More pronounced nephrotoxic effects noticed in arsenic exposed diabetic group as evidenced by increased BUN, serum creatinine and relative kidney weight along with decreased level of reduced glutathione and glutathione peroxidase activity compared to non-arsenic exposed diabetic group.

Roy and Roy (2011) evaluated the effect of *Psidium guajava* (@ 50 mg/kg b.wt) leaf extract on arsenic (@ 20 mg/kg in drinking water) for 45 days in Wistar rats. Arsenic exposure lead to significant rise in glucose, serum urea nitrogen and serum creatinine whereas non-significant decrease in total protein, calcium and phosphorus levels. Therefore, it was concluded that kidney damage caused by arsenic can be repaired upto some extent by $AEPG_{50}$. Singh *et al.* (2012) studied the effect of arsenic (0.02 mg/litre) in drinking water for 7, 14 and 21 days respectively in albino rats. After stipulated period, the body weight, organ weight and their ratio reduced significantly while there was significant increase in SGOT, SGPT, ACP and ALP. Similarly Tandan *et al.* (2012) investigated the effects of co-administration of *P. guajava* was most effective not only in reducing arsenic-exposed haematological and biochemical alterations but also in depleting arsenic from blood and soft tissues following arsenic exposure. Balasubramanian and Kumar (2013) observed that the arsenic exposure is responsible for significant alteration in activity of SGOT, SGPT and Alkaline phosphatase in comparison to control in *Heteropneustes fossilis* while treatment with zeolite could significantly bring recovery of the conditions in the fish.

Gora *et al.* (2014) studied that oral administration of sodium arsenite @ 10 mg/kg for 28 days resulted in a significant decrease in Hb, TEC and TLC, significant increase of serum glucose, cholesterol, calcium and significant increase in arsenic accumulation in tissues. They concluded that supplementation of TPE (500 mg/kg) daily orally for 28 days has shown protection against arsenic induced toxicity by its protective effect. Khan *et al.* (2013) observed that As-treated birds showed a significant decrease in serum total proteins while serum enzymes, urea and creatinine were significantly increased. The alkaline phosphatase and lactate dehydrogenase were completely ameliorated whereas proteins, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine were partially ameliorated in birds treated with arsenic along with ascorbic acid as control birds.

Charles (2014) concluded that arsenic trioxide at 20 mg/kg body weight twice a day for 7 days induces marked renal and liver damage characterized by a significant increase in plasma urea, creatinine, AST, ALT and ALP values in arsenic treated rats as compared to normal control rats. Kalia *et al.* (2014) investigated the protective role of the

bamboo leaves methanolic extract (BLME) against sodium arsenite (@ 5.55 mg/kg/day) in albino rats for 15 days. Arsenic exposure produced significant rise in liver cell injury markers (SGOT and SGPT) and oxidative stress markers (AOPP and TBARS) but there was significant reduction in antioxidant enzymes (CAT, SOD and GPx). Bamboo leaves extract at three different doses (50, 250 and 500 mg/kg b.wt) for 15 days after arsenic treatment altered most of parameters of oxidative stress caused by arsenic. Bamboo leaves extract eliminate the effect of arsenic induced oxidative damage by being a potent free radical scavenger. Chowdhury *et al.* (2016) found immunosuppression, leukopenia as well as marked increase in serum ALT and AST of arsenic intoxicated rats.

2.2.4. Alterations in Antioxidant Parameters

Lipid peroxidation has been documented to a sensitive parameter of repeated arsenic exposure. Forty day exposure of arsenic as sodium arsenate at 0.05 ppm caused lipid peroxidation in the brain of rats (Chaudhuri *et al.*, 1999). Maiti and Chatterjee (2000) reported that liver and kidneys have different adaptive cellular protective mechanisms against arsenic exposure. The kidneys were observed to be more vulnerable to arsenic treatment in male Wistar rats that have been exposed to arsenite (3.33 mg/kg/day) for 14 days. Significantly increased lipid peroxidation and decreased SOD, CAT activities were recorded in kidneys of the rats. Male wistar rats given 100 ppm arsenic for 10 weeks showed marked elevation in ROS level in blood, depletion of GSH and these changes were accompanied by significant decline in blood haemoglobin level (Flora *et al.*, 2005).

Oral administrations of sodium arsenite (2 mg/kg, for 3 weeks) in rats showed significant but marginal increase in hepatic LPO and CAT activity along with decrease in GSH level, while no marked effect on kidney oxidative stress (Modi *et al.*, 2006). Wang *et al*. (2006) studied the mechanism of arsenic- induced oxidative stress in growing pigs, involved lipid peroxidation, depletion of glutathione and decreased activities of some enzymes such as SOD, CAT, GP_X, GR and GST, which are associated with free radical metabolism.

Rats exposed to arsenic (5 mg/kg) via, drinking water for 30 days induced oxidative stress in plasma, liver, kidney, lungs, testes and brain with increased LPO and decreased GST, SOD, CAT and content of sulfhydryl group (El-Demerdash *et al*., 2009).

Jain *et al.* (2011) demonstrated that arsenic exposure produces a significant decrease in hepatic GSH levels, SOD and catalase activities and an increase in GST and TBARS levels. Silymarin or narigenin administration increased GSH levels and was beneficial in the recovery of altered SOD and catalase activity besides significantly reducing blood and tissue arsenic concentration. Chattopadhyay *et al.* (2012) studied the protective role of Vitamin B12 with folic acid against arsenic-induced hepatotoxicity in female rats. Ingestion of sodium arsenite contaminated water (0.4 ppm/100 g b.wt/day) in combination with Vitamin B12 plus folic acid (0.07 and 4 µg respectively/100 g b.wt/day) for 24 days to wistar rats offered a significant protection against arsenic-induced distorted liver function, damaged histoarchitecture, elevated oxidative stress and DNA fragmentation of hepatic tissues. Arsenic alone exposure reduced hepatic SOD, CAT activities and the level of non-protein soluble thiol (NPSH) with a concomitant rise in LPO and conjugated dienes (CD) in liver. Vitamin supplementation restrained the increase of LPO and CDs by restoring CAT, SOD and NPSH levels.

Muthumani and Miltonprabhu (2012a) evaluated the protective effect of silibinin (SB @ 75 mg/kg b.wt) on arsenic (As @ 5mg/kg b.wt) induced oxidative stress in the testis of rats for 28 days. As-exposure significantly increased the levels of testicular oxidative stress markers (TBARS, LOOH and PC) and significantly reduced the testicular enzymatic antioxidant enzyme activities of SOD, CAT, GPx, GST, GR, G6PD and non-antioxidants GSH, total sulfhydryl groups (TSH). Co-administration of SB with As was found to protect against adverse changes in the reproductive organ, enhance plasma reproductive hormone levelalong with increased membrane bound ATPase, antioxidant activities and reduced lipid peroxidation levels.

Sudha (2012) concluded that administration of arsenic (@ 100 ppm) generate ROS by decreasing the activation of antioxidant enzymes (SOD, CAT, GST, GP_x, LPO), thereby causing stress in the testes of rats while co-administration of arsenic with α-tocopherol (400 mg/kg b. wt) reversed the oxidative stress generated in testes of rats. Khan *et al.* (2013) reported that administration of arsenic (40 ppm in drinking water for 28 days) caused increase in lipid peroxidation and As content with concomitant reduction in glutathione and activities of catalase, superoxide dismutase along with histopathological impairments in testes. Curcumin co-treatment (100 mg/kg body weight

orally for 28 days) was found to be effective in reversing arsenic-induced all these effects and could be able to antagonize reproductive toxicity.

Sumedha and Miltonprabhu (2014) investigated the protective effect of diallyl trisulfide (DATS @ 80 mg/kg b.wt) on arsenic (As @ 5mg/kg b.wt) induced testicular oxidative stress in male rats for 28 days. Short term exposure to As resulted in reduced testicular weight, sperm count, plasma hormonal concentration and significant increase levels of oxidative stress markers with a significant fall in the enzymatic antioxidant, non-enzymatic enzymes and ATPases enzymes. Pre-administration of DATS to As-treated rats were found to protect against adverse changes in the reproductive organ weight, sperm count, enhance plasma hormone level along with restored antioxidant enzymes, non-antioxidant enzymes and ATPases enzymes.

Lakshmi *et al.* (2015) concluded that wheat grass extract possessed remarkable effect against arsenic induced organ toxicity in male albino rats mediated by alleviation of arsenic induced oxidative stress by enhancing the anti-oxidant defence mechanism and also by detoxification of free radicals generated in the body.

2.3. Interaction studies

Adult male rats were pre-exposed to two different doses of nicotine (0.75 and 3 mg/kg intra-peritoneally) for 7 days followed by 30 days of arsenic exposure (50 ppm sodium arsenite in drinking water). Nicotine pre-exposure resulted in an increased brain arsenic accumulation and a decreased liver arsenic accumulation. Arsenic also caused a significant oxidative stress in blood, brain and liver of exposed rats. Glutathione-S-tranferase was inhibited by both arsenic and nicotine but no such inhibition was noted in arsenic-treated animals pre-exposed to nicotine (Pachauri and Flora, 2013).

Jain *et al.* (2015) evaluated that male rats at very low dose of arsenic (25 ppm in drinking water) or nicotine (0.25 mg/kg s.c) for a period of 5 months and post-exposure various biochemical variables indicative of oxidative stress and apoptosis evaluated. Almost all glutathione linked enzymes showed marked alteration in individual as well as co-exposure treated groups. While serum creatinine and apoptosis indicator, Lactate dehydrogenase (LDH) were significantly increased in both treatments, an additive effect was noted in co-exposure group. A similar trend was also seen in brain and liver but not

in kidneys. It was concluded that arsenic and nicotine exhibited significant toxicity during individual exposure whereas co-exposure to these toxins showed variable conditions (indicative of both synergism and antagonism) in male rats. Kayode *et al*. (2015) studies the effects of sodium arsenite (100 mg/L in drinking water) and di-(2-ethylhexyl) phthalate {DEHP} (100 mg/kg b. wt. by gavage) individually and in combination on oxidative stress indices in rats. Exposure to either arsenic or DEHP resulted in significant rise of advanced oxidation protein product (AOPP) and lipid hydroperoxides (LOOH) concentrations in plasma, liver, kidney and brain while there was significant fall in total antioxidative capacity (TAC) in kidney and brain of rats. The combined exposure resulted in moderate additive effect on AOPP and LOOH level in liver and kidney respectively.

Dwivedi and Flora (2015) studied the combined exposure of arsenic (25 ppm in drinking water) and dichlorovos (2.5 mg/kg, orally) for 56 days on biochemical variables, indicative of lipid peroxidation, antioxidant enzyme system and AChE activity in erythrocytes of rats. While arsenic cause significant rise in AChE, dichlorovos produced marked depletion. Arsenic and dichlorovos exposure produced significant rise in reactive oxygen species (ROS), MDA levels and glutathione linked enzymes (GSH, GP_x, GST and GR) while catalase activity remained unchanged. Therefore, it was concluded that combined exposure to arsenic and dichlorovos may lead to synergistic effects on certain biochemical indicators of oxidative stress like ROS, GSH and MDA, suggesting a more pronounced induction of lipid peroxidation of erythrocytes (Flora *et al.,* 2014). Yardimci *et al.* (2014) studied the effect of imidacloprid alone (170 mg/kg) or in combination with piperonyl butoxide (100 mg/kg) or menadione (25 mg/kg) for 12 and 24 hr. The liver and kidney were evaluated for GPx, GST, CAT, GSH, total protein content and lipid peroxidation levels. Imidacloprid displayed its pro-oxidative and neurotoxic effects predominantly in kidney of male rats after 24 hr of exposure. Co-exposure (90 min. pre-treatment) with piperonyl butoxide or menadione revealed tissue-specific effect of imidacloprid on total cholinesterase activity. Increased cholinesterase activity in kidney could be an adaptive responsive to imidacloprid-induced oxidative stress. In the male rat liver, co-exposure with piperonyl butoxide or menadione caused pro-oxidative effects, while no such effects were observed with Imidacloprid alone or menadione alone.

CHAPTER – III

MATERIALS AND METHODS

3.1 Experimental animals

The study on effects of imidacloprid and arsenic were conducted on healthy male wistar rats weighing 180 to 200 gm, which were procured from Indian Institute of Integrative Medicine (IIIM), CSIR Lab, Jammu. The animals were provided standard pellet ration and clean drinking water *ad-libitum*. All the animals were maintained under standard managemental conditions. A daily cycle of 12 hr of light and 12 hr of darkness was provided to animals. Prior to start of experiment, the rats were acclimatized in the laboratory conditions for a period of 15 days. All the experimental animals were kept under constant observation during the entire period of study.

3.2 Insecticide used: Imidacloprid (17.8% SL) used in the present study was commercially obtained from Mahindra and Mahindra Ltd. Agribusiness, Mumbai, India.

3.3 Chemicals used: All the chemicals used in the study were of extra pure quality and purchased from Hi-media, S. D. Fine Chem. Pvt. Ltd., Qualigens Chem. (Mumbai) and Merck (Mumbai). Diagnostic kits of Erba Mannheim and Recon brand were used for estimating different blood biochemical parameters and were supplied by Transasia Bio- Medicals Ltd, Solan (H.P) and Recon diagnostics Pvt. Ltd, Vadodara respectively.

3.4 Plan of Work: In the present investigation, subacute toxicity of imidacloprid and arsenic were studied for biochemical alterations and antioxidant stress in wistar rats. Rats of 180-200 gm body weight were divided into eight groups of six rats each and were subjected to various daily oral treatment regimens for 28 days. Group I served as control receiving only distilled water orally, Group II received imidacloprid at the rate of 16.9 mg/kg b. wt orally, Group III, IV and V received arsenic at the rate of 50,

100 and 150 ppb respectively in distilled water. Group VI, VII and VIII, V received arsenic at the rate of 50, 100 and 150 ppb respectively in distilled water along with imidacloprid at the rate of 16.9 mg/kg b. wt orally. The detailed experimental design is presented here:

Groups	Treatment	Dose rate	Route of administration (For 28 days)
I	Control	-	Only distilled water
II	Imidacloprid	16.9 mg/kg	Oral gavage
III	Arsenic	50 µg/L	Daily in drinking water
IV	Arsenic	100 µg/L	Daily in drinking water
V	Arsenic	150 µg/L	Daily in drinking water
VI	Imidacloprid + Arsenic	16.9 mg/kg + 50 µg/L	Oral gavage + Arsenic daily in drinking water
VII	Imidacloprid + Arsenic	16.9 mg/kg + 100 µg/L	Oral gavage + Arsenic daily in drinking water
VIII	Imidacloprid + Arsenic	16.9 mg/kg + 150 µg/L	Oral gavage +Arsenic daily in drinking water

3.5 Collection and Processing of samples

At the end of 28 days of daily treatment with imidacloprid and arsenic alone and in combination, the animals were anaesthetised with diethyl ether. Blood samples were collected from heart directly by cardiac puncture using capillary tubes containing heparin @ 10 IU/ml of blood. Immediately after collection of blood, samples were centrifuged at 3000 rpm for 15 min and the plasma was harvested in clean sterile glass test tubes and was immediately stored under refrigerator conditions (-4^{o}C) pending biochemical analysis and antioxidant parameters on the same day.

After blood collection, the animals of all groups were killed by cervical dislocation. The organs were examined for any gross abnormality. Organs like liver, kidney and testes were removed, cleaned free from extraneous material, 1 gm of tissue was taken in 10 ml ice-cold 0.1M potassium phosphate buffer (pH 7.4) and its homogenate was

prepared using tissue homogeniser. The homogenates were centrifuged at 4000 rpm for 10-15 minutes and the supernatant was collected and used for determination of activities of enzymatic and non-enzymatic components of oxidative stress.

The red blood cells were washed with normal saline solution thrice before preparing the RBC lysate. Erythrocyte sediment obtained after harvesting of plasma was diluted with normal saline solution in the ratio of 1:1 on v/v basis and normal saline was gently mixed with erythrocyte sediment. The diluted erythrocytes were centrifuged for 10 min. After centrifugation, the supernatant was discarded along with buffy coat and again NSS was added to the RBC on v/v basis, mixed gently and centrifuged. This process was repeated thrice. After final washing, 1% hemolysate (100 µl washed RBC + 9.9 ml 0.1M PBS) in phosphate buffer solution (pH 7.4) were prepared. 1 percent lysate was used for the estimation of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S- transferase (GST), glutathione reductase (GR) and lipid peroxidation (MDA).

3.6 Determination of blood biochemical parameters:

Standard kits procured from Recon diagnostics Pvt. Ltd. and Transasia Bio-Medicals were used for estimation of biochemical estimation. Plasma Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were determined by using standard kits from Transasia Bio-Medicals. The principle reaction of the calorimetric determination of AST and ALT activity is based on the reaction of aspartate or alanine with α-ketoglutarate to form oxaloacetate or pyruvate respectively. The oxaloacetate or pyruvate formed was measured by monitoring the concentration of oxaloacetate or pyruvate hydrazone formed with 2,4- dinitrophenyl hydrazine. Plasma Alkaline phosphatase (ALP) and Acid phosphatase (ACP) activity was measured at 405 nm by the formation of para-nitrophenol from para-nitrophenyl phosphate as substrate. Urea in blood reacts with water in presence of urease and forms ammonia which again reacts with α-ketoglutarate and NADH in presence of glutarate dehyrogenase to form glutamate and NAD which was measured spectrophotometrically at 500-520 nm. Plasma Creatinine reacts with alkaline picrate to produce an orange yellow colour, the absorbance is directly related to creatinine

concentration and was measured spectrophotometrically at 500-520 nm. Total protein was estimated by Biuret method, in which plasma forms a blue coloured complex when treated with cupric ions in alkaline medium. The intensity of the blue colour is proportional to the protein concentration.

3.7 Oxidative stress parameters: Oxidative stress parameters were estimated in blood and different tissue samples of control and treatment groups of experimental animals.

3.7.1 Glutathione peroxidase (GPx): The activity of glutathione peroxidase in erythrocytes was assayed by the method of Hafeman *et al.* (1974).

Reagents

1. 20 mM Glutathione (9.2 mg GSH dissolved in 15 ml of water).
2. 0.4 M Sodium phosphate buffer (pH 7), containing 4 x 10^{-4} M EDTA.
3. 0.01M Sodium azide**:** 65 mg sodium azide was added to 100 ml of distilled water.
4. 1.2 mM Hydrogen peroxide**:** 0.012 ml 30 per cent H_2O_2 was added to 100 ml of distilled water.
5. 0.4 M Disodium hydrogen phosphate (Na_2HPO_4)**:** 7.12 g Na_2HPO_4 was dissolved in 100 ml distilled water.
6. Metaphosphoric acid precipitating solution**:** 1.67 g m-phosphoric acid, 0.2 g EDTA and 30 g NaCl were added to 100 ml of distilled water.
7. DTNB reagent**:** 40 mg 5-5-dithiobis (2-nitrobenzoic acid) was dissolved in 100 ml of trisodium citrate solution.

Procedure

To 0.1 ml erythrocyte lysate or 10 percent tissue supernatant, 1 ml of GSH, 1 ml of phosphate buffer and 0.5 ml of sodium azide were added successively and volume was made to 4 ml with distilled water. After each addition the contents were thoroughly mixed. After five minute of pre-incubation 1 ml of H_2O_2 (pre-warmed to 37°C) was added. After 1 min interval, 1 ml of aliquots of incubation mixture was removed to which 4 ml of m-phosphoric acid precipitation solution was added. Glutathione in protein free

filtrate was determined by mixing 2 ml of filtrate with 2 ml of Na_2HPO_4 and 1 ml of DTNB reagent and OD was recorded at 412 nm within two minutes after mixing. The zero time GSH concentration was determined in the same manner using an aliquot from a sample treated similarly but contained water instead of H_2O_2.

Activity of GPx= 10 log Co / C

Co = concentration of GSH at zero time.

C = concentration of GSH after one min incubations

3.7.2 Assay of catalase (CAT): The activity of catalase in erythrocyte lysate and tissue homogenate was determined according to the method described by Aebi (1983).

Reagents: 50 mM Phosphate buffer, pH 7.0 and Hydrogen peroxide, 30 mM: 0.34 ml of 30% H_2O_2 was diluted with buffer. The optical density of diluted H_2O_2 at 240 nm should be around 1.5. Buffered H_2O_2 solution was always prepared fresh.

Procedure:

Test	**Blank**
2ml. Phosphate buffer ⇩ Add 20µl sample ⇩ Add 1ml H_2O_2 ⇩ Read absorbance at 240 nm every 10 seconds interval for 1min. (There is decrease in absorbance)	2ml. Phosphate buffer ⇩ Set zero base

The results were expressed as µmol of H_2O_2 decomposed/min/mg of protein or g tissue using 36 as molar extinction coefficient of H_2O_2

Calculation

Catalase (µmol H_2O_2 utilized/ min/ mg of protein) =

$$\frac{\Delta OD/time}{0.67} \times \frac{\text{total volume of reaction mixture}}{\text{amount of sample taken}} \times \frac{1}{\text{mg of protein in 0.01 ml}}$$

3.7.3 Determination of blood glutathione: The blood glutathione (GSH) was determined by the method of Beutler (1975).

Reagents: Precipitating solution: Dissolve 1.67 g glacial metaphosphoric acid, 0.2 g disodium salt of EDTA and 30 g sodium chloride in 100 ml of distilled water. This solution is stable for approximately 3 weeks at 4^0C. A fine precipitate, which may form, probably consists of EDTA and does not interfere in test. Phosphate solution (0.3M $Na_2HPO_4.2H_2O$): Dissolve 13.35 g disodium hydrogen phosphate dihydrate in 250 ml distilled of water. DTNB reagent: 40 mg of 5-5'-dithiobis-(2-nitrobenzoic acid) in 100 ml of 1% sodium citrate. The DTNB reagent is stable for at least 3 months at 4^0C. Standard GSH solution: 60 mg of reduced glutathione was dissolved in 100 ml distilled water. The solution is stable for 4 weeks at 4^0C.

Procedure:

Test	Blank
0.2ml. whole blood ⇩ Add 1.8ml Distilled H_2O ⇩ Add 3ml.Precipitating solution (Stand for 5 min.) ⇩ Centrifuge at 3000 rpm. For 15 min. ⇩ Take 1ml.supernant ⇩ Add 4ml. phosphate solution ⇩ Add 0.5ml. DTNB (At the time of recording) ⇩ O.D.at 412 nm.	2ml. whole blood ⇩ Add 3ml Precipitating solution (Stand for 5 min.) ⇩ Centrifuge at 3000 rpm. For 15 min. ⇩ Take 1ml.supernant ⇩ Add 4ml. phosphate solution ⇩ Add 0.5ml. DTNB (At the time of recording) ⇩ Set zero base

A standard curve was prepared using different concentrations of glutathione in distilled water.

3.7.4 Assay of superoxide dismutase (SOD): The activity of superoxide dismutase (SOD) in erythrocyte lysate and tissue homogenate was determined by the method of Marklund and Marklund (1974).

Reagents: Pyrogallol, 0.6 mM: Dissolve 76 mg of pyrogallol in 100 ml of water, store in brown bottle. The solution was prepared fresh prior to use. EDTA, 6mM: Dissolve 223 mg EDTA disodium salt in 100 ml distilled water. Tris-HCl buffer 100 mM: Dissolve 1.21 g Tris in 80 ml of distilled water. Adjust pH to 8.2 with 10 mM HCl and make volume to 100 ml.

Procedure:

	Control	**Test**	**Blank**
Tris HCl buffer	1.5ml.	1.5ml.	1.5ml.
EDTA	0.5ml.	0.5ml.	0.5ml.
Pyrogallol	1ml.	1ml.	___
Enzyme preparation (1 % lysate or tissue homogenate)	___	20µl	___

Read absorbance at 420 nm. Every 30 seconds up to 4 min. The unit of enzyme activity is defined as the amount of enzyme causing 50% inhibition of auto oxidation of pyrogallol observed in control.

3.7.5 Assay of glutathione-S-transferase (GST): The activity of glutathione-S-transferase (GST) in erythrocyte lysate and tissue homogenate was determined by the method of Habig *et al.* (1974).

Reagents: Potassium phosphate buffer, 0.3 M, pH 6.5 GSH, 30 mM: - 46 mg of reduced glutathione was dissolved in 5 ml of distilled water. CDNB solution, 30 mM: - 30 mg of 1-chloro-2, 4 dinitrobenzene in 5 ml of 95% ethyl alcohol.

Procedure:

Test	Blank
2.8ml Phosphate buffer ⇩ 0.1ml.GSH ⇩ Add 20µl sample ⇩ 0.1ml CDNB solution ⇩ Read absorbance at 340 nm every 30 seconds interval for 3min.	Take Phosphate buffer in the cuvette ⇩ Set zero base

The unit of enzyme activity is defined as the amount of enzyme that catalyzes the formation of 1 µmol conjugate of GSH and CDNB per min per mg of Hb.

3.7.6 Glutathione reductase (GR): The assay of glutathione reductase (GR) was performed according to the method described by Carlberg and Mannervik (1985).

Reagents

1. Potassium phosphate buffer, 0.2 M, pH 7.0, containing 2 mM EDTA
2. NADPH, 2Mm in 10Mm Tris-HCl, pH 7.0
3. GSSG, 20mM in water: 62 mg of oxidized glutathione in 5ml of distilled water.

Procedure

Test	Blank
2.6ml Phosphate buffer ⇩ 0.15ml NADPH ⇩ 0.15ml GSSG ⇩ Add 0.1ml erythrocyte lysate ⇩ Read absorbance at 340nm every 30 seconds interval for 4 min.	2.6ml Phosphate buffer ⇩ Set zero base

A unit of glutathione reductase activity is defined as the amount of enzyme that catalyzes the oxidation of 1 µmol of NADPH/ min using 6.22×10^3 as molar extinction coefficient of NADPH.

3.7.7 Total thiol level: The total thiol in plasma was estimated as per the method described by Prakash *et al.* (2009). In brief, reaction mixture contained 900µl of ethylene diamine tetra acetic acid (2mM in 0.2 M Na_2HPO_4), 20µl of 5-5'-dithiobis, 2-nitrobenzoic acid (10mM in 0.2 M Na_2HPO_4) and 100µl of fresh plasma. The reaction mixture was incubated at room temperature for 5 min and the absorbance was read at 412 nm in UV visible spectrophotometer (UV-1601, Shimadzu). A reagent blank without sample and sample blank without DTNB were prepared in the same manner. Concentrations of total thiols (mM) were determined using reduced glutathione as a standard (Motchnik *et al.*, 1994).

3.7.8 Lipid peroxidation: The activity of lipid peroxidation in erythrocytes and tissues was determined according to method described by Shafiq-ur-Rehman (1984). Membrane peroxidative damage in erythrocytes or in tissues was determined in terms of MDA (malondialdehyde) production, using thiobarbituric acid (TBA). Briefly to 1ml of 1 per cent erythrocyte lysate or 10% tissue homogenate, 1 ml of 10% TCA was added. After vortexing, the mixture was centrifuged at 3000rpm for 10 min. The supernatant was collected and sediment was rejected. To 1 ml of supernatant 1 ml of 0.67 % TBA was added and it was kept in boiling water bath for 10 min. Then it was cooled and diluted with 1 ml of distilled water. The blank was made by adding all the reagents excepting 1ml of packed erythrocytes substituted with equal volume of distilled water. The absorbance was read at 535 nm in UV-visible spectrophotometer. The amount of lipid peroxidation was expressed as n mol MDA formed/ ml packed cells or was expressed as n mol MDA formed/g in tissue. The extinction coefficient (EC) of 1.56 X 10^5/M/cm was used for calculation.

3.7.9 Advanced oxidation protein product (AOPP): The advanced oxidation protein product in plasma and tissues were estimated as per the method of Witko-Sarsat *et al.*

(1996). In brief, the reaction mixture contained 160µl of sample and 640µl of PBS. Then, 40µl of potassium iodide (1.16 M) and 80µl of glacial acetic acid were added to the reaction mixture. The reaction mixture was incubated at room temperature for 5 min. and absorbance was read at 340 nm in UV visible spectrophotometer (UV-1601, Shimadzu). A reagent blank without sample was prepared in the same manner. Concentration of advanced oxidation protein product (µM) was determined using Chloramine-T as a standard.

3.8 Histopathological studies: The histopathological studies were carried out according to Drury and Wallington (1980). Briefly, a small pieces of liver, kidney and testes were immediately fixed in 10% formalin. These formalin fixed tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin (H&E), and examined under a light microscope for histopathological assessment.

3.9 Statistical analysis: Standard statistical procedures were followed and the data collected during the experiment was subjected to analysis of variance (ANOVA) using statistical software SPSS and the significance were tested using Duncan Multiple Range Test (Duncan, 1955). The significance was assayed at 5% ($P < 0.05$) levels.

CHAPTER IV

RESULTS

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Biochemical alterations and oxidative stress induced by subacute toxicity of imidacloprid (IMI) and arsenic alone and in combination was studied in male wistar rats. Male wistar rats having body weight 180-200 gm were randomly divided into eight groups of six rats each. Group I served as the control receiving only distilled water orally whereas group II received IMI @ 16.9 mg/kg b. wt. orally, group III, IV and V received arsenic @ 50, 100 and 150 ppb respectively in drinking water while group VI, VII and VIII received combined administration of IMI and arsenic @ 16.9 mg/kg + 50 ppb, 16.9 mg/kg + 100 ppb and 16.9 mg/kg + 150 ppb respectively.

4.1. Effect of repeated oral administration of imidacloprid and arsenic alone and in combination in wistar rats.

4.1.1. Biochemical alterations

4.1.1.1. Plasma aminotransferases: The effect of subacute oral exposure of imidacloprid and arsenic alone and in combination on mean values of aminotransferases (SGPT and SGOT) activity in groups are presented in table 1 and its graphical representation shown in figure 2(a).

A statistically significant ($P<0.05$) increase in SGOT levels was observed in group II (145.30±4.13) but no significant change was observed in group III (121.78±4.65) as compared to the control group (112.23±6.02). In contrast, group IV and V showed significant ($P<0.05$) increased levels of SGOT activity after repeated exposure for 28 days in comparison to control group. Group VI, VII and VIII also showed significant ($P<0.05$) increased SGOT activity but showed non-significant difference among themselves.

Significant ($P<0.05$) increase in SGPT level was observed in group II (81.66±2.28) as compared to control group (52.15±2.77). Group III, IV and V also showed significant rise

in SGPT activity but there was non-significant increase among these arsenic exposed groups. Group VI, VII and VIII also showed significant (P<0.05) increase in SGPT activity as compared to control group.

4.1.1.2. Plasma phosphatases: The effect of repeated oral exposure of imidacloprid and arsenic alone and in combination on mean plasma phosphatases (ACP and ALP) activity in all groups are presented in table 1 and its graphical representation shown in figure 2(b) and 2(c).

A statistically significant (P<0.05) increase in acid phosphatase (ACP) activity was observed in group II (71.70±3.86) as compared to control group (45.19±4.74). Arsenic exposed groups (Group III, IV and V) also showed a significant (P<0.05) rise in ACP activity after repeated exposure of 28 days. Also, significant (P<0.05) increase in ACP activity was observed in group VI, VII and VIII as compared to control as well as imidacloprid alone treated group. Whereas non-significant alterations in alkaline phosphatase (ALP) were observed in group II, III, IV and V as compared to control group (286.89±16.48). Similarly, there was non-significant increase in ALP activity within Group VI and VII but significant (P<0.05) increase was observed in group VIII (344.83±17.45) as compared to the control group.

4.1.1.3. Blood urea nitrogen (BUN) & Creatinine (CR): The effect of subacute oral exposure of imidacloprid and arsenic alone and in combination on mean BUN and CR levels in different groups are presented in table 2 and its graphical representation shown in figure 3(a) and 3(b) respectively.

Increase in BUN levels (P<0.05) was observed in group II (42.06±3.33) after repeated exposure of 28 days as compared to the control group (21.99±2.50). Also, there was significant (P<0.05) increase in BUN levels in arsenic exposed groups (Group III, IV and V). In addition, group VI, VII and VIII also showed significant (P<0.05) rise in BUN levels as compared to the control group.

A statistically significant (P<0.05) increase in CR levels were observed in imidacloprid exposed group (0.82±0.05) as compared to the control group (0.53±0.06). There was significant (P<0.05) increase in CR levels in arsenic-exposed groups (group III, IV and V) after repeated oral administration. Also, a significant (P<0.05) increase in CR levels were observed in groups VI, VII and VIII as compared to control group.

4.1.1.4. Total Plasma Proteins (TPP) & plasma albumin: The effect of repeated oral administration of imidacloprid and arsenic alone and in combination on mean total plasma proteins and plasma albumin concentration are presented in table 3 and its graphical representation shown in figure 4(a).

A statistically non-significant rise in plasma protein levels was observed in group II (6.76±0.25) but there was non-significant decrease in plasma proteins level in group III (5.97±0.15) after repeated exposure of arsenic as compared to control group (6.49±0.20). In contrast, a significant ($P<0.05$) fall in total protein levels were observed in group IV and V. Also, there was non-significant fall in total plasma proteins levels within group VI and VII whereas Group VIII (5.73±0.15) showed significant ($P<0.05$) decrease in total plasma proteins levels as compared to the control group.

Non-significant increase was observed in group II (4.50±0.24) while there was non-significant fall in plasma albumin levels in group III (3.76±0.26) as compared to control group (4.25±0.22). But there was significant ($P<0.05$) fall in plasma albumin levels in groups IV and V as compared to control group. Similarly, there was non-significant fall in albumin levels in group VI and VII but there was significant ($P<0.05$) decrease in group VIII (3.43±0.21) as compared to control group.

4.1.1.5. Plasma globulin and A/G (Albumin/Globulin) ratio: Subacute oral exposure of imidacloprid and arsenic alone and in combination on mean plasma globulin and A/G ratio in treated groups are shown in table 3 and its graphical representation shown in figure 4(b).

A non-significant increase was observed in group II (2.26±0.11) while arsenic-exposed groups (Group III, IV and V) showed non-significant decrease in plasma globulin levels. Also, there was non-significant increase in globulin levels group VI, VII and VIII as compared to control group. Non-significant increase was observed in group II (2.02±0.16) but there was non-significant decrease in plasma AG ratio in arsenic-exposed groups (Group III, IV and V). Similarly, there was no significant difference in group VI, VII and VIII as compared to control group.

Table 1: Effect of repeated oral administrations of IMI and arsenic alone and in combination on hepatic biomarkers in wistar rats.

Groups	SGOT	SGPT	ACP	ALP
I. Control	$112.23^{a}\pm6.02$	$52.15^{a}\pm2.77$	$45.19^{a}\pm4.74$	$286.89^{a}\pm16.48$
II. Imidacloprid(IMI)	$145.30^{c}\pm4.13$	$81.66^{cd}\pm2.28$	$71.70^{bc}\pm3.86$	$317.94^{ab}\pm15.90$
III. Arsenic (50 ppb)	$121.78^{ab}\pm4.65$	$65.76^{b}\pm4.47$	$64.67^{b}\pm3.05$	$295.26^{ab}\pm17.67$
IV. Arsenic (100 ppb)	$126.62^{b}\pm5.23$	$69.86^{b}\pm4.57$	$70.28^{bc}\pm3.40$	$307.99^{ab}\pm15.97$
V. Arsenic (150 ppb)	$132.74^{b}\pm4.09$	$73.67^{bc}\pm3.42$	$76.94^{bc}\pm6.01$	$313.02^{ab}\pm18.34$
VI. IMI + As (50 ppb)	$148.22^{c}\pm3.17$	$88.17^{de}\pm3.24$	$80.11^{cd}\pm6.41$	$329.58^{ab}\pm19.42$
VII. IMI + As (100ppb)	$149.05^{c}\pm3.93$	$93.16^{ef}\pm4.07$	$83.99^{cd}\pm5.11$	$338.41^{ab}\pm17.52$
VIII. IMI + As (150ppb)	$156.93^{c}\pm2.33$	$100.13^{f}\pm2.51$	$91.40^{d}\pm3.01$	$344.83^{b}\pm17.45$

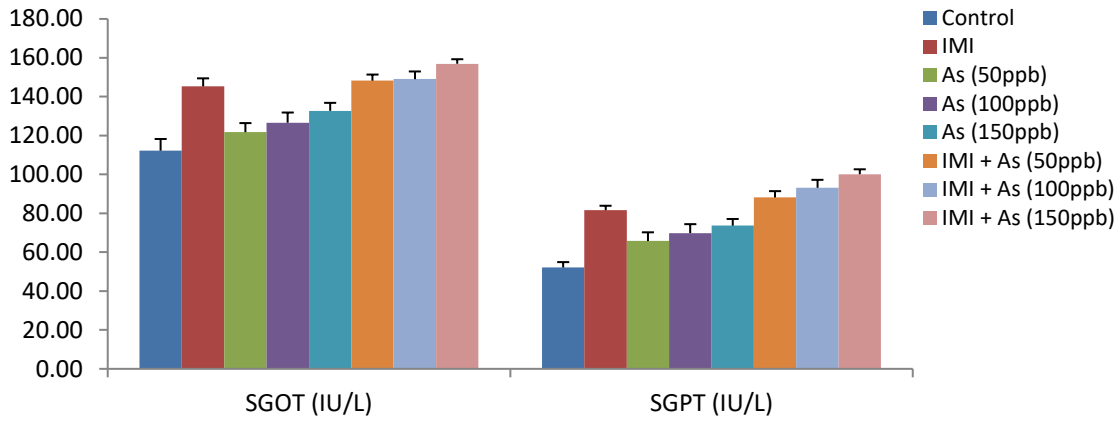
Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscript (a,b,c,d,e,f) in a column are statistically different from one another at 5% level of significance.

Table 2: Effect of repeated oral administrations of IMI and arsenic alone and in combination on renal biomarkers in wistar rats.

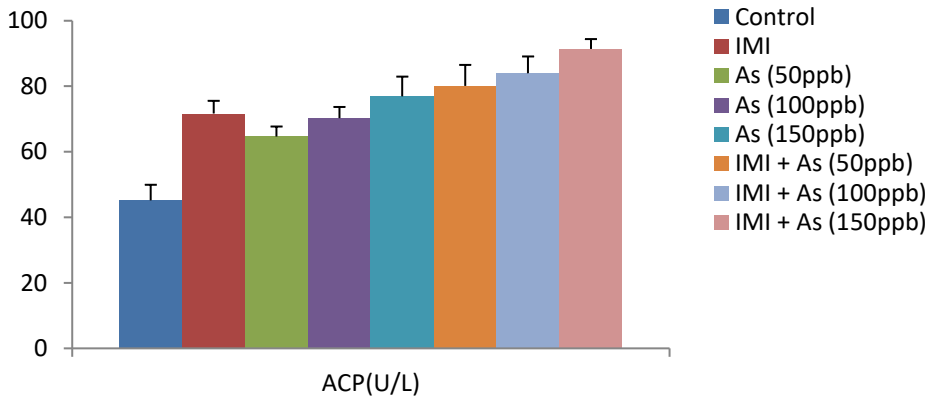
Groups	BUN	Creatinine (CR)
I. Control	$21.99^{a}\pm2.50$	$0.53^{a}\pm0.06$
II. Imidacloprid(IMI)	$42.06^{bc}\pm3.33$	$0.82^{b}\pm0.05$
III. Arsenic (50 ppb)	$34.33^{b}\pm2.31$	$0.79^{b}\pm0.08$
IV. Arsenic (100 ppb)	$43.61^{c}\pm2.39$	$0.91^{b}\pm0.04$
V. Arsenic (150 ppb)	$51.94^{d}\pm3.02$	$0.97^{bc}\pm0.06$
VI. IMI + As (50 ppb)	$62.11^{e}\pm3.02$	$1.03^{bc}\pm0.09$
VII. IMI + As (100ppb)	$75.01^{f}\pm2.81$	$1.19^{c}\pm0.09$
VIII. IMI + As (150ppb)	$85.37^{g}\pm3.04$	$1.53^{d}\pm0.13$

Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscript (a,b,c,d,e,f,g) in a column are statistically different from one another at 5% level of significance.

(a).



(b).



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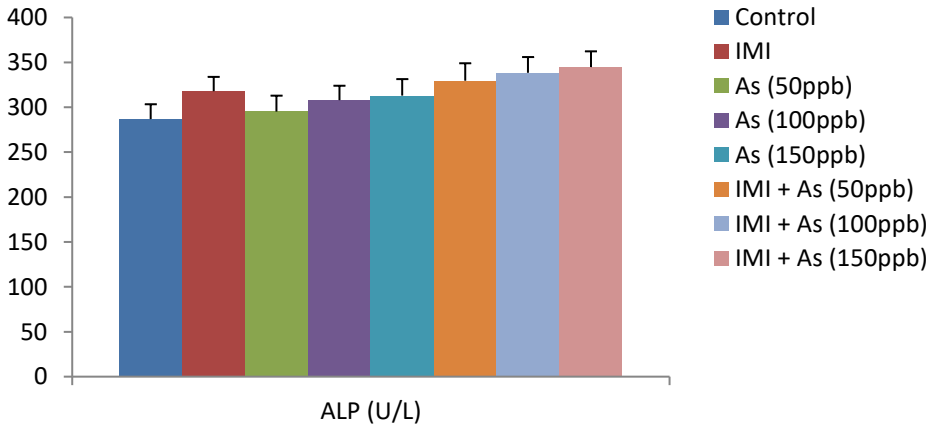
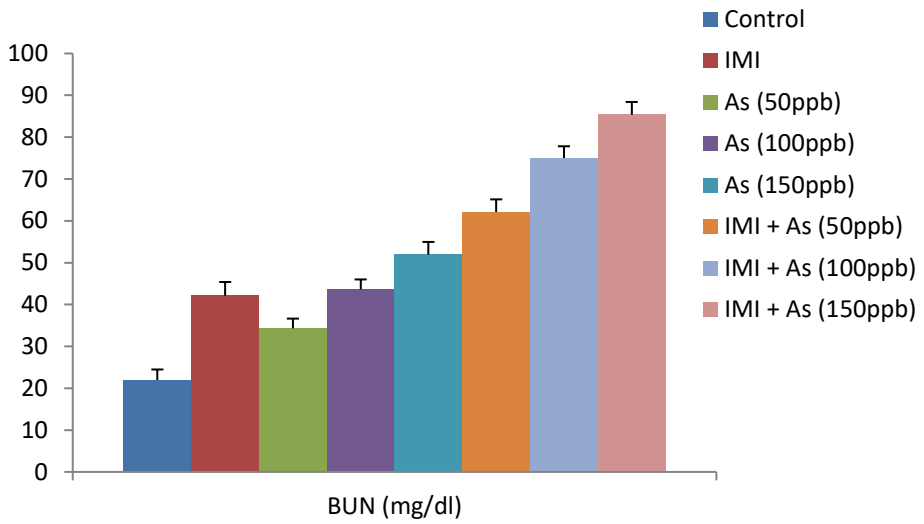


Figure 2. Effect of repeated oral administrations of IMI and arsenic alone and in combination on plasma (a) SGOT and SGPT (b) ACP and (c) ALP activity (U/L) in wistar rats.

(a).



(b).

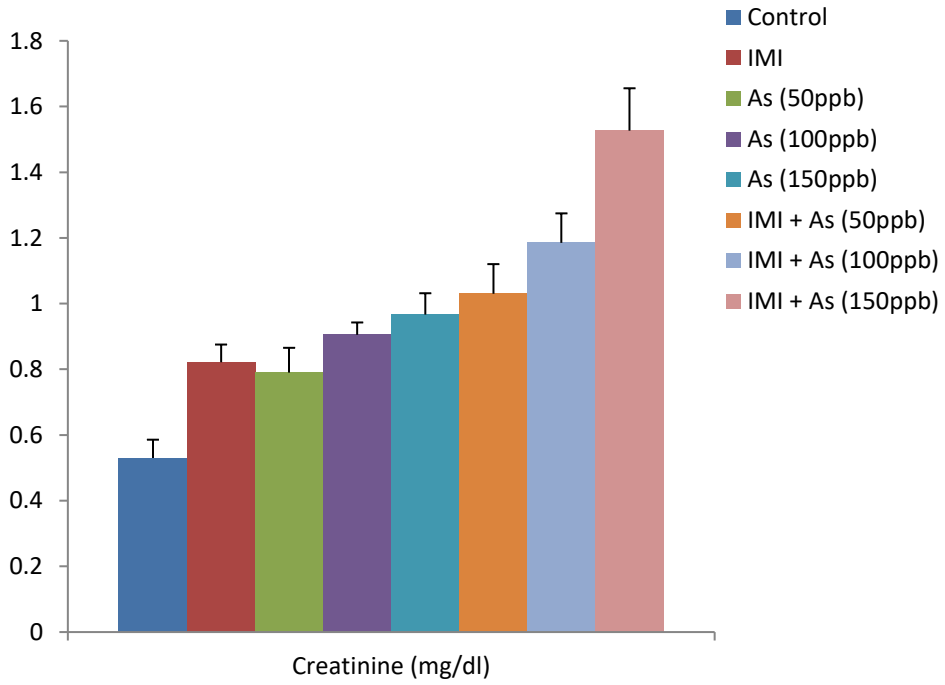


Figure 3. Effect of repeated oral administrations of IMI and arsenic alone and in combination on (a) Blood urea nitrogen (BUN) and (b) plasma creatinine (CR) levels (mg/dl) in wistar rats.

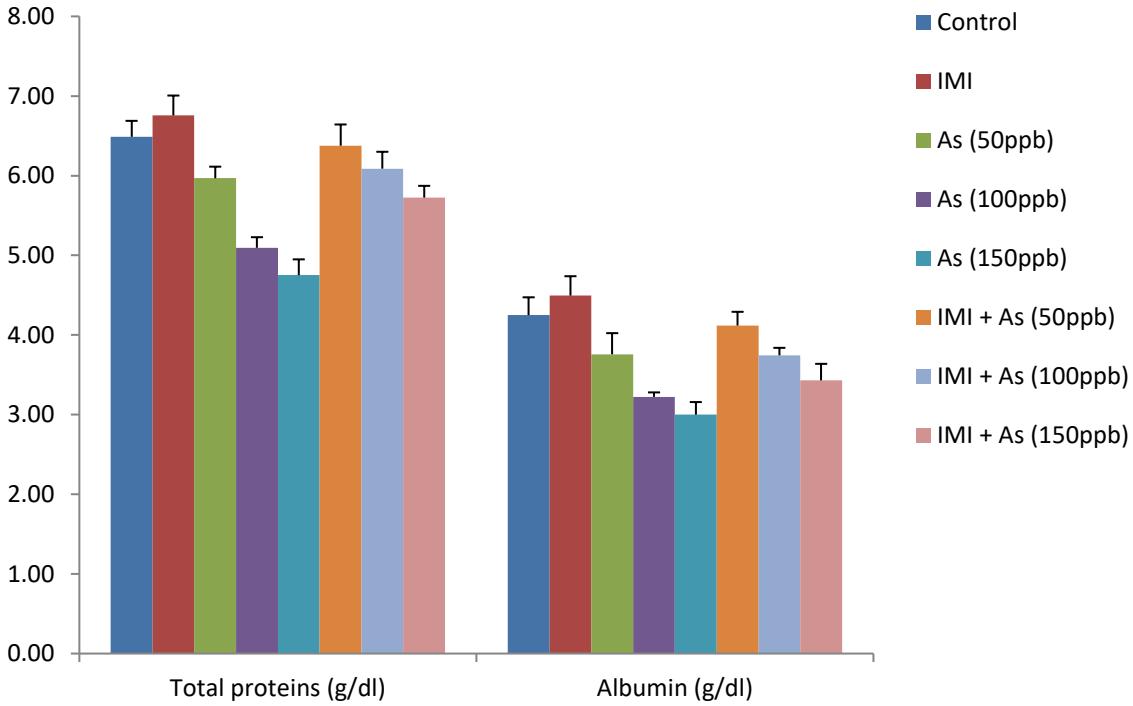
Table 3: Effect of repeated oral administrations of IMI and arsenic alone and in combination on plasma protein profile in wistar rats.

Groups	Total Proteins	Albumin	Globulin	A/G ratio
I. Control	6.49^{cd}±0.20	4.25^{cd}±0.22	2.24^{ab}±0.16	1.97^{a}±0.23
II. Imidacloprid(IMI)	6.76^{d}±0.25	4.50^{d}±0.24	2.26^{ab}±0.11	2.02^{a}±0.16
III. Arsenic (50 ppb)	5.97^{bc}±0.15	3.76^{bc}±0.26	2.21^{ab}±0.22	1.85^{a}±0.33
IV. Arsenic (100 ppb)	5.10^{a}±0.13	3.22^{ab}±0.06	1.87^{ab}±0.17	1.80^{a}±0.18
V. Arsenic (150 ppb)	4.75^{a}±0.20	3.00^{a}±0.16	1.75^{a}±0.14	1.77^{a}±0.16
VI. IMI + As (50 ppb)	6.38^{cd}±0.27	4.12^{cd}±0.17	2.26^{ab}±0.14	1.85^{a}±0.11
VII. IMI + As (100ppb)	6.09^{bc}±0.21	3.75^{bc}±0.09	2.34^{b}±0.23	1.73^{a}±0.27
VIII. IMI + As (150ppb)	5.73^{b}±0.15	3.43^{ab}±0.21	2.29^{ab}±0.20	1.60^{a}±0.27

Values are given as mean ± SE of 6 animals unless otherwise stated.

Values having different superscript (a,b,c,d) in a column are statistically different from one another at 5% level of significance.

(a).



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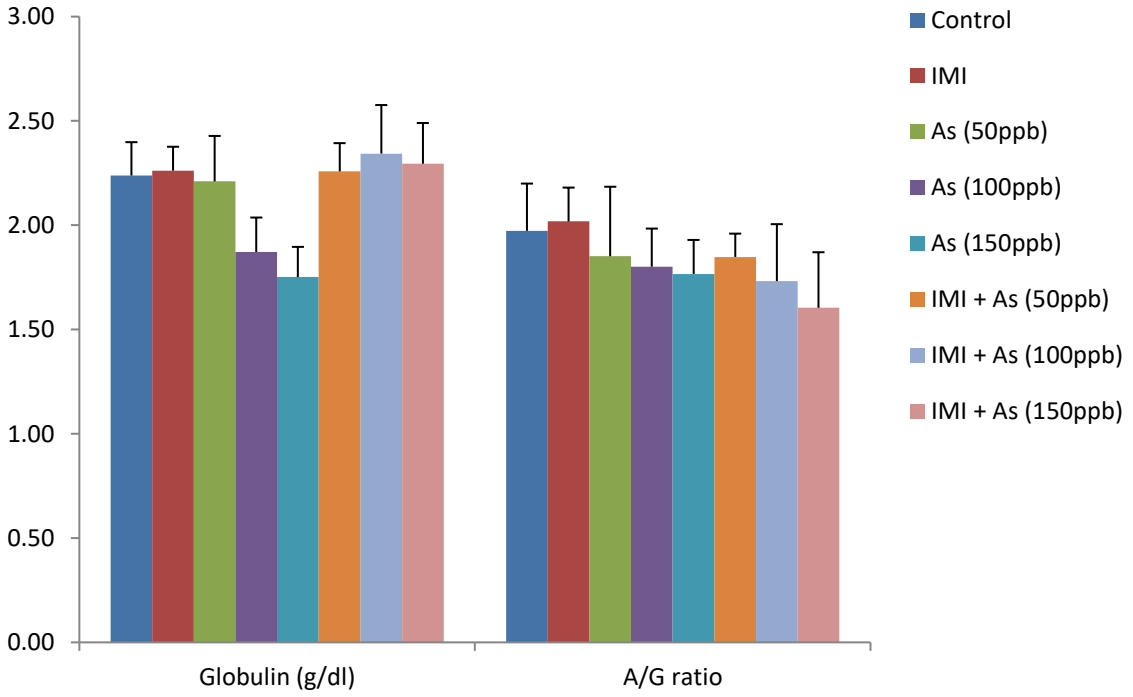


Figure 4. Effect of repeated oral administrations of IMI and arsenic alone an in combination on plasma (a) proteins and albumin (g/dl) (b) globulin (g/dl) and A/G ratio in wistar rats.

4.1.2. Antioxidant parameters in RBC

4.1.2.1. Non-enzymatic components of antioxidant system

4.1.2.1.1. Haemoglobin (Hb): The effect of subacute oral exposure of imidacloprid and arsenic alone and in combination on mean haemoglobin level in different groups are presented in table 4 and its graphical representation shown in figure 5(a). Non significant alteration in the level of Hb was observed in all treated groups as compared to control group (13.83±1.15).

4.1.2.1.2. Total thiols (TTH): Significant ($P< 0.05$) decrease in plasma TTH level was observed in group II (0.052±0.006) as compare to the control group (0.110±0.007). Similarly, a significant fall in TTH level was recorded in group III, IV and V. In addition, there was significant ($P< 0.05$) decrease in plasma TTH level in group VI, VII and VIII after repeated oral exposure in combination as compared to control as well as imidacloprid alone treated group. The mean values of TTH in different groups following repeated oral administration of imidacloprid and arsenic alone and in combination in wistar rats are shown in table 4 and its graphical representation shown in figure 5(b).

4.1.2.1.3. Reduced Glutathione (GSH): The GSH level in different groups following repeated oral administration of imidacloprid and arsenic alone and in combination are presented in table 4 and its graphical representation shown in figure 5(c). A significant ($P<0.05$) decrease was noticed in imidacloprid-exposed group (2.86±0.16) as compared to the control group. Also, there was significant ($P<0.05$) fall in GSH level in group III, IV and V as compared to control group. Similarly, a significant ($P<0.05$) decrease was recorded in group VI, VII and VIII as compared to the control group (4.86±0.21). Combined exposure of As (150ppb) along with IMI significantly reduced GSH levels as compared to As (50ppb) along with IMI.

4.1.2.1.4. Lipid peroxidation (MDA levels): The levels of MDA (end product of lipid peroxidation) following repeated oral administration of imidacloprid and arsenic alone and in combination, are expressed as nmol MDA formed/g of Hb/hr and are presented in table 5 and its graphical representation shown in figure 6(a). A statistically significant ($P<0.05$) increase in MDA levels were observed in group II (4.36±0.26) as compared to control group (2.17±0.18). Similarly, group III, IV and V showed significant ($P<0.05$) increase in MDA levels as compared to control. Also, there was significant ($P<0.05$) rise

in MDA formed in Group VI, VII and VIII as compared to control group as well as IMI alone treated group.

4.1.2.1.5. Protein oxidation (AOPP): The alteration in the levels of protein oxidation i.e. levels of AOPP (µM) following repeated oral administration of imidacloprid and arsenic alone and in combination are presented in table 5 and its graphical representation shown in figure 6(b). A significant ($P<0.05$) increase in AOPP levels was observed in group II (78.46±2.74) but in group III (69.91±3.96), there was non-significant rise in comparison to control group (60.31±1.33). Significant ($P<0.05$) increase in AOPP levels in group IV, V, after subacute exposure of arsenic as compared to control group. Significant ($P<0.05$) increase is also observed in group VIII after subacute exposure of arsenic (150ppb) along with IMI as compared to control group.

4.1.2.2. Enzymatic antioxidant parameters

4.1.2.2.1. Glutathione-S-Transferase (GST): The activity of GST is expressed as µmol of GSH-CDNB conjugate formed/min/mg Hb. Significant ($P<0.05$) decrease in GST activity was observed in group II (0.19±0.024) also there was significant fall in group III, IV and V as compared to control group (0.43±0.037). There was significant ($P<0.05$) fall in GST activity in group VI, VII and VIII in comparison to control group as well as IMI alone administered group. The alteration in activity of GST in different groups of wistar rats are presented in table 6 and its graphical representation shown in figure 7(a).

4.1.2.2.2. Glutathione reductase (GR): The activity of GR is expressed as nmol of NADPH/min and the values of GR in different treated groups are presented in table 6 and its graphical representation shown in figure 7(a). Significant ($P<0.05$) reduction in GR activity in group II (0.514±0.039) was observed but group III (0.0804±0.094) showed non-significant decrease in comparison to control group (0.871±0.076). Significant fall in the activity of GR was observed in increased administered levels of arsenic. There was significant ($P<0.05$) decrease in blood GR activity in group IV and V as compared to control. Similarly, a significant decrease in GR activity was observed in group VI, VII and VIII in comparison to control group.

4.1.2.2.3. Glutathione peroxidase (GPx): The activity of GPx is expressed as Units/mg of Hb. The alteration in activity of GPx following repeated oral administration of imidacloprid and arsenic alone and in combination on wistar rats are presented in table 6

and its graphical representation shown in figure 7(b). A significant (P<0.05) decrease was observed in IMI-exposed group (3.37±0.34) as well as arsenic-exposed groups (Group III, IV and V) in comparison to control group (8.04±0.65). Similarly, there was significant (P<0.05) fall in GPx activity in group VI, VII and VIII as compared to control group.

4.1.2.2.4. Superoxide dismutase (SOD): The activity of SOD enzyme is expressed as Units/mg of Hb and the values of SOD in different groups following repeated oral administration of imidacloprid and arsenic alone and in combination are presented in table 7 and its graphical representation shown in figure 7(c). A significant (P<0.05) decrease in SOD activity was observed in group II after repeated exposure of IMI as compared to control. Similarly a significant (P<0.05) decrease in SOD activity was observed in different groups (III, IV and V) of arsenic as compared to control. There was significant (P<0.05) fall in SOD activity in combined exposed groups (VI, VII and VIII) as compared to control group (55.20±4.76).

4.1.2.2.5. Catalase (CAT): The mean values of CAT activity were presented in table 7 and its graphical representation shown in figure 7(c). The results on CAT activity were expressed as µmol H_2O_2 decomposed/min/mg Hb. A significant (P< 0.05) decrease in CAT activity was observed in group II (34.48±3.39) compared to control. Similarly, there was significant (P< 0.05) fall in CAT activity in group III, IV and V after repeated arsenic exposure. Also, a significant (P< 0.05) decrease was observed in CAT activity in combination groups (VI, VII and VIII) as compared to control group animals (58.70±3.09).

Table 4: Effect of repeated oral administrations of IMI and arsenic alone and in combination on haemoglobin (Hb), total thiols (TTH) and reduced glutathione (GSH) in blood of wistar rats.

Groups	Hb	TTH	GSH
I. Control	$13.83^{a}\pm1.15$	$0.110^{f}\pm0.007$	$4.86^{d}\pm0.21$
II. Imidacloprid (IMI)	$13.90^{a}\pm0.847$	$0.052^{bcd}\pm0.006$	$2.86^{c}\pm0.16$
III. Arsenic (50 ppb)	$13.97^{a}\pm0.726$	$0.083^{e}\pm0.009$	$2.61^{c}\pm0.16$
IV. Arsenic (100 ppb)	$14.13^{a}\pm1.01$	$0.067^{de}\pm0.013$	$2.51^{c}\pm0.18$
V. Arsenic (150 ppb)	$14.33^{a}\pm0.507$	$0.058^{cd}\pm0.003$	$2.45^{c}\pm0.14$
VI. IMI + As (50 ppb)	$12.74^{a}\pm0.745$	$0.042^{abc}\pm0.005$	$1.87^{b}\pm0.16$
VII. IMI + As (100ppb)	$12.53^{a}\pm0.838$	$0.031^{ab}\pm0.006$	$1.79^{ab}\pm0.14$
VIII. IMI + As (150ppb)	$12.12^{a}\pm0.900$	$0.025^{a}\pm0.006$	$1.33^{a}\pm0.12$

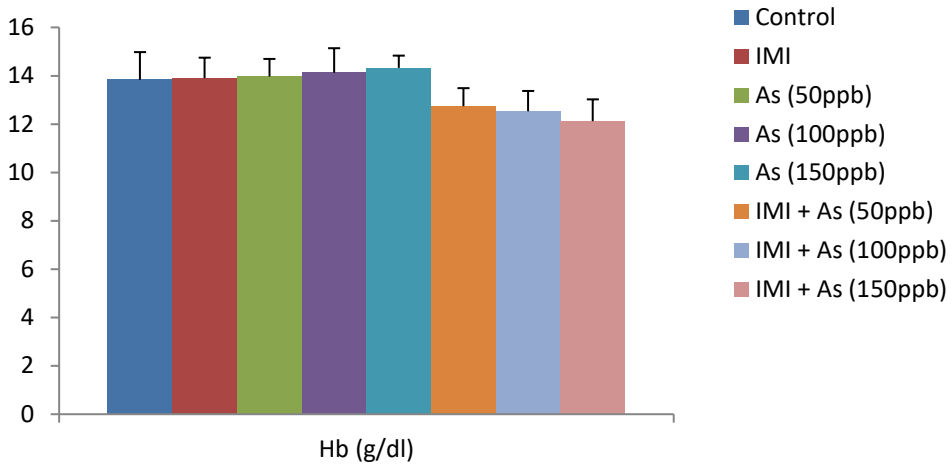
Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscript (a,b,c,d,e,f) in a column are statistically different from one another at 5% level of significance.

Table 5: Effect of repeated oral administrations of IMI and arsenic alone and in combination on levels of malondialdehyde (MDA) and advanced oxidation protein product (AOPP) in blood of wistar rats.

Groups	MDA	AOPP
I. Control	$2.17^{a}\pm0.18$	$60.31^{a}\pm1.33$
II. Imidacloprid(IMI)	$4.36^{b}\pm0.26$	$78.46^{bc}\pm2.74$
III. Arsenic (50 ppb)	$4.02^{b}\pm0.17$	$69.91^{ab}\pm3.96$
IV. Arsenic (100 ppb)	$4.99^{bc}\pm0.36$	$75.65^{bc}\pm3.63$
V. Arsenic (150 ppb)	$5.89^{cd}\pm0.24$	$79.98^{bc}\pm4.07$
VI. IMI + As (50 ppb)	$6.54^{d}\pm0.33$	$84.87^{cd}\pm4.86$
VII. IMI + As (100ppb)	$8.59^{e}\pm0.55$	$87.34^{cd}\pm4.08$
VIII. IMI + As (150ppb)	$9.24^{e}\pm0.47$	$93.74^{d}\pm3.96$

Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscript (a,b,c,d,e) in a column are statistically different from one another at 5% level of significance.

(a).



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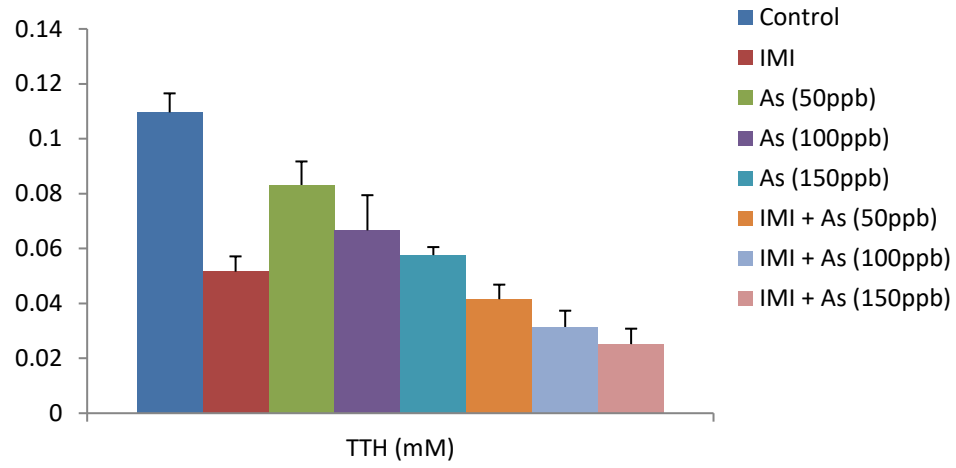

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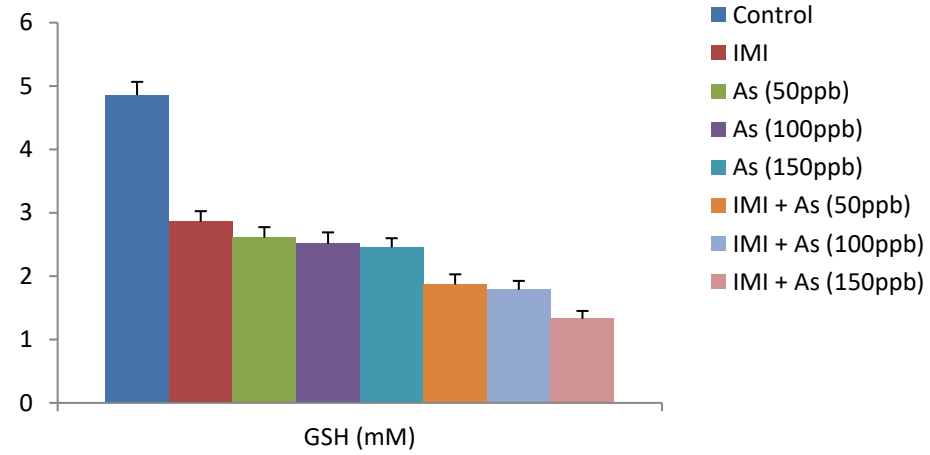
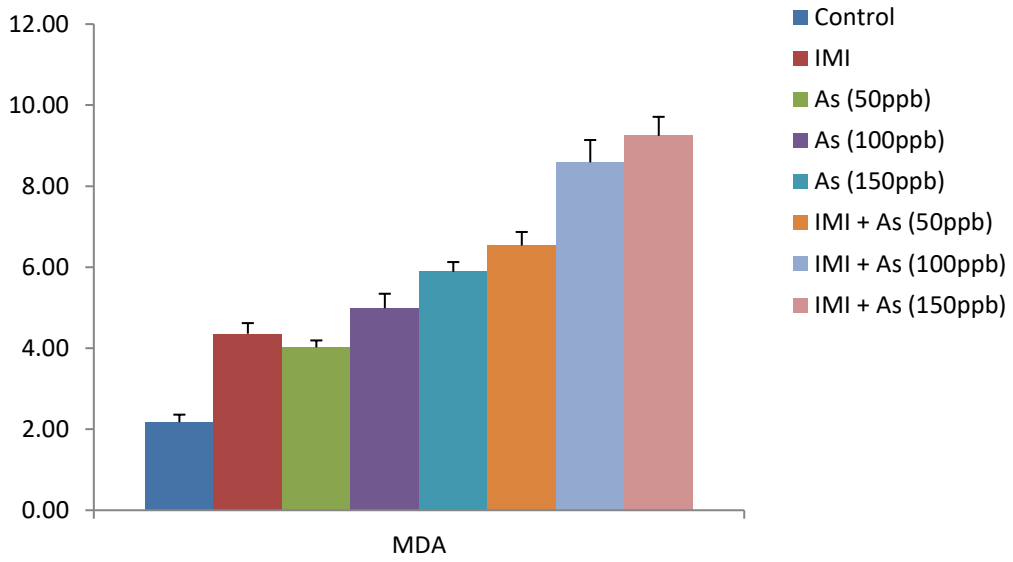


Figure 5. Effect of repeated oral administrations of IMI and arsenic alone and in combination on (a) Haemoglobin (Hb) (b) total thiols (TTH) and (c) blood glutathione (GSH) levels in blood of wistar rats.

(a).



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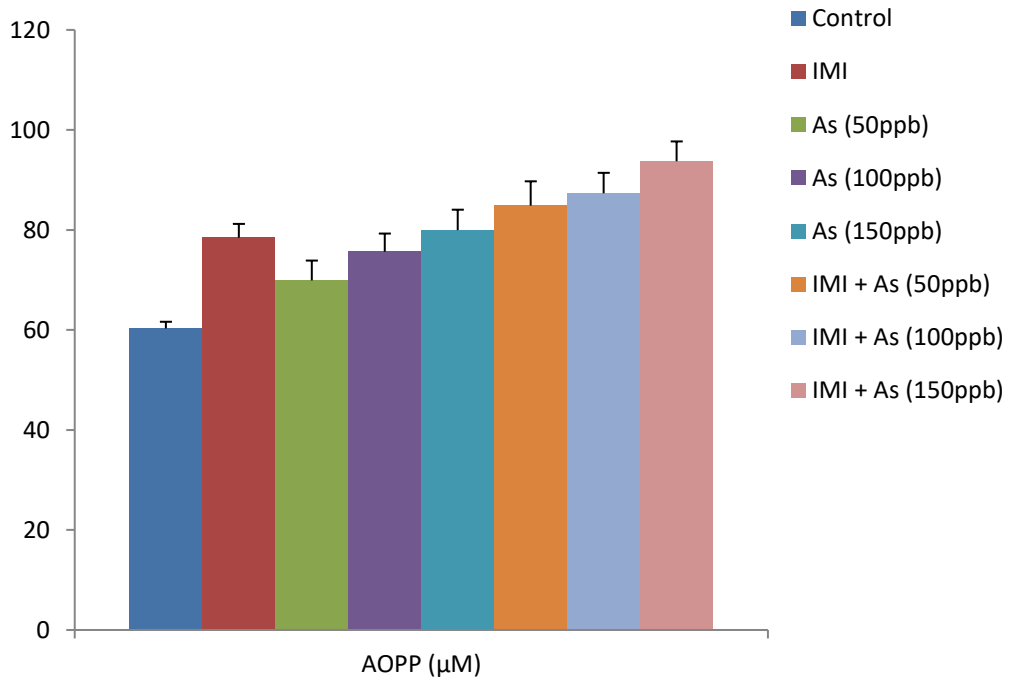


Figure 6. Effect of repeated oral administrations of IMI and arsenic alone and in combination on (a) Malondialdehyde (MDA) and (b) Protein oxidation (AOPP) levels in blood of wistar rats.

Table 6: Effect of repeated oral administrations of IMI and arsenic alone and in combination on Glutathione-s-transferase (GST), Glutathione reductase (GR) and Glutathione peroxidase (GPx) in blood of wistar rats.

Groups	GST	GR	GPx
I. Control	$0.43^{d}\pm0.037$	$0.871^{d}\pm0.076$	$8.04^{f}\pm0.65$
II. Imidacloprid(IMI)	$0.19^{c}\pm0.024$	$0.514^{bc}\pm0.039$	$3.37^{cde}\pm0.34$
III. Arsenic (50 ppb)	$0.23^{c}\pm0.027$	$0.804^{d}\pm0.094$	$4.25^{e}\pm0.47$
IV. Arsenic (100 ppb)	$0.18b^{c}\pm0.024$	$0.619^{c}\pm0.065$	$3.77^{de}\pm0.52$
V. Arsenic (150 ppb)	$0.12^{ab}\pm0.008$	$0.490^{bc}\pm0.064$	$2.63^{bcd}\pm0.17$
VI. IMI + As (50 ppb)	$0.10^{a}\pm0.014$	$0.386^{b}\pm0.036$	$2.31^{abc}\pm0.44$
VII. IMI + As (100ppb)	$0.09^{a}\pm0.012$	$0.334^{ab}\pm0.037$	$1.70^{ab}\pm0.21$
VIII. IMI + As (150ppb)	$0.06^{a}\pm0.005$	$0.194^{a}\pm0.030$	$1.19^{a}\pm0.23$

Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscript (a,b,c,d) in a column are statistically different from one another at 5% level of significance.

Table 7: Effect of repeated oral administrations of IMI and arsenic alone and in combination on Superoxide dismutase (SOD) and Catalase (CAT) in RBC of wistar rats.

Groups	SOD	CAT
I. Control	$55.20^{d}\pm4.76$	$58.70^{d}\pm3.09$
II. Imidacloprid(IMI)	$27.21^{b}\pm2.17$	$34.48^{bc}\pm3.39$
III. Arsenic (50 ppb)	$35.43^{c}\pm1.52$	$39.04^{c}\pm2.98$
IV. Arsenic (100 ppb)	$27.31^{b}\pm2.13$	$34.99^{bc}\pm3.99$
V. Arsenic (150 ppb)	$24.87^{ab}\pm1.58$	$29.72^{abc}\pm2.49$
VI. IMI + As (50 ppb)	$23.11^{ab}\pm1.84$	$32.84^{bc}\pm3.39$
VII. IMI + As (100ppb)	$21.25^{ab}\pm1.94$	$28.89^{ab}\pm2.02$
VIII. IMI + As (150ppb)	$18.49^{a}\pm1.20$	$23.33^{a}\pm1.89$

Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscript (a,b,c,d,e,f) in a column are statistically different from one another at 5% level of significance.

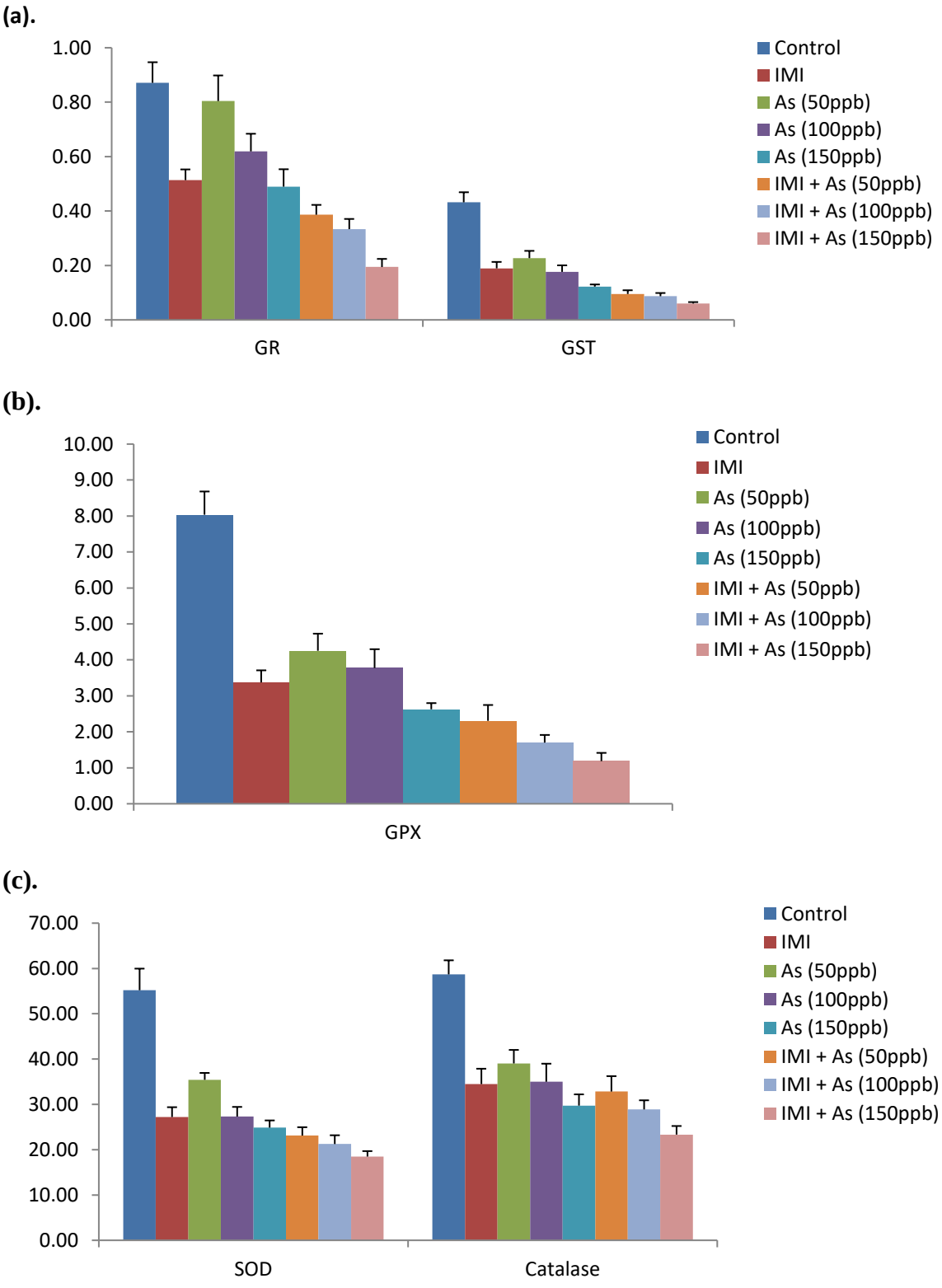


Figure 7. Effect of repeated oral administrations of IMI and arsenic alone and in combination on (a) GST and GR (b) GPx and (c) SOD and CAT activity in blood of wistar rats.

4.1.3. Antioxidant system in hepatic tissue

4.1.3.1. Non-enzymatic antioxidant parameters

4.1.3.1.1. Total thiols (TTH): The mean values of TTH in different groups following repeated oral administration of imidacloprid and arsenic alone and in combination in wistar rats are expressed as mM and are presented in table 8. A significant ($P<0.05$) reduction in mean total thiols level was observed in Group II (2.86±0.330), III, IV and V as compared to control group (5.22±0.174). Similarly, a significant ($P<0.05$) decrease in total thiols level was observed in Group VI, VII and VIII as compared to control group as well as IMI administered group.

4.1.3.1.2. Lipid peroxidation (MDA levels): A significant ($P<0.05$) increase in MDA levels were observed in IMI alone administered group (47.64±1.99) as compared to the control group (29.76±1.82). Also, there was significant ($P<0.05$) increase in MDA levels in Group III, IV and V after repeated subacute administration for 28 days. Similarly, a significant ($P<0.05$) rise in MDA levels were observed in group VI, VII and VIII as compared to control group as well as individually administered IMI and arsenic 50ppb and arsenic 100ppb group. The mean values of lipid peroxidation levels are expressed as nmol MDA/g of tissue/hr in hepatic tissue of wistar rats following repeated oral administration of IMI and arsenic alone and in combination are presented in table 8.

4.1.3.1.3. Protein oxidation (AOPP): The results on protein oxidation (AOPP) levels in hepatic tissue following repeated oral administration of imidacloprid and arsenic alone and in combination are expressed as mM and are presented in table 8. As compared to control group there was significant ($P<0.05$) increase in AOPP levels in IMI exposed group (2.05±0.083). Similarly, a significant ($P<0.05$) increase was also observed in arsenic exposed groups (Group III, IV and V) as compared to the control group (1.82±0.024). In addition, there was also significant ($P<0.05$) rise in protein oxidation products levels in group VI, VII and VIII as compared to control group.

4.1.3.2. Enzymatic antioxidant parameters

4.1.3.2.1. Glutathione-S-Transferase (GST): Mean values of GST in different treated and control group are expressed as µmol of GSH-CDNB conjugate formed /min/g of tissue and presented in table 9. A significant ($P<0.05$) reduction in GST activity was observed in group II (126.93±8.81) after repeated IMI exposure as compared to the

control group (189.85±7.72). Similarly, there was significant (P<0.05) fall in GST activity in group III, IV and V. Also, a significant (P<0.05) decrease in group VI, VII and VIII was observed as compared to control group.

4.1.3.2.2. Glutathione reductase (GR): The activity of GR in hepatic tissue is expressed as nmol of NADPH/min and the values of GR in different groups following repeated oral administration of imidacloprid and arsenic alone and in combination are presented in table 9. **S**ignificant (P<0.05) decrease in GR levels were observed in imidacloprid exposed group (29.74±2.02), similarly decrease was also observed in Group III, IV and V as compared to the control group (39.41±2.84). In addition, there was also significant fall in glutathione reductase activity in group VI, VII and VIII as compared to control group as well as IMI administered group.

4.1.3.2.3. Glutathione peroxidase (GP_X): The activity of GPx is expressed as Units/g of tissue. The alteration in activity of GPx in hepatic tissue of wistar rats following repeated oral administration of imidacloprid and arsenic alone and in combination on wistar rats are presented in table 9. A significant (P<0.05) decrease in glutathione peroxidase activity was observed in group II (29.74±2.02) as compared to the control group (39.41±2.84). There was also significant (P<0.05) reduction in group III, IV and V after repeated oral exposure of different doses of arsenic. Similarly, a significant decrease (P<0.05) in GPx activity were also observed in group VI, VII and VIII as compared to control group as well as IMI alone administered group.

4.1.3.2.4. Superoxide dismutase (SOD): The effect of repeated oral administration of imidacloprid and arsenic alone and in combination on mean SOD activity in hepatic tissue of all groups are expressed as Units/g of tissue and are presented in table 10.

Significantly (P<0.05) reduced activity of SOD was observed in group II (223.83±12.72) and similar significant reduction (P<0.05) in activity were also seen in group III, IV and V in comparison to the control group (350.77±16.61). Similarly, there was significant (P<0.05) decrease in group VI, VII and VIII as compared to control group.

4.1.3.2.5. Catalase (CAT): The effect of subacute oral administration of imidacloprid and arsenic alone and in combination on CAT activity in hepatic tissue of all groups are expressed as µmol H_2O_2 decomposed/min/g of tissue and are presented in table 10. A significant (P<0.05) fall in CAT activity were observed in group II (2839.93±58.22) as

compared to the control group (4173.89±63.89). There was also significant ($P<0.05$) decrease in Group III, IV and V in CAT activity of hepatic tissue. Similarly, a significant ($P<0.05$) reduction in CAT activity were observed in group VI, VII and VIII as compared to control.

4.1.3.3. Histopathological alterations in hepatic tissue:

The histopathological alterations in the liver on repeated oral administrations of imidacloprid and arsenic alone as well as combination in wistar rats are shown in Plate 1 and 2. In control group, there was normal histological appearance with different hepatic lobules containing a central vein and hepatocytes, lined in a cord like fashion around it, clearly delineating portal tracts with portal vein, hepatic artery, bile duct and lymphatics (Plate 1a). Liver sections of rats administered IMI and arsenic (50 ppb) appeared histologically normal with mild alterations in the normal hepatic architecture (Plate 1b and 1c). Increasing the dose of arsenic induced mild to moderate degenerative changes in liver in a dose dependent manner and also included mild congestion and accumulation of edema in the portal triad of arsenic (150 ppb) exposed group. Group VI rats revealed severely dilated and congested central veins, presence of severe vacuolar degeneration and disruption of hepatic cords, necrosis of hepatocytes, haemorrhages and mild infiltration of inflammatory cells (Plate 2a). Also, the necrosis of hepatocytes around central vein involved less number of hepatocytes whereas this pattern extends upto nearly the entire width of the lobule sparing only few hepatocytes in the periphery around the portal tracts in a dose dependent manner in group VII and VIII. In group VII, severe degeneration and coagulative type of necrosis was noticed (Plate 2b). The necrotic areas were being infiltrated by low number of mononuclear cells chiefly macrophages and lymphocytes. Affected hepatocytes had deeply eosinophilic cytoplasm and nuclear changes included pyknosis, karyorrhexis and karyolysis. Necrosis in the combination groups had a clear centrilobular pattern. This pattern increased in severity with increasing dose of arsenic in the combination groups. Necrosis of liver was very severe in group VIII when compared to any other group including group VII. Majority of the hepatocytes in liver sections in this group were necrotic and total disruption of liver parenchyma occurred (Plate 2c).

Table 8: Effect of repeated oral administrations of IMI and arsenic alone and in combination on Total thiols (TTH), Malondialdehyde (MDA) and Advanced oxidation protein product (AOPP) levels in hepatic tissue of wistar rats.

Groups	TTH	MDA	AOPP
I. Control	$5.22^{e}\pm0.174$	$29.76^{a}\pm1.82$	$1.82^{a}\pm0.024$
II. Imidacloprid (IMI)	$2.86^{cd}\pm0.330$	$47.64^{c}\pm1.99$	$2.05^{bc}\pm0.083$
III. Arsenic (50 ppb)	$3.13^{d}\pm0.440$	$39.20^{b}\pm1.62$	$1.98^{b}\pm0.024$
IV. Arsenic (100 ppb)	$2.77^{cd}\pm0.058$	$65.72^{d}\pm3.47$	$2.03^{b}\pm0.040$
V. Arsenic (150 ppb)	$2.38^{bc}\pm0.047$	$69.30^{de}\pm2.25$	$2.11^{bc}\pm0.039$
VI. IMI + As (50 ppb)	$2.07^{ab}\pm0.059$	$74.67^{e}\pm3.40$	$2.17^{cd}\pm0.033$
VII. IMI + As (100ppb)	$1.73^{a}\pm0.040$	$87.36^{f}\pm2.73$	$2.27^{de}\pm0.048$
VIII. IMI + As (150ppb)	$1.49^{a}\pm0.049$	$95.95^{g}\pm3.36$	$2.33^{e}\pm0.027$

Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscript (a,b,c,d,e,f,g) in a column are statistically different from one another at 5% level of significance.

Table 9: Effect of repeated oral administrations of IMI and arsenic alone and in combination on Glutathione-s-transferase (GST), Glutathione reductase (GR) and Glutathione peroxidase (GPx) activity in hepatic tissue of wistar rats.

Groups	GST	GR	GPx
I. Control	$189.85^{e}\pm7.72$	$39.41^{e}\pm2.84$	$52.94^{e}\pm2.20$
II. Imidacloprid(IMI)	$126.93^{cd}\pm8.81$	$29.74^{cd}\pm2.02$	$34.58^{d}\pm1.47$
III. Arsenic (50 ppb)	$141.35^{d}\pm9.93$	$31.56^{d}\pm2.56$	$37.09^{d}\pm1.67$
IV. Arsenic (100 ppb)	$137.75^{d}\pm10.77$	$27.91^{cd}\pm2.40$	$32.35^{cd}\pm1.78$
V. Arsenic (150 ppb)	$112.65^{c}\pm10.87$	$24.38^{bc}\pm2.53$	$29.21^{c}\pm1.75$
VI. IMI + As (50 ppb)	$80.23^{b}\pm5.32$	$21.46^{ab}\pm1.29$	$21.75^{b}\pm1.36$
VII. IMI + As (100ppb)	$59.35^{ab}\pm2.91$	$19.30^{ab}\pm1.06$	$17.02^{a}\pm1.43$
VIII. IMI + As (150ppb)	$41.38^{a}\pm3.91$	$15.79^{a}\pm1.08$	$12.99^{a}\pm1.06$

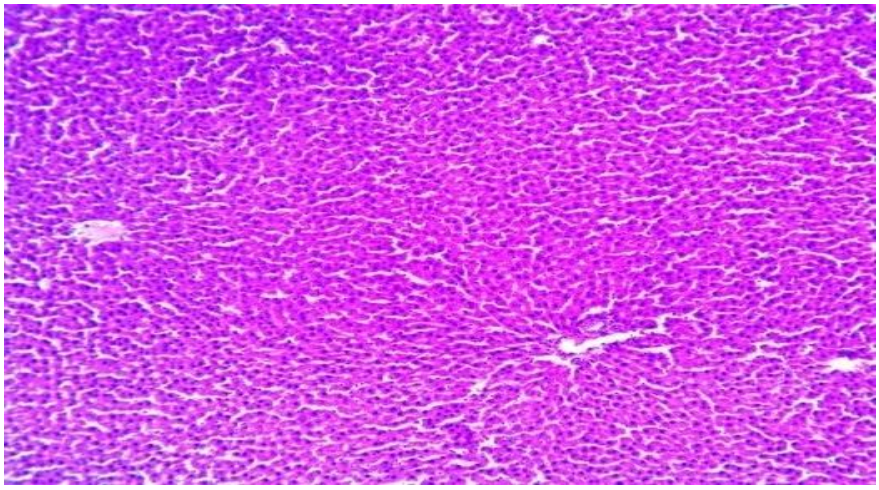
Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscript (a,b,c,d,e) in a column are statistically different from one another at 5% level of significance.

Table 10. Effect of repeated oral administrations of IMI and arsenic alone and in combination on Superoxide dismutase (SOD) and Catalase (CAT) activity in hepatic tissue of wistar rats.

Groups		SOD	CAT
I.	Control	$350.77^{f}\pm16.61$	$4173.89^{g}\pm63.89$
II.	Imidacloprid(IMI)	$223.83^{de}\pm12.72$	$2839.93^{d}\pm58.22$
III.	Arsenic (50 ppb)	$233.29^{e}\pm13.27$	$3484.71^{f}\pm69.32$
IV.	Arsenic (100 ppb)	$186.44^{cd}\pm11.50$	$3019.78^{e}\pm64.40$
V.	Arsenic (150 ppb)	$165.48^{bc}\pm11.82$	$2723.89^{d}\pm58.60$
VI.	IMI + As (50 ppb)	$149.04^{bc}\pm13.86$	$2460.83^{c}\pm58.71$
VII.	IMI + As (100ppb)	$134.25^{ab}\pm14.08$	$1788.81^{b}\pm55.14$
VIII.	IMI + As (150ppb)	$106.85^{a}\pm11.10$	$1433.58^{a}\pm59.37$

Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscript (a,b,c,d,e,f,g) in a column are statistically different from one another at 5% level of significance.

(a).



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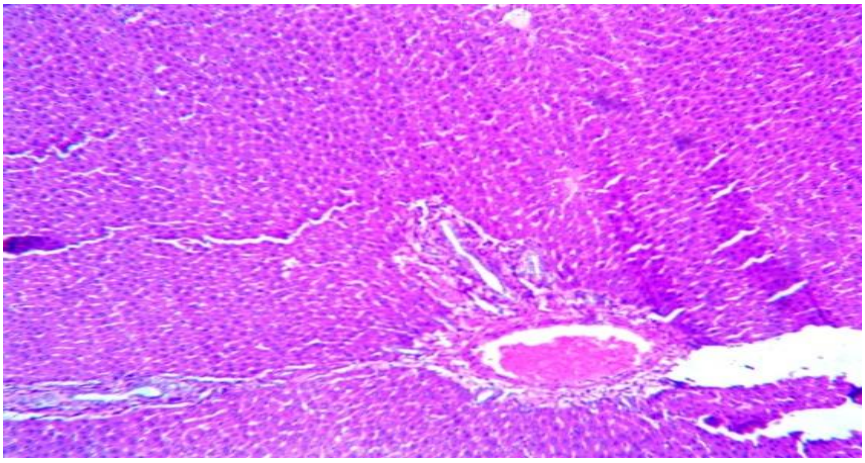

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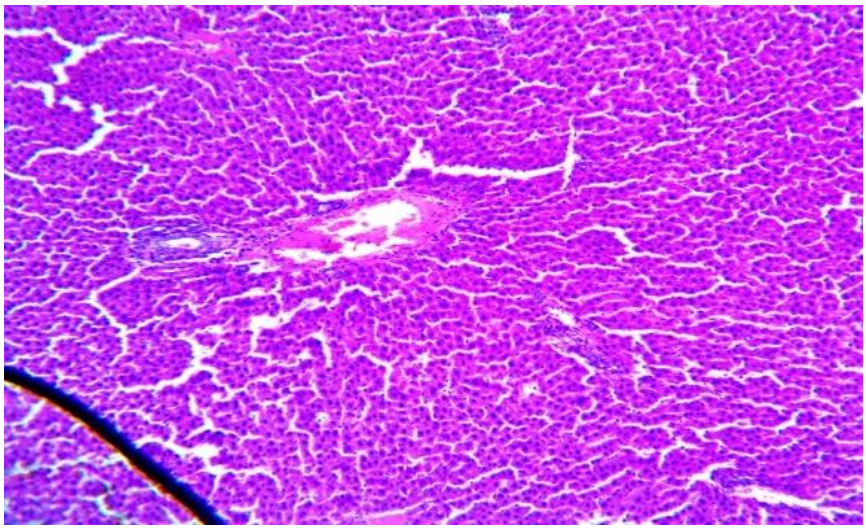
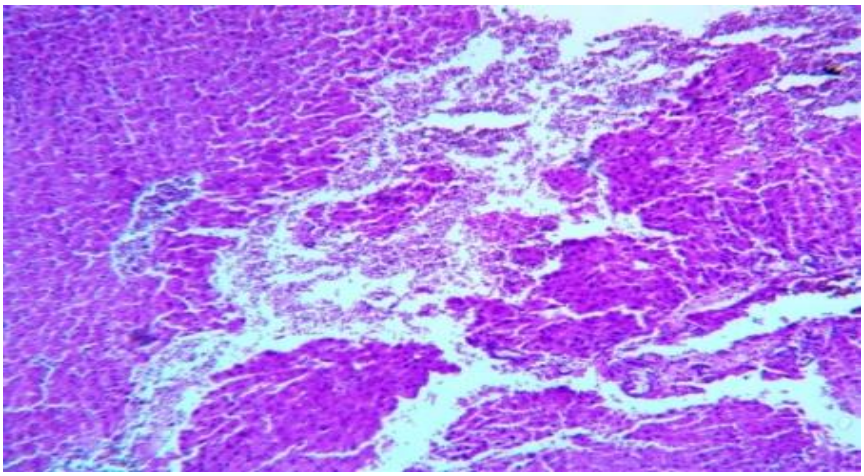


Plate 1: Photomicrograph of H & E (10X) stained sections of formalin fixed liver (a) Group I: Normal histological appearance of liver in control rats (b) Group II and III indicate mild changes in the hepatic arrangement in wistar rats.

(a).



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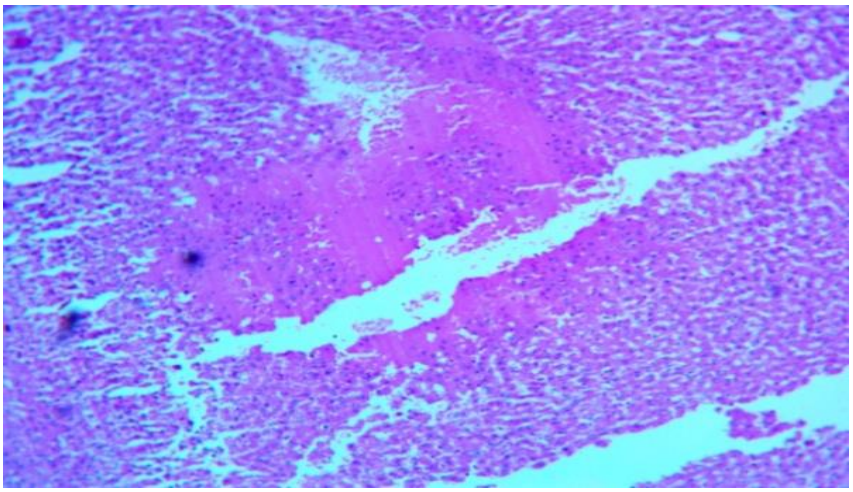

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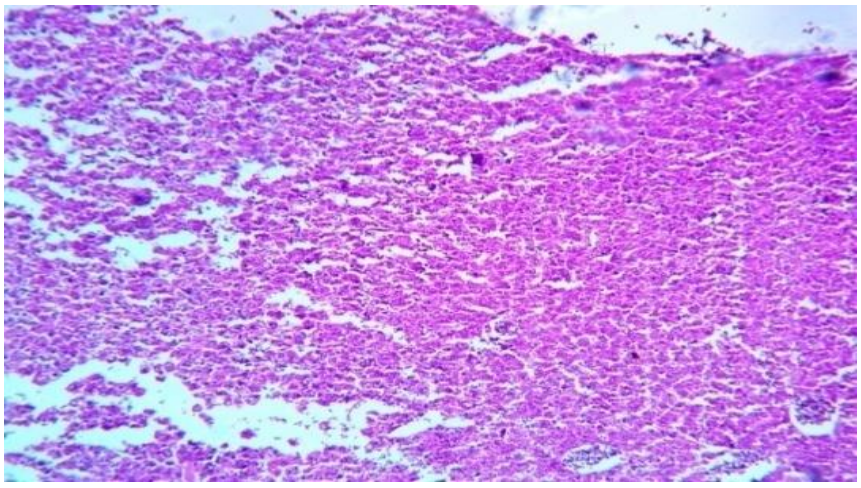

Plate 2: Photomicrograph of H & E (10X) stained sections of formalin fixed liver (a) Group VI showing haemorrhages and necrosis of hepatocytes (b) Group VII: Coagulative necrosis of hepatocytes (c) Group VIII: Severe necrosis of hepatocytes in wistar rats.

4.1.4. Antioxidant system in renal tissue

4.1.4.1. Non-enzymatic antioxidant parameters

4.1.4.1.1. Total thiols (TTH): The mean values of TTH levels in renal tissue of different groups following repeated oral administration of imidacloprid and arsenic alone and in combination in wistar rats are expressed as mM and are presented in table 11. A significant ($P<0.05$) decrease in mean total thiols level was observed in Group II (2.45±0.340) as compared to control group (3.65±0.141). There was also significant ($P< 0.05$) fall in total thiols content in group III, IV and V. Similarly, a significant ($P< 0.05$) decrease in total thiols level was observed in Group VI, VII and VIII as compared to control group.

4.1.4.1.2. Lipid peroxidation (MDA levels): A significant ($P<0.05$) increase in MDA levels were observed in group II (43.83±2.14) as compared to control group (21.65±2.45). Also, there was significant ($P< 0.05$) increase in MDA levels in Group III, IV and V after repeated subacute administration for 28 days. Similarly, a significant ($P< 0.05$) rise in MDA levels were observed in group VI, VII and VIII as compared to control group. The results on lipid peroxidation levels in renal tissue of rats following repeated oral administration of IMI and arsenic alone and in combination, are expressed as nmol MDA produced/g of tissue/hr and presented in table 11.

4.1.4.1.3. Protein oxidation (AOPP): Data presented in table 11 shows the effect of repeated oral administration of IMI and arsenic alone and in combination on product of protein oxidation (mM) levels in renal tissue of wistar rats. A significant ($P<0.05$) increase in AOPP levels were observed in imidacloprid exposed group (1.99±0.036) as compared to control group (1.60±0.046). Similarly, a significant ($P< 0.05$) increase was also observed in arsenic-exposed groups (Group III, IV and V). Co-administration of both IMI and arsenic also shows significant ($P< 0.05$) rise in AOPP level in group VI, VII and VIII as compared to control group and IMI alone administered group.

4.1.4.2. Enzymatic antioxidant parameters

4.1.4.2.1. Glutathione-S-Transferase (GST): Alterations in the levels of GST following repeated oral exposure of imidacloprid and arsenic alone and in combination in renal tissue of wistar rats are presented in table 12. GST activities are expressed as µmol of GSH-CDNB conjugate formed/min/g of tissue. A significant ($P<0.05$) decrease in GST

activity was observed in group II (42.85±4.51) after repeated imidacloprid exposure for 28 days as compared to control group (92.55±4.58). Similarly, there was significant (P< 0.05) fall in GST activity in group III, IV and V. Also, a significant (P< 0.05) decrease in group VI, VII and VIII was observed as compared to control group.

4.1.4.2.2. Glutathione reductase (GR): The activity of GR in renal tissue is expressed as nmol of NADPH/min and the values of GR in different groups following repeated oral administrations of imidacloprid and arsenic alone and in combination are presented in table 12. Significantly (P<0.05) decrease activity of GR were observed in imidacloprid exposed group (39.26±2.89) and also in different doses of arsenic administration alone as compared to the control group (56.11±3.34). In addition, there was also significant (P<0.05) fall in glutathione reductase activity in group VII and VIII as compared to control group as well IMI alone administered group.

4.1.4.2.3. Glutathione peroxidase (GPx): The GPx activity in renal tissue is expressed as Units/g of tissue. The alteration in activity of GPx in renal tissue following repeated oral administration of imidacloprid and arsenic alone and in combination on wistar rats are presented in table 12. Significant (P<0.05) decrease in glutathione peroxidase activity were observed in group II (22.51±2.26). There was also significant (P< 0.05) reduction in group III, IV and V after repeated oral administration of arsenic for 28 days. Similarly, a significant (P< 0.05) decrease in GPx activity were also observed in group VI, VII and VIII as compared to control group (45.19±3.67). Repeated co-administrations of IMI along with arsenic (100 and 150ppb) shows significant reduction in the activity of GPx as compared to IMI or arsenic (150ppb) alone administrated group as well as control group.

4.1.4.2.4. Superoxide dismutase (SOD): The effect of repeated oral administration of imidacloprid and arsenic alone and in combination on mean SOD activity (Units/g of tissue) in renal tissue of different groups are presented in table 13. SOD activity was significant (P<0.05) decrease in group II (241.92±12.81), III, IV and V in comparison to control group (378.08±14.4). Similarly, there was significant decrease (P< 0.05) in group VI, VII and VIII as compared to control as well as IMI alone administered group.

4.1.4.2.5. Catalase (CAT): The effect of subacute oral administration of imidacloprid and arsenic alone and in combination on mean catalase activity in renal tissue are expressed as µmol H_2O_2 decomposed/min/g of tissue and are presented in table 13. A

significant (P<0.05) fall in catalase activity were observed in group II (2145.15±59.20) as compared to control group (3352.99±84.27). There was also significant (P< 0.05) decrease in Group III, IV and V in catalase activity of renal tissue. Similarly, a significant (P< 0.05) fall in catalase activity was observed in group VI, VII and VIII as compared to control, IMI and arsenic alone exposed group.

4.1.4.3. Histopathological alterations in renal tissue

The architecture alterations in the renal tissue on repeated oral administrations of imidacloprid and arsenic alone as well as combination in wistar rats are shown in Plate 3 and 4. In control the histology of kidney sections were normal with usual appearance of glomeruli and tubules (Plate 3a). Rats in group II and III had no appreciable histological abnormalities in the kidney (Plate 3b and 3c). Rats in group IV and V had mild vacuolar degeneration of tubular epithelium and renal pathological changes in these groups overall were less severe than as compared to that found in group VI. Rats in group VI showed moderate inter-tubular congestion and haemorrhages along with mild degenerative changes in the tubular epithelium (Plate 4a). In group VII, degenerative changes were characterized by cytoplasmic vacuolar degeneration, necrosis and mild infiltration of phagocytic cells in the tubular epithelium (Plate 4b). Other hallmarks of progressive nephropathy were seen like the presence of hyaline casts and thickened basement membrane. Also, tubular epithelium was markedly attenuated in many areas. The severity of degenerative and necrotic changes in tubular epithelial cells were appreciably higher in group VIII as compared to all other groups. Multifocally, integrity of basement membrane of tubules was found to be disrupted and necrotized along with mild infiltration of phagocytic cells was also seen. Other significant histopathological changes in kidneys of group VIII included inter-tubular congestion, haemorrhage, edema along with degeneration and necrosis in kidney sections of rats (Plate 4c).

Table 11: Effect of repeated oral administrations of IMI and arsenic alone and in combination on Total thiols (TTH), Malondialdehyde (MDA) and Advanced oxidation protein product (AOPP) levels in renal tissue of wistar rats.

Groups		TTH	MDA	AOPP
I.	Control	$3.65^{d}\pm0.141$	$21.65^{a}\pm2.45$	$1.60^{a}\pm0.046$
II.	Imidacloprid (IMI)	$2.45^{c}\pm0.340$	$43.83^{b}\pm2.14$	$1.99^{cd}\pm0.036$
III.	Arsenic (50 ppb)	$3.16^{d}\pm0.290$	$37.82^{b}\pm1.85$	$1.84^{b}\pm0.040$
IV.	Arsenic (100 ppb)	$2.55^{c}\pm0.026$	$59.63^{c}\pm1.89$	$1.93^{bc}\pm0.031$
V.	Arsenic (150 ppb)	$2.30^{bc}\pm0.052$	$67.69^{d}\pm2.24$	$2.02^{cd}\pm0.050$
VI.	IMI + As (50 ppb)	$1.94^{bc}\pm0.120$	$74.38^{d}\pm2.36$	$2.08^{d}\pm0.041$
VII.	IMI + As (100ppb)	$1.72^{ab}\pm0.214$	$85.46^{e}\pm2.61$	$2.20^{e}\pm0.037$
VIII.	IMI + As (150ppb)	$1.32^{a}\pm0.177$	$108.52^{f}\pm3.72$	$2.24^{e}\pm0.031$

Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscript (a,b,c,d,e,f) in a column are statistically different from one another at 5% level of significance.

Table 12: Effect of repeated oral administrations of IMI and arsenic alone and in combination on Glutathione-s-transferase (GST), Glutathione reductase (GR) and Gluatathione peroxidase (GPx) activity in renal tissue of wistar rats.

Groups		GST	GR	GPx
I.	Control	$92.55^{f}\pm4.58$	$56.11^{e}\pm3.34$	$45.19^{f}\pm3.67$
II.	Imidacloprid(IMI)	$42.85^{cd}\pm4.51$	$39.26^{cd}\pm2.89$	$22.51^{cd}\pm2.26$
III.	Arsenic (50 ppb)	$61.73^{e}\pm4.34$	$44.71^{d}\pm2.17$	$32.67^{e}\pm2.09$
IV.	Arsenic (100 ppb)	$49.45^{d}\pm3.71$	$42.10^{d}\pm3.36$	$25.52^{d}\pm2.25$
V.	Arsenic (150 ppb)	$44.15^{d}\pm2.95$	$37.03^{cd}\pm2.38$	$18.30^{bc}\pm1.58$
VI.	IMI + As (50 ppb)	$33.43^{bc}\pm2.78$	$32.90^{bc}\pm2.31$	$16.46^{bc}\pm1.50$
VII.	IMI + As (100ppb)	$27.95^{ab}\pm1.57$	$25.87^{ab}\pm1.03$	$13.95^{ab}\pm1.20$
VIII.	IMI + As (150ppb)	$21.05^{a}\pm1.60$	$23.98^{a}\pm2.00$	$9.56^{a}\pm0.692$

Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscript (a,b,c,d,e,f) in a column are statistically different from one another at 5% level of significance.

Table 13: Effect of repeated oral administrations of IMI and arsenic alone and in combination on Superoxide dismutase (SOD) and Catalase (CAT) activity in renal tissue of wistar rats.

Groups		SOD	CAT
I.	Control	378.08^{e}±14.94	3352.99^{g}±84.27
II.	Imidacloprid(IMI)	241.92^{d}±12.81	2145.15^{e}±59.20
III.	Arsenic (50 ppb)	266.99^{d}±18.24	2840.30^{f}±79.25
IV.	Arsenic (100 ppb)	232.47^{cd}±13.57	2141.05^{e}±77.87
V.	Arsenic (150 ppb)	194.52^{bc}±13.87	1700.15^{d}±73.24
VI.	IMI + As (50 ppb)	171.64^{ab}±14.35	1488.44^{c}±67.49
VII.	IMI + As (100ppb)	147.95^{a}±12.68	1294.78^{b}±44.51
VIII.	IMI + As (150ppb)	133.70^{a}±14.68	1077.24^{a}±34.81

Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscript (a,b,c,d,e,f,g) in a column are statistically different from one another at 5% level of significance.

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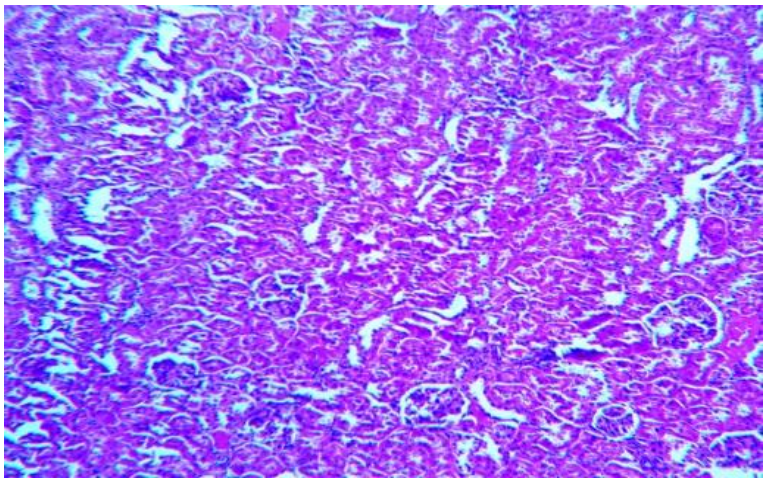

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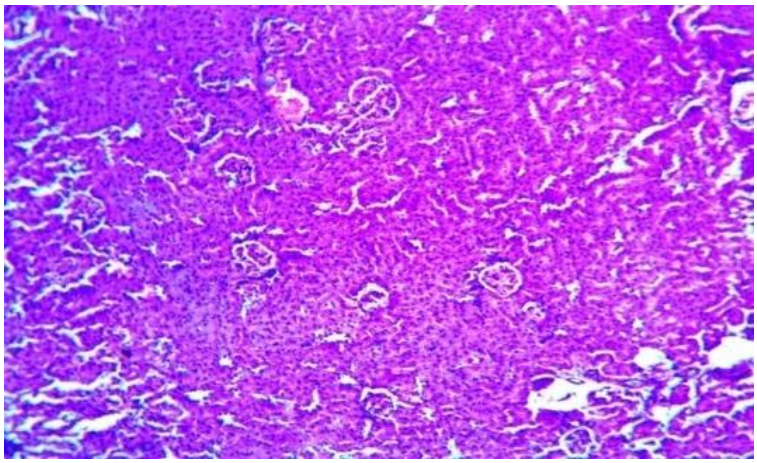

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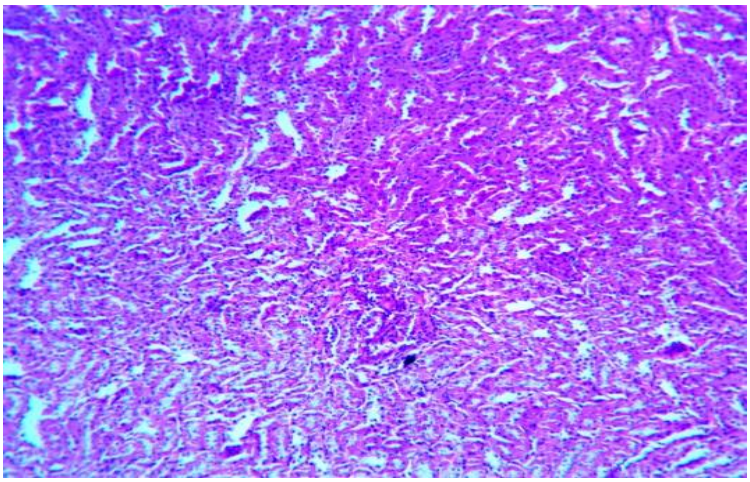
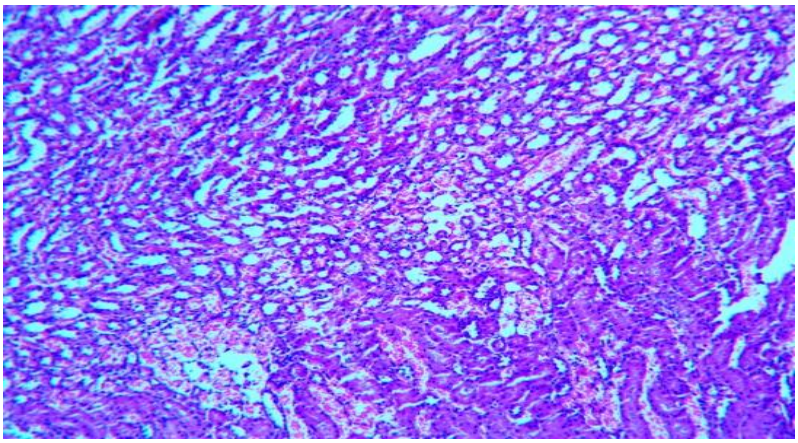


Plate 3: Photomicrograph of H & E (10X) stained sections of formalin fixed kidney **(a)** Control animals showing normal renal parenchyma **(b & c)** Group II and III: Mild alterations in histological appearance of renal parenchyma with slight degenerative changes in wistar rats.

(a).



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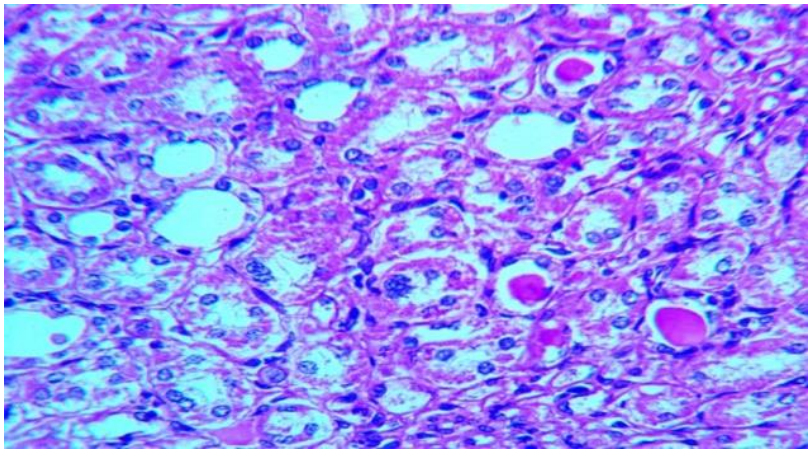

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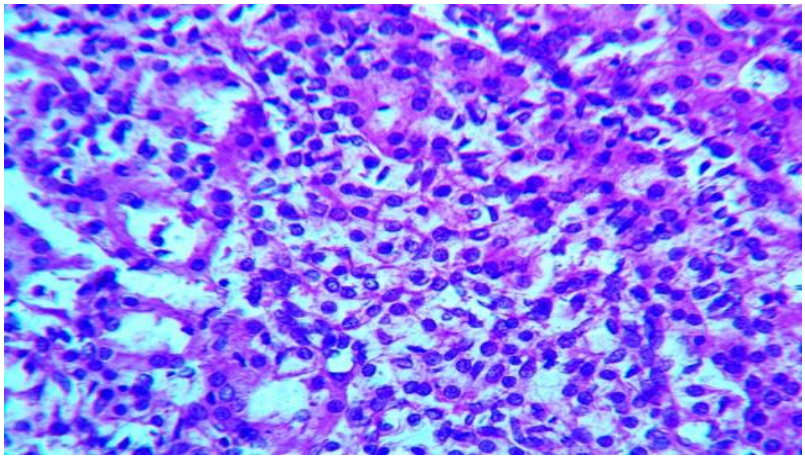


Plate 4: Photomicrograph of H & E (10X) stained sections of formalin fixed kidney **(a)** Group VI: Haemorrhages and congestion in renal interstitial tissues **(b)** Group VII: Vacuolar degeneration of tubular epithelium and presence of hyaline casts in tubular lumen of rats **(c)** Group VIII: Necrosis of tubular epithelium in wistar rats.

4.1.5. Antioxidant system in testes

4.1.5.1. Non-enzymatic antioxidant parameters

4.1.5.1.1. Total thiols (TTH): The mean values of TTH in testes of different groups following repeated oral administration of IMI and arsenic alone and in combination in wistar rats are expressed as mM and are presented in table 14. A significant ($P< 0.05$) decrease in mean total thiols level was observed in Group II (3.26±0.105), there was also significant ($P< 0.05$) fall in total thiols content in group III, IV and V in comparison to control group (4.76±0.540). Similarly, a significant ($P< 0.05$) decrease in total thiols level was observed in Group VI, VII and VIII as compared to control group.

4.1.5.1.2. Lipid peroxidation (MDA levels): The results on lipid peroxidation levels following repeated oral administration of imidacloprid and arsenic alone and in combination, are expressed as nmol MDA formed/g of tissue/hr and are presented in table 14. Significant ($P<0.05$) increase in MDA levels were observed in group II (51.76±2.36) as compared to control group (23.48±1.49). Also, there was significant ($P< 0.05$) increase in MDA levels in Group III, IV and V after repeated subacute administration for 28 days. Similarly, a significant ($P< 0.05$) rise in MDA levels were observed in group VI, VII and VIII as compared to control group.

4.1.5.1.3. Protein oxidation (AOPP): The levels of AOPP (mM) in testes of wistar rats following repeated oral administration of IMI and arsenic alone and in combination are presented in table 14. A significant ($P<0.05$) increase in AOPP levels were observed in imidacloprid exposed group (2.02±0.063) in comparison to the control group (1.47±0.029). Similarly, a significant ($P< 0.05$) increase was also observed in arsenic-exposed groups (Group III, IV and V). In addition, there was also significant ($P< 0.05$) increase in AOPP levels in group VI, VII and VIII as compared to control, IMI and arsenic alone exposed group.

4.1.5.2. Enzymatic antioxidant parameters

4.1.5.2.1. Glutathione-S-Transferase (GST): Data presented in table 15 shows the effect of repeated oral administration of IMI and arsenic alone and in combination on GST activity (µmol of GSH-CDNB conjugate formed/min/g of tissue) in testes of wistar rats. A significant ($P<0.05$) decrease in GST activity was observed in group II (38.65±2.83) after repeated imidacloprid exposure for 28 days. Similarly, there was

significant (P< 0.05) fall in GST activity in group III, IV and V in comparison to the control group (59.03±3.34). Also, a significant (P< 0.05) decrease in group VI, VII and VIII was observed as compared to control group.

4.1.5.2.2. Glutathione reductase (GR): The activity of GR in testes of wistar rats is expressed as nmol of NADPH/min and the values of GR in different groups following repeated oral administration of imidacloprid and arsenic alone and in combination are presented in table 15. Significant (P<0.05) decrease in GR activity were observed within imidacloprid exposed group (15.72±1.73), similar significant (P< 0.05) decrease was observed in Group III, IV and V as compared to control group (25.56±1.88). In addition, there was also significant fall in glutathione reductase activity in group VI, VII and VIII as compared to control group.

4.1.5.2.3. Glutathione peroxidase (GP_X): The activity of GPx is expressed as Units/g of tissue. The alteration in activity of GPx in testis of wistar rats following repeated oral administration of imidacloprid and arsenic alone and in combination on wistar rats are presented in table 15. Glutathione peroxidase activity was significant (P<0.05) decrease in group II (17.26±1.30) as compared to control group (26.89±2.99). There was also significant (P<0.05) fall in group III, IV and V after repeated oral exposure of arsenic for 28 days. Similarly, a significant (P<0.05) decrease in GPx activity was also observed in co-administered groups viz. VI, VII and VIII as compared to control group and IMI alone administered group.

4.1.5.2.4. Superoxide dismutase (SOD): The effect of repeated oral administration of imidacloprid and arsenic alone and in combination on mean SOD activity in testis of all groups are expressed as Units/g of tissue and are presented in table 16. A significant (P<0.05) decrease in SOD activity was observed in group II (136.44±14.02) as compared to control group (196.80±13.12). SOD activity also showed significant (P<0.05) fall in group III, IV and V. Similarly, there was significant (P<0.05) decrease in group VI, VII and VIII as compared to control group.

4.1.5.2.5. Catalase (CAT): The effect of subacute oral administration of imidacloprid and arsenic alone and in combination on mean CAT activity in testicular tissue of all groups are expressed as µmol H_2O_2 decomposed/min/g of tissue and are presented in table 16. A significant (P<0.05) fall in CAT activity were observed in group II

(1152.61±42.63) as compared to control group (1694.41±62.69). There was also significant (P< 0.05) decrease in the catalase activity in Group III, IV and V. Similarly, a significant (P< 0.05) fall in catalase activity was observed in group VI, VII and VIII as compared to control group.

4.1.5.3. Histopathological alterations in testes

The histopathological alterations in the testes on repeated oral administrations of imidacloprid and arsenic alone as well as combination in wistar rats are shown in Plate 5 and 6.In normal rats, no microscopic lesion of pathological significance was observed in testes and revealed typical appearance of rat testicular parenchyma with seminiferous tubules and interstitial tissue in paraffin sections. In control group, testes had normal morphological features comprising of seminiferous tubules with central tubular lumen surrounded by basement membrane. All stages of spermatogenesis could be appreciably seen and mature spermatozoa were present in the lumen. Interstitial tissue contained leydig cells, macrophages and lymphatics (Plate 5a). In imidacloprid exposed group, the testicular parenchyma did not show any significant histological alterations when compared to control, other than mild edematous fluid accumulation in the interstitial spaces (Plate 5b). Testes in rats belonging to group III had appreciable but only mild degenerative changes in few seminiferous tubules (Plate 5c). Pathological changes in this group were generally found to be of greater severity than the group of rats receiving imidacloprid administration only. In group V rats, degenerative and necrotic changes of germ cells were appreciable which were not seen in rats administered lower doses of arsenic. On the other hand, rats in group VI had severe degenerative changes in seminiferous tubules. The number of tubules was markedly reduced along with degeneration and depletion of germ cells of all the lineages was seen (Plate 6a). With increasing dose of toxicants, the Histopathological changes became more severe in the testes. The severity of pathological changes increased in combination groups with increasing dose of arsenic. Severe testicular necrosis was seen in group VII characterized by tubular shrinkage, decreased lumen diameter along with generalized necrosis and depletion of germ cells (Plate 6b). More severe changes were appreciable in group VIII where tubules became severely necrotic with only remnants of fibrillar necrotic debris seen in testicular sections (Plate 6c).

Table 14: Effect of repeated oral administrations of IMI and arsenic alone and in combination on total thiols (TTH), Malondialdehyde (MDA) and Advanced oxidation protein product (AOPP) levels in testes of wistar rats.

Groups		TTH	MDA	AOPP
I.	Control	$4.76^{c}\pm0.540$	$23.48^{a}\pm1.49$	$1.47^{a}\pm0.029$
II.	Imidacloprid (IMI)	$3.26^{ab}\pm0.105$	$51.76^{cd}\pm2.36$	$2.02^{d}\pm0.063$
III.	Arsenic (50 ppb)	$3.96^{bc}\pm0.151$	$38.05^{b}\pm2.37$	$1.59^{ab}\pm0.040$
IV.	Arsenic (100 ppb)	$3.52^{ab}\pm0.302$	$47.55^{c}\pm1.98$	$1.70^{bc}\pm0.054$
V.	Arsenic (150 ppb)	$3.23^{ab}\pm0.341$	$58.93^{d}\pm2.93$	$1.82^{c}\pm0.062$
VI.	IMI + As (50 ppb)	$2.88^{ab}\pm0.229$	$71.92^{e}\pm4.02$	$2.11d^{e}\pm0.037$
VII.	IMI + As (100ppb)	$2.75^{a}\pm0.425$	$82.50^{f}\pm4.63$	$2.20^{e}\pm0.067$
VIII.	IMI + As (150ppb)	$2.40^{a}\pm0.457$	$88.84^{f}\pm3.81$	$2.25^{e}\pm0.048$

Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscript (a,b,c,d,e,f) in a column are statistically different from one another at 5% level of significance.

Table 15. Effect of repeated oral administrations of IMI and arsenic alone and in combination on Glutathione-s-transferase (GST), Glutathione reductase (GR) and Glutathione peroxidase (GPx) activity in testis of wistar rats.

Groups		GST	GR	GPx
I.	Control	$59.03^{f}\pm3.34$	$25.56^{f}\pm1.88$	$26.89^{e}\pm2.99$
II.	Imidacloprid(IMI)	$38.65^{d}\pm2.83$	$15.72^{bcd}\pm1.73$	$17.26^{cd}\pm1.30$
III.	Arsenic (50 ppb)	$50.65^{e}\pm3.64$	$21.58^{ef}\pm1.44$	$19.68^{d}\pm1.19$
IV.	Arsenic (100 ppb)	$41.48^{d}\pm1.90$	$19.30^{de}\pm1.50$	$16.24^{cd}\pm1.24$
V.	Arsenic (150 ppb)	$30.50^{c}\pm1.83$	$16.51^{cd}\pm1.33$	$13.65^{bc}\pm0.923$
VI.	IMI + As (50 ppb)	$24.28^{bc}\pm1.73$	$13.75^{bc}\pm1.42$	$10.02^{ab}\pm0.806$
VII.	IMI + As (100ppb)	$20.65^{ab}\pm1.30$	$11.59^{ab}\pm1.09$	$8.75^{a}\pm0.899$
VIII.	IMI + As (150ppb)	$14.60^{a}\pm0.350$	$8.50^{a}\pm0.967$	$6.01^{a}\pm0.753$

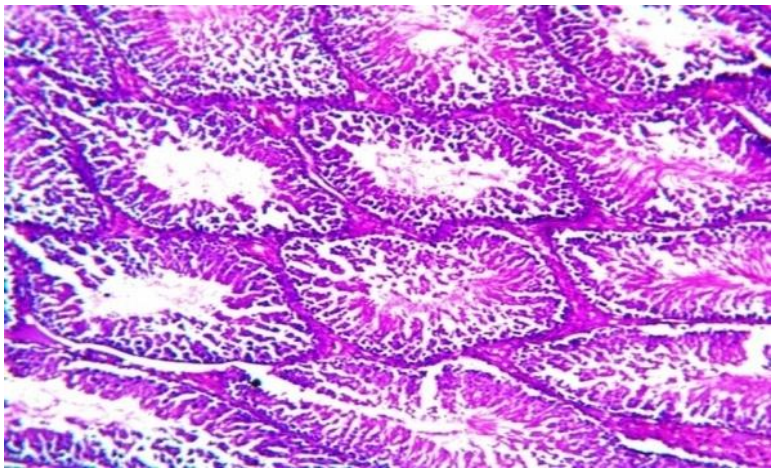
Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscript (a,b,c,d,e,f) in a column are statistically different from one another at 5% level of significance.

Table 16. Effect of repeated oral administrations of IMI and arsenic alone and in combination on Superoxide dismutase (SOD) and Catalase (CAT) activity in testis of wistar rats.

Groups		SOD	CAT
I.	Control	$196.80^{d}\pm13.12$	$1694.41^{e}\pm62.69$
II.	Imidacloprid (IMI)	$136.44^{bc}\pm14.02$	$1152.61^{d}\pm42.63$
III.	Arsenic (50 ppb)	$160.96^{cd}\pm15.02$	$1256.72^{d}\pm58.52$
IV.	Arsenic (100 ppb)	$146.71^{bc}\pm13.59$	$1189.93^{d}\pm51.57$
V.	Arsenic (150 ppb)	$135.89^{bc}\pm14.95$	$1020.15^{c}\pm32.86$
VI.	IMI + As (50 ppb)	$128.90^{bc}\pm12.97$	$744.78^{b}\pm36.45$
VII.	IMI + As (100ppb)	$117.26^{ab}\pm12.86$	$687.69^{b}\pm33.43$
VIII.	IMI + As (150ppb)	$84.25^{a}\pm9.90$	$544.78^{a}\pm34.73$

Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscript (a,b,c,d,e) in a column are statistically different from one another at 5% level of significance.

(a).



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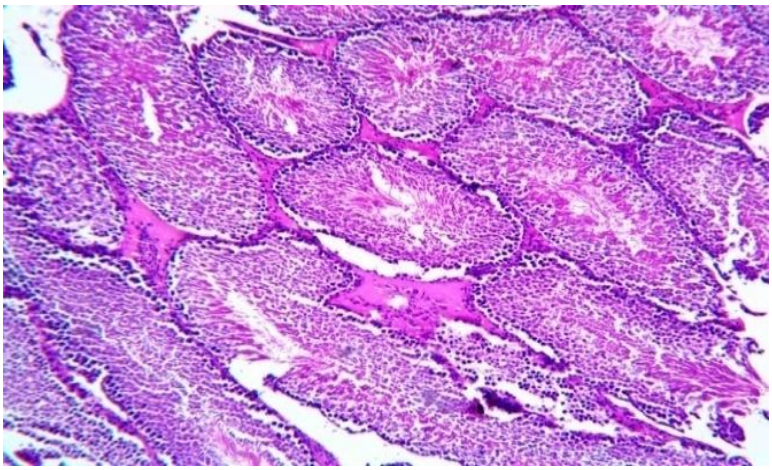

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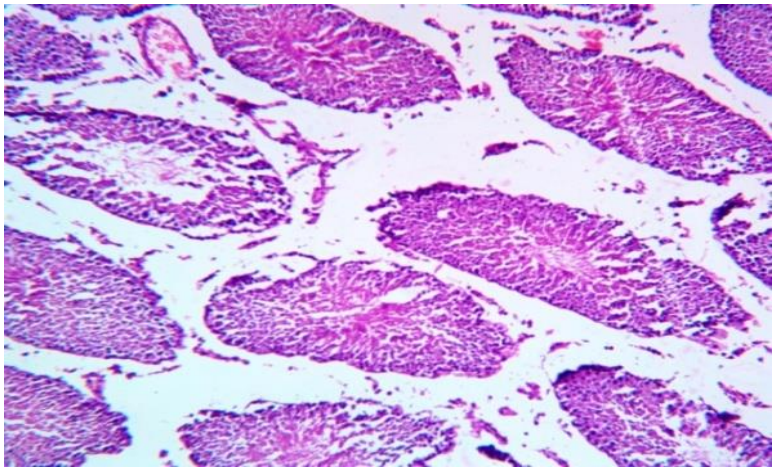
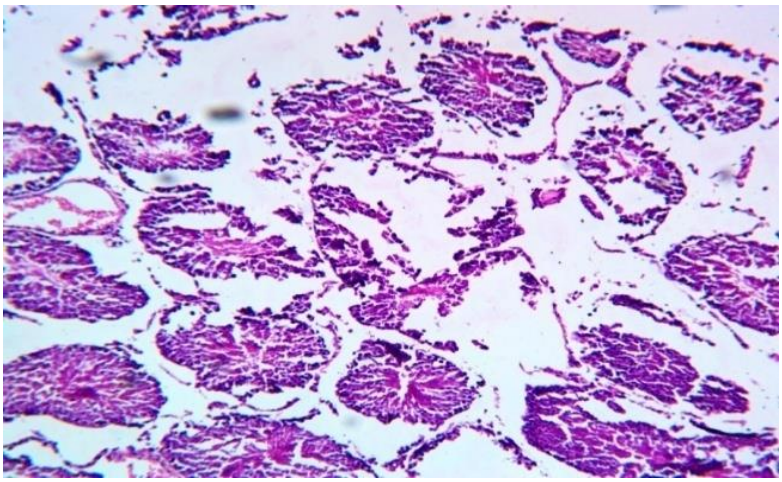
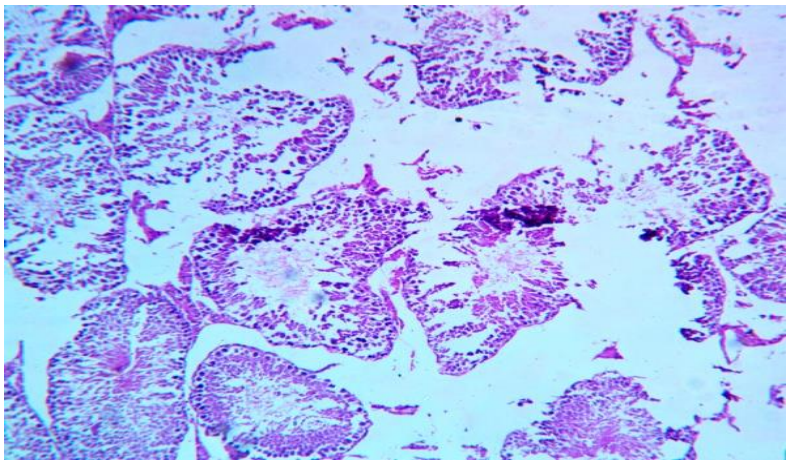


Plate 5: Photomicrograph of H & E (10X) stained sections of Formalin fixed testes **(a)** Control: normal testicular parenchyma with seminiferous tubules and interstitial tissue **(b)** IMI administered group: mild edema in the interstitial spaces **(c)** Arsenic group: mild degeneration and necrosis in germ cells of testes in wistar rats.

(a).



(b).



(c).

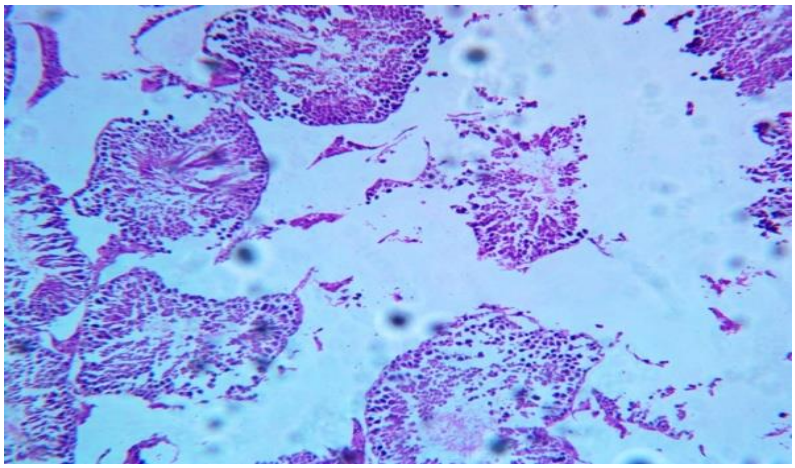


Plate 6: Photomicrograph of H& E (10X) stained sections of Formalin fixed testes **(a)** Group VI: Disruption of seminiferous tubules and necrosis of germ cells **(b)** Group VII: Severely necrotic seminiferous tubules **(c)** Group VIII: Fibrillar debris representing the remnants of seminiferous tubules in wistar rats.

Chapter- V

DISCUSSION

Humans and animals interact with environment on a daily basis and as a consequence, are exposed to large quanta of synthesized chemicals present in the food they eat, the air they breathe and the water they drink. Exponential increase in the production, use and disposition of chemicals has a profound impact on the environment and create unforeseen hazards to human well being (Desjardins, 1985; Chia, 2000). The ever increasing trend of population indices since last few decades has put tremendous pressure on land and has lead to a shift in cultivation of recalcitrant varieties to high yielding varieties and this is followed by sizeable variety of invading pests (Giridhar and Indira, 1997). To subvert these pests, variety of pesticides came into use, one of these are insecticides, which are hailed as miracles of modern technology. Pesticide toxicity incidents have more than doubled in the last 10 years. Each year 3 million people are poisoned by pesticides with 2,20,000 deaths (WHO, 1997). With greater availability of potential chemicals, the burgeoning number and complexity of intoxication are of greater concern shared by the treating physicians as well as toxicologists. Therefore, present study was designed to evaluate the effects of IMI and arsenic on various biochemical and antioxidant parameters in male wistar rats.

5.1. Effects on Hepatic Biomarkers

5.1.1. Biochemical alterations

5.1.1.1 Plasma aminotransferases: Alteration in the activities of SGOT and SGPT is very common in liver and muscle damage (Hall, 1992). In the present study, SGOT and SGPT levels were found to be increased in wistar rats exposed to IMI and arsenic alone as well as in combination groups. Increased levels of aminotransferases in blood have been used as markers for altered permeability of plasma membrane (Ramazzotto and Carlin, 1978) and cellular damage (Drotman and Lawhorn, 1978). SGOT is found in high

levels in heart and liver, while SGPT is most active in liver and their levels are elevated following tissue damage in which cellular enzymes are dumped into blood. Liver being a predominant organ for metabolism of xenobiotics, is also the first organ exposed to ingested toxicants due to portal blood supply. Therefore, toxic responses have been reported to occur more in liver as compared to other organs (Dar, 2009). Alterations in the levels of these enzymes indicate the status of liver. Similar increase in levels of these enzymes were also reported by Kammon *et al.* (2010), Soujanya *et al.* (2013), Kaur *et al.* (2006), Bhardwaj *et al*. (2010) and Mohany *et al.* (2012) on IMI exposure in rats.

In the present study, there was also significant rise in plasma SGOT and SGPT in arsenic exposed groups as compared to control group. Similar results have been reported by Nandi *et al.* (2006), Singh and Rana (2007) in rats, Khan *et al.* (2013) in chicken, Lakshmi *et al*. (2015) and Chowdhury *et al.* (2016) in rats. Charles (2014) concluded that arsenic trioxide induced marked renal and hepatic damage characterized by a significant increase in plasma urea, creatinine, AST, ALT and ALP values. Co-administration of both the toxicants produced further increase in plasma aminotransferases levels as compared to individual administration of either toxicant to the rats and similar results have been reported by Raina *et al.* (2015).

The increased levels of specific enzymes (SGOT and SGPT) could be due to degeneration and necrosis of hepatocytes, which attributes an increased permeability of cell membrane that results in the release of transaminases into the blood stream. These findings can be correlated with the histological changes in the present study. Hepatotoxicity observed in this study may be attributed to reactive oxygen species (ROS) induced by imidacloprid and arsenic as evident from significantly reduced concentration of total thiols level in toxic groups. The present findings, therefore, suggest that the insecticide and heavy metals has the potential of causing damage to liver at varying extent.

5.1.1.2 Plasma phosphatases: Alkaline phosphatase (AKP), a zinc containing enzyme (Clampitt and Hart, 1978) present in liver, biliary tracts, small intestine, bones, lungs and kidney. Its elevated levels in blood may indicate damage to liver and other organs due to non-specific irritation (Forbes, 2001). In the present study, a significant elevation in the AKP level in the imidacloprid and arsenic administered groups reflects the degree of

damage to organs, such as liver, muscles and intestines. These enzymes catalyze the transfer the phosphatases to suitable acceptor alcohols. One or all the organs producing AKP have experienced varying degree of pathological changes as a result of imidacloprid and arsenic exposure. Acid phosphatase is a lysosomal enzyme that gets stimulated in cases of imminent and prevailing tissue damage (Wilson *et al.*, 1970). The increase in its activity may be the result of labialization of lysosomal system (Johal *et al.*, 2003). A significant increase was observed in plasma ACP and AKP levels in imidacloprid treated rats when compared to control group (Kaur *et al.* 2006; Kammon *et al.* 2010; Mohany *et al.* 2012; Vohra and Khera 2015). In the present study, there was also significant rise in plasma ACP and AKP in arsenic-exposed groups as compared to control group. Similar results have been also reported in fish (Balasubramanian and Kumar, 2013), mice (Sharma *et al.* (2007) and rats (Patel and Kalia 2013; Charles, 2014; Lakshmi *et al*. 2015).

5.1.1.3 Effects on Protein Profile: Plasma proteins are a significant indicator of the health condition, metabolic and production features of the organism because of their numerous roles in the physiology. Therefore, plasma proteins have an exceptional significance in homeostasis as they play important roles in the maintenance of colloidal osmotic pressure. At birth, plasma protein levels of most animals are quite low due to minimal quantities of immunoglobin and low albumin. Modifications of total proteins concentration can result from variations of concentrations of albumin, globulins or both. An increased albumin or globulin concentration, however, does not always produce detectable increase in total protein concentrations (Thrall *et al*., 2004).

The IMI administrations produce non-significant increase in total plasma proteins and similar changes were also reported by Abbassy *et al.* (2014b) as well as Vohra and Khera (2015). Balani *et al.* (2011) evaluated the toxic effects of sub-acute oral administrations of Imidacloprid in male white leghorn chicken and reported an increase in ALT but no change in AST, serum total proteins, globulins, albumin and serum creatinine levels. In the present study, significant fall in total proteins level was observed in higher doses of arsenic exposed rats. These finding are in accordance with the results of Tandan *et al.* (2012), Lakshmi *et al.* (2015) in rats, Tanju and Madhuri (2013) and Khan *et al*. (2013) in chickens.

Hypoproteinemia observed in arsenic-exposed groups in the present study may be due to increased catabolism of proteins and their decreased synthesis. The decreased levels of biochemical parameters might be attributed to decreased feed consumption, increased catabolism of protein, hepatic and renal dysfunction as evidenced in histopathological observations of liver and kidney in the present study. Plasma albumin measures as considerable diagnostic value in laboratory animals because it relates general nutritional status, the integrity of the vascular system and liver function. Albumin participates in metabolism and performs transport functions of substances necessary for life activities like fatty acids, minerals and vitamins (Andreeva, 1999). In the present study, there was no significant change in globulins and A/G ratio (Table 3 and Figure 4). A/G ratio is important in the interpretation of diagnostic significance, since it changes at different pathological stages (Kaneko *et al.*, 1997).

5.1.2 Effect on antioxidant parameters in blood

Oxidative stress is defined as disturbance of the equilibrium between pro- and antioxidants system. An imbalance between pro-oxidant (reactive oxygen species, ROS) and antioxidant mechanisms in cells cause oxidative stress. However, antioxidant defence system which consists of superoxide dismutase, catalase, glutathione-s-transferase, glutathione reductase and glutathione peroxidase offers protection to cells against ROS. Excess generation of ROS in cells is known to damage DNA, lipids and proteins resulting in several biological effects ranging from alterations in signal transduction, gene expression and mutagenesis to apoptosis. ROS is believed to be involved in the aetiology of adverse effects of many toxicants.

Erythrocytes serve as the principal vehicle for effective transport of O_2 and CO_2 between the lungs and tissues. Erythrocyte survival in the circulation depends on certain factors that affect their mechanical behaviour. Peroxidation of membrane phospholipids is one of them. Erythrocytes could be a target for the metal and insecticide induced oxidative damage and the consequences could be potentially life-threatening, as this may clinically be manifested as anaemia, hypoxia and several other disorders. Erythrocytes possess non-enzymatic and enzymatic antioxidant systems, which detoxify ROS that are generated during oxidative stress.

5.1.2.1 Effect on non-enzymatic antioxidant parameters in blood

5.1.2.1.1 Haemoglobin (Hb): Erythrocytic indices such as total erythrocyte count and Hb concentration are important indicators of the functional state of the erythron (Schalm *et al*., 1975). Erythrocyte counts reflect the total number of red blood cells per unit volume of circulating blood while Hb level indicates the oxygen carrying capacity of blood (Coles, 1986). In the present study, the rats in all groups did not show any significant changes in their Hb levels during repeated subacute exposure with IMI and arsenic alone as well as in combination groups.

A non-significant increase in haemoglobin content was observed after imidacloprid exposure in wistar rats. These results are in agreement with the observations of previous studies on the haematological effects of insecticides on mammalians by Bhardwaj *et al.* (2010) and Balani *et al.* (2011), Premlata *et al.* (2006) and Abbassy *et al.* (2014b). Contrary to our observation, reduction in Hb levels have been observed in rats treated with phentoate, flumeturon, dichlorovos and diazinon (Punia, 1978; Ahmed *et al*., 1980; Chou *et al*., 1989), guinea pigs administered with fenuron (Perlovskaya *et al*., 1971) and in BALB/c mice treated with imidacloprid (Kavani, 2007). A non-significant increase in haemoglobin was observed after repeated oral administration of arsenic at different dose levels in drinking water. These results are in accordance with Jain *et al*. (2011) and Chowdhury *et al.* (2016).

5.1.2.1.2 Total thiols (TTH) level: A significant decline was observed in TTH level in the IMI administered animals. Present findings of decline in TTH levels are in agreement with the studies of Mecdad *et al.* (2011) and Mehri *et al.* (2016). Similarly, repeated subacute exposure of arsenic in rats caused a significant fall in TTH levels as compared to the control group. This finding is in consistence with the studies of El-Demerdash (2004), Mousa *et al*. (2002) and El-Demerdash *et al.* (2009).

Thiols are the organic compounds that contain single or multiple sulphydryl (-SH) group. They are the endogenous molecules that assist aerobic cells in maintaining a reducing state, despite an oxidizing environment (Chung *et al.*, 2005). Thiols are extraordinarily efficient antioxidants protecting cells against the consequences of damage induced by free radicals, due to their ability to react with the latter (Atmaca, 2004). Among all the antioxidants that are available in the body, thiols constitute the major portion of total

body antioxidants and they play a significant role in defence against ROS and act as a source of –SH group for the metabolism. Total thiols composed of both intracellular and extracellular thiols either in the free form as oxidized or reduced glutathione, or thiols bound to proteins. Among the thiols that are bound to proteins, albumin makes the major portion of the protein bound thiols, which bind to sulhydryl group at its cysteine- 34 portion. Both intracellular and extracellular redox states of thiols play a critical role in the determination of protein structure and function, regulation of enzymatic activity of transcription factors and antioxidant protection (Wlodek, 2002). Decreased levels of thiols has been noted in various medical disorders including chronic renal failure and other disorders related to kidney, cardiovascular disorders, stroke and other neurological disorders, diabetes mellitus, alcoholic cirhossis and various other diseases.

5.1.2.1.3 Blood Glutathione (GSH): Glutathione is a tripeptide comprising of glutamic acid, glycine and cystine, found in extracellular as well as intracellular fluids (Beutler, 1975). GSH is a potent antioxidant, which prevents free radical damage and helps detoxification by conjugation with chemicals. In addition, GSH is pivotal to the cellular antioxidant defences by acting as an essential cofactor for antioxidant enzymes including GP_X and GST (Hayes *et al*., 2005). In the present study, a significant fall in GSH level has been observed due to imidacloprid treatment in rats. Similar results have been reported by Achudume (2012) in rats and Ince *et al*. (2013) in mice. Similarly in the present study, a significant fall in blood glutathione levels were also observed after repeated exposure of different doses of arsenic and these results are in consonance with Wang *et al.* (2006) and Jain *et al.* (2011). Co-exposure to both the toxicants produce further reduction in GSH levels as compared to individual administration of either toxicant alone and similar results have also been observed by Baba *et al.* (2013) and Ahmad *et al*. (2014). Under oxidative stress, GSH is depleted by GSH related enzymes viz. GST, GPx etc to detoxify the peroxides produced due to increased lipid peroxidation. Decrease in glutathione results in the impairments of mechanisms of metabolic detoxification (Verma and Srivastava, 2001).

5.1.2.1.4 Lipid peroxidation (MDA levels): Erythrocytes are more vulnerable for oxidative insults (Eristland, 2000). Malondialdehyde (MDA) is the end point of lipid peroxidation process and indicator of oxidative deterioration of polyunsaturated lipids

during oxidative insults. Glutathione (GSH) an important antioxidant, protecting the membrane from oxidative insult, is thus considered a critical determinant for the threshold of tissue injury caused by environmental chemicals. Several enzymatic antioxidant defences designed to scavenge reactive oxygen species (ROS) in the eukaryotic cells protect them from oxidative injury. Balance between several antioxidant species and ROS appears to be more important for the overall protection of cells. Lipid peroxidation has been measured by quantifying the level of thiobarbituric acid reactive substances (TBARS) which indicate the physical status of membrane lipids (Pipicelli *et al*., 2005).

Repeated oral administration of imidacloprid in rats caused a significant increase in lipid peroxidation levels. These findings are in agreement with the studies of Pathak *et al.* (2010), Achudume (2012), Mecdad *et al.* (2011), Sharma *et al.* (2014a), Ince *et al.* (2013) in mice. Different doses of arsenic also significantly increased lipid peroxidation levels have been reported in rats and similar results have been observed by Kowalczky *et al.* (2002), Wang *et al.* (2006) in pigs and Aflanie (2015) in rats. Co-exposure of both toxicants further enhances the levels of MDA in rats which may be due to increased levels of generation of free radicals in the blood. Free radicals reacting with lipids cause peroxidation, resulting in the release of products such as malondialdehyde, hydrogen peroxide and hydroxyl radicals. An increase in lipid peroxides indicates serious damage to cell membranes, inhibition of several enzymes, cellular functions and cell death (Pompella *et al*., 1991). LPO has been shown to cause profound alterations in the structure and functions of the cell membrane, including decreased membrane fluidity, increased membrane permeability, inactivation of membrane-bound enzymes including ATPases and loss of essential fatty acids (De-Zwart and Meerman, 1998).

5.1.2.1.5 Advance oxidation protein product (AOPP): AOPP is a dityrosine containing cross linked protein products and it excludes the protein aggregates that are formed by disulphide bonds as a result of oxidative stress. Measurement of AOPP reflects the reactive species generation and the ROS induced protein oxidation (Witko-Sarsat *et al.*, 1996). It is reported that AOPP generated by different oxidation patterns lead to the production of either hydrogen peroxide or nitric oxide (Servettaz *et al*., 2007). Nitric oxide can interact with superoxide anion-radical forming reactive nitrogen species like

peroxynitrile. These reactive nitrogen species secondarily promote important reactions such as nitrosation, oxidation or nitration, leading to impaired cellular functions and enhanced inflammatory reactions (Iwakiri and Groszmann, 2007).

In the present study, there was significant rise in protein oxidation products after IMI exposure of rats. Metabolism of IMI produces free radicals/intermediate reactive molecules which may be responsible for the increased protein oxidation in rats. Similar results have also been also reported by Pathak *et al.* (2010) and Soleimani *et al.* (2008). Similarly, there was significant increase in protein oxidation after subacute oral exposure of different doses of arsenic in rats. These results are in accordance with Kowalczky *et al.* (2002), Aflanie (2015) and Kayode *et al.* (2015). Combined administration of both the toxicants produced higher level of protein oxidation as compared to individual administration of either toxicant alone. Therefore, AOPP is a good oxidative stress marker, which originates under oxidative and carbonyl stress and increased global inflammatory activity (Suhartono *et al.*, 2014).

5.1.2.2. Effect on enzymatic antioxidant parameters in blood

5.1.2.2.1 Glutathione-S-Transferase (GST): Glutathione related enzymes such as GR, GST and GP_x function either directly or indirectly as antioxidants. Repeated oral administration of imidacloprid and arsenic in wistar rats caused a significant decrease in GST levels. Present findings of decreased GST activity on IMI exposure are in agreement with the studies of Mecdad *et al.* (2011) and Sharma *et al.* (2014a). Similarly, repeated subacute exposure of arsenic in rats caused a significant fall in GST activity as compared to the control group. This finding is in consistence with the studies of Miltonprabhu and Sumedha (2014) and Wang *et al.* (2006). Co-exposure of both the toxicants produced more reduction in GST activity as compared to either toxicant and these results are in consonance with the studies of Dubey *et al.* (2012) and Baba *et al.* (2013). GSTs are a major group of enzymes that constitute 10% cytosolic protein in some mammalian organs. It catalyzes the conjugation of reduced glutathione via the sulfhydryl group to electrophilic centres on a wide variety of substances. In addition, GST also binds with varying affinities to a variety of hydrophobic compounds such as polycyclic aromatic hydrocarbons and other xenobiotics such as pyrethroids (Singh *et al.*, 2009). This activity is useful in the detoxification of the endogenous compounds such as peroxidized lipids as

well as metabolism of xenobiotics. This catalytic activity of combined glutathione with electrophiles helps in excretion of toxicant or their reactive metabolites from the cells and protects the tissues from oxidative insults (Hayes and Paiford, 1995).

5.1.2.2.2 Glutathione reductase (GR): The activity of GPx and GR play a key role in the protection of cells from free radicals generated during intoxication. GPx and GR are involved in normalizing the cellular ratio of reduced and oxidized glutathione (GSH and GSSG) above 300:1 (Alpert and Gilbert, 1985). Inhibition of GPx and GR activity diminishes the intracellular glutathione levels and leads to the reduced scavenging potential of cell from free radicals resulting in oxidative stress. Thus, co-ordinated activity of GPx and GR maintains the intracellular redox status (Ketterer, 1986). GSH together with the GPx can detoxify free radicals into non-toxic products (Sinha *et al.*, 2008). Several experimental studies demonstrated that arsenical compounds are potent inhibitors of both GPx and GR (Avani and Rao, 2008). In the present study, imidacloprid caused a significant reduced the GR levels in erythrocyte of exposed rats. These findings are in agreement with Mecdad *et al.* (2011), Achudume (2012) and Sharma *et al.* (2014a). Also, a significant decline in GR activity was observed after arsenic exposure in rats in comparison to the control group. This is in accordance with the findings of Miltonprabhu and Sumedha (2014) and Wang *et al.* (2006). Co-administration of both IMI and arsenic produced decrease in GR activity much lower as compared to the individual administration of either IMI or arsenic alone indicate the more stress levels in on co-administration of toxicants.

5.1.2.2.3 Glutathione peroxidase (GPx): GPx is a selenium (Se) containing enzyme which reduces H_2O_2 utilizing GSH to forming GSSG and thereby serves as an alternative means of detoxifying activated oxygen radicals. Thus the activity of GPx is also dependent upon reduced glutathione level. Decreased GSH in present study might be the reason for decreased GP_X activity. In animal cells, especially in human erythrocytes, the principal enzyme for H_2O_2 detoxification is GP_x, as catalase has a much lower affinity for H_2O_2 than GP_X (Izawa *et al*., 1996). The decrease of this enzyme activity is observed in the present study reflects alterations in the normal redox mechanisms during IMI and arsenic treatment, and was more pronounced upon combined exposure in rats. Although GPx and catalase share the substrate H_2O_2, the glutathione redox cycle is a major

protective mechanism against low levels of oxidant stress, whereas catalase becomes more important in protecting against severe oxidative stress (Yan and Harding, 1997). In the present study, there was a significant decrease in the activities of GP_x in erythrocytes of imidacloprid and arsenic-exposed rats. GPx has a well-established role in protecting the cells against oxidative injury. GPx is a non-specific enzyme for H_2O_2 and lack of substrates specificity extends a range of substrates from H_2O_2 to organic hydroperoxides (Chance *et al*., 1979). Therefore, the excess of H_2O_2 and lipid peroxides are effectively scavenged by GPx activity. A significant decline was observed in GPx activity in the IMI exposure and similar decreased GPx activity were also observed in studies of Abbassy *et al.* (2014a), Sharma *et al.* (2014a). Similarly, repeated subacute exposure of arsenic in rats caused a significant fall in GPx levels as compared to the control. This finding is in consistence with Miltonprabhu and Sumedha (2014), Wang *et al.* (2006) and Dubey *et al.* (2012). Combined administration of both toxicants produced more significant fall in GPx activity and these results have also been reported by Ahmad *et al.* (2014), Baba *et al.* (2013) and Dubey *et al.* (2012).

5.1.2.2.4 Superoxide dismutase (SOD): SOD is the first line of defence against the action of superoxide (O_2^-, H_2O_2) and other ROS. Superoxide radicals are produced in mitochondria and endoplasmic reticulum as a consequence of auto-oxidation of electron transport chain components. These superoxide free radicals are generated during the monovalent reduction of oxygen and are toxic to biological systems. The major enzyme that protects against superoxide protection in the body is superoxide dismutase which disproportionates the superoxide to H_2O_2 and oxygen (McCord and Fridovich, 1969). Decreased SOD activity in the present study is suggestive of excess free radicals generation which impairs natural defence mechanism of this enzyme. A significant decrease in SOD activity in IMI exposed rats was observed and similar reports have also been observed in rats (Achudume, 2012; Sharma *et al.*, 2014a) and mice (Ince *et al.*, 2013). In the current study, a significant decline in SOD levels in arsenic-administered group of rats as compared to their control group. These findings are in agreement with Miltonprabhu and Sumedha (2014), Wang *et al.* (2006), Dubey *et al.* (2012), Okediran *et al.* (2017).

Co-administration of both IMI and arsenic produced more rapid fall in SOD activity in comparison to individual administration of either toxicant. These findings are in accordance with Ahmad *et al.* (2014), Baba *et al.* (2013) and Dubey *et al.* (2012). Superoxide dismutase has an antitoxic effect against the superoxide anion. SOD accelerates the dismutation of superoxide to H_2O_2 which is removed by catalase (Usoh *et al*., 2005). Thus, SOD acts as a primary defense and prevents further generation of free radicals. The decreased SOD activity in organs suggests that the accumulation of superoxide anion radical might be responsible for increased lipid peroxidation following arsenic treatment. The free radicals are produced by arsenic treatment was also indicated by Yamanaka *et al*. (1990) and they suggested that free radical species are generated by the reaction of molecular oxygen with dimethylarsine, a metabolite of dimethyl arsenic acid.

5.1.2.2.5 Catalase (CAT): Catalase is an antioxidant enzyme and its decreased activity implies Fenton reaction mediated conversion of more H_2O_2 to the ultimate toxicant, OH^- (Klassen, 1996). CAT acts as a preservative antioxidant and plays an important in protection against the deleterious effects on lipid membrane (Dinkova-Kostova, 2002). The efforts of the endogenous antioxidant enzymes to remove the continuously generated free radicals initially due to an induction but on prolonged exposure, enzyme depletion occurs due to oxidative damage (Kalra *et al.*, 1994). In the present study, IMI caused a significant decline in catalase activity of the experimental group of rats as compared to the control group. These finding are in agreement with studies of Achudume (2012), Sharma *et al.* (2014a) in rats and Ince *et al.* (2013) in mice. Similarly, repeated subacute exposure of arsenic in rats caused a significant fall in CAT activity as compared to the control group. This finding is in consistence with the studies of Miltonprabhu and Sumedha (2014), Wang *et al.* (2006), Dubey *et al.* (2012) and Okediran *et al.* (2017). Combined administration of both toxicants produced more significant fall in CAT activity and these results have also been reported by Baba *et al.* (2013), Dubey *et al.* (2012) and Ahmad *et al.* (2014). Catalase is a haem-containing enzyme that catalyzes the dismutation of hydrogen peroxide into H_2O and O_2. The enzyme is found in all aerobic eukaryotes and is important for the removal of H_2O_2 generated in peroxisomes by oxidases, involved in β-oxidation of fatty acids, the glyoxylate cycle (photo-respiration)

and purine metabolism. Stress conditions in which there is a large free radical generation also result in the depletion in catalase activity (Hertwig and Feirabend, 1992), thus justifying the decreased activity of the enzyme.

5.1.3. Effect on antioxidant parameters in Hepatic tissue

5.1.3.1 Effect on non-enzymatic parameters: Repeated oral administration of IMI produced significant reduction in the total thiols level in the hepatic tissue of wistar rats and these results are in accordance with the findings of Achudume *et al.* (2010), Kapoor *et al.* (2010), Madkour (2012) and Heikal *et al.* (2013). Similarly, subacute oral administration of different doses of arsenic alone leads to fall in TTH levels of the hepatic tissue of wistar rats and these findings are in agreement with El-Demerdash *et al.* (2009), Jain *et al.* (2011), Muthumani (2013) and Patel and Kaila (2013). Combined administration of both IMI and arsenic produced more significant fall in TTH level and these findings are in consonance with studies of Jain *et al.* (2015) and El-Murr *et al*. (2016). The reduction in TTH levels in hepatic tissue may be due to the increased utilization in scavenging the free radicals/reactive metabolites generated on metabolism of IMI and arsenic in hepatic tissue. This may be attributed to direct utilization of GSH as an antioxidant in terminating the free radical reaction resulting in GSH exhaustion during oxidative stress, which was evident from significant alterations in hepatic biomarkers and corresponding histological changes in the liver sections of rats. A significant increase in the lipid peroxidation product and protein oxidation product was observed in the IMI alone administered group and these results are similar with Xu *et al.* (2015), Heikal *et al.*(2013), Madkour (2012) and Al-Awthan *et al.* (2012). Also, the subacute oral administration of arsenic produced significant rise in MDA and AOPP levels in the wistar rats. Similar observations were also recorded by Nain and Smits (2012), Mitra *et al.* (2013), Muthumani and Miltonprabhu (2012b), Patel and Kalia (2013) and Reckziegel *et al.* (2016). Co-administrations of both the toxicants produced more significant increase in lipid and protein oxidation levels as compared to individual administration of either toxicant. These results are in accordance with Raina *et al.* (2015), Jain *et al.* (2015) and El-Murr *et al.* (2016). The increased levels of MDA and AOOP in hepatic tissues indicate the increased membrane peroxidation which is responsible for altered membrane

permeability leading to leakage of cytosolic enzymes from the hepatocytes. The excess free radicals cause cellular and sub-cellular damage and thereby, disrupting the cellular functions which may be responsible for a variety of hepatic disorders (Kushnareva *et al.*, 2002).

5.1.3.2 Effect on enzymatic parameters: Cellular antioxidant system consists of enzymatic and non-enzymatic components. In the present study, IMI also caused a significant reduction in the antioxidant enzymatic components (GST, GR, GPx, SOD and CAT) after repeated administration of IMI alone and these results are in consonance with the studies of Kapoor *et al.* (2010), Achudume *et al.* (2010), Madkour (2012), Ince *et al.* (2013) and Lohiya *et al.* (2017). Also, repeated oral administration of arsenic produces fall in the antioxidant enzymes activity in the hepatic tissue of wistar rats. This finding is in consistence with Wang *et al.* (2006), Muthumani and Miltonprabhu (2012b), Muthumani (2013), Patel and Kalia (2013), Jain *et al.* (2011) and Mitra *et al.* (2013). Combined administration of both IMI and arsenic to wistar rats produced more significant decrease in antioxidant enzymatic activity. These findings have also been reported by Raina *et al.* (2015), Jain *et al.* (2015) and El-Murr *et al.* (2016). The reduction in endogenous antioxidant system of hepatic tissue may occur either due to oxidative stress or excessive ROS generation and or interference in antioxidant defence components. The liver is the most susceptible organ to toxic responses as compared to other organs because live is the main organ responsible for metabolism and is also first major organ to be exposed to orally ingested toxins.

5.1.3.3 Histopathology alterations in liver: In the present study, IMI treated rats showed mild alterations in the normal hepatic architecture viz. slight congestion, para tubular inflammation, dilation of the central vein and sinusoids with few numbers of inflammatory cells in the hepatic parenchyma. Similarly, Mohany *et al.* (2012) reported that animals treated with 0.21 mg/kg IMI for 4 weeks showed heavily congested central vein and blood sinusoids, widely distributed pyknotic nuclei and leukocytes infiltration in the hepatic tissue of exposed rats. IMI at $1/10^{th}$ of LD_{50} treatment resulted in dilatations of central vein and sinusoids between hepatocytes (Toor *et al.*, 2013). High doses of IMI (20 mg/kg/day) resulted in mild focal necrosis with swollen cellular nuclei and cytoplasmic lesions in rat liver and slight degeneration of tubules in the kidney of the female rats

(Kapoor *et al*., 2010). IMI produced similar histopathological lesions in liver, kidneys and brain of Japenese quail exposed to toxicant for 6 weeks (Omiama, 2004) and in layer chickens exposed to 139 mg/kg IMI (Kammon *et al.*, 2010). In the present study, different histopathological lesions were observed in the liver which became more severe with the increasing doses of arsenic administration like mild hepatic alteration at low doses of arsenic while mild to moderate degenerative changes along with accumulation of edema at higher doses of arsenic exposure. The findings are in agreement with studies of Javaid *et al.* (2008) in mice due to arsenic toxicity. Devaraju *et al.* (2010) reported several changes in the liver occurred such as nuclear degeneration, cytoplasmic degeneration and emptied portal vein, binucleated condition and also exhibition of vacuoles in hepatocytes.

Ferzand *et al.* (2008) have also mentioned histological disturbance caused by arsenic containing water in mice and revealed mild to severe type of necrosis and degenerative changes in the kidney and liver of mice. In the combination groups, co-administration of IMI and arsenic produced severe degeneration, coagulative type of necrosis and necrosis of hepatocytes which became more severe with the increasing dose of arsenic in the combination groups. The present study revealed that IMI and arsenic treated rats showed different pathological lesions in the liver tissue. The liver is the centre for detoxifying any foreign compounds entering the body. So, it is uniquely exposed to a variety of endogenous and exogenous products. These include environmental toxins and chemicals present in food or drinking water. This study suggested that toxic responses occur relatively more frequently in the liver compared with other organs mainly because the liver is a predominant organ for the metabolism and is also the first organ to be exposed to ingested toxins, due to its portal blood supply (Popp and Cattley, 1991).

5.2 Effects on Renal Biomarkers

5.2.1 Biochemical alterations: Blood levels of urea and creatinine, end products of protein metabolism are primary indicator for renal dysfunctions in mammals. Marked increase in plasma BUN and creatinine, as noticed in this study indicate renal dysfunctions and these finding are confirms in histopathological changes in the kidney on IMI or arsenic administered rats. Necrotic and degenerative changes caused disturbance

in the transport system of biochemical constituents in the kidney leads to accumulation of these indicators in plasma (Janardhan *et al*., 1988 and Garba *et al*., 2007). Urea level can be increased by many other factors such as dehydration, anti-diuretic drugs and diet, while creatinine is, therefore, more specific to kidney, since kidney damage is the only significant factor that increases plasma creatinine level (Well *et al*., 1986 and Nwanjo *et al.*, 2005). Insecticide and heavy metal induced increase in BUN levels observed in the present study may be due to the effect of these xenobiotics on liver function, as urea is the end-product of protein catabolism (Coles, 1986). Elevated blood urea is correlated with an increased protein catabolism in mammalian body or from more efficient conversion of ammonia to urea as a result of increased synthesis of enzyme involved in urea production (Rodwell, 1979).

The renal dysfunction was evaluated from increased BUN (marker for proximal tubule injury) and creatinine levels (marker for glomerular injury). In present study, the elevation in plasma BUN and creatinine suggested an extensive renal damage in imidacloprid and arsenic exposed rats. Increased levels of blood urea are generally correlated with increased level of protein catabolism in mammalian body or from more efficient conversion of ammonia to urea as a result of increased synthesis of enzymes involved in urea production (Rodwell, 1979). Also high levels of blood urea results from either increased breakdown of tissue/dietary protein or impaired excretion (Bush, 1991). Creatinine is derived mainly from the catabolism of creatine found in muscle tissue and its catabolism to creatinine occurs at a steady rate. Creatinine in also present in tissues (brain, blood etc) as a high energy compound phospho-creatinine excreted in small quantities in urine (Bloch *et al*., 1941). Increase in creatinine concentration may be due to impaired renal function and is considered as functional evidence of imidacloprid and arsenic-induced nephrotoxicity. Metabolism of xenobiotics being energetic process would utilize phosho-creatinine for energy for energy generation and therefore, possibly explaining the increased level of creatinine in the present study. Increase in creatinine levels may be the indicator of the degeneration of the kidneys, heart muscle and other muscles. Creatinine is useful in early detection of nephrotoxicity induced by exogenous compounds. Therefore, significant increases in BUN and creatinine levels noticed in this study are a classical sign that the kidney was affected by imidacloprid and arsenic

administration. Renal dysfunction and nephrotoxicity induced by the toxicants in present investigation are consequences of oxidative stress. In the present study, the results of elevation in BUN and creatinine as a consequence of Imidacloprid exposure are in accordance with Bhardwaj *et al.* (2010), Soujanya *et al.* (2013) and Hussein *et al.* (2014). Similarly, results of significant rise in renal markers due to arsenic exposure are also obtained by Patel and Kalia (2010), Khan *et al.* (2013), Charles (2014) and Lakshmi *et al.* (2015).

Combined administration of both the toxicants produced more significant rise in BUN and plasma creatinine levels indicative of more severe renal damage as compared to individual administration of either toxicant. These findings are in accordance with Baba *et al.* (2016). The elevation of plasma levels of BUN and creatinine are considered as significant markers of renal dysfunction (Almdal and Vilstrup, 1988). Elevation of BUN and creatinine in plasma of treated rats may be attributed to reduction in glomerular filtration in the kidney and also reflect dysfunction of the kidney tubule (Walmsley and White, 1994).

5.2.2 Effect on antioxidant parameters in renal tissue

5.2.2.1 Effect on non-enzymatic parameters:

Free radicals (RNS/ROS) directly act on cell components, including lipids, proteins, and DNA, and altered their functional activity. Glutathione a non-protein thiols (-SH group) plays a major role in the detoxification of extra and intracellular RNS/ROS, and therefore is an indispensible component of the cellular antioxidant defences. Further its concentration is also responsible for maintaining redox state of protein thiols involved in DNA synthesis and repair. Repeated oral administration of IMI produced significant reduction in the TTH levels in the renal tissue of rats and these results are in accordance with the findings of El-Saad (2011) and Marouani *et al.* (2017). Similarly, subacute oral administration of different doses of arsenic alone leads to fall in TTH levels of the renal tissue of wistar rats and these findings are in agreement with Wang *et al.* (2006), El-Demerdash *et al.* (2009) and Patel and Kalia (2010). Combined administration of both IMI and arsenic produced more significant fall in TTH levels and these findings are in consonance with studies of Jain *et al.* (2015). The depletion of TTH levels in renal tissue suggests that there was increased peroxidation in kidneys due to induction of oxidative

stress that was evidenced from significant alterations in renal biomarkers and corresponding histological alterations in kidney.

A significant increase in the lipid and protein oxidation product was observed in the IMI alone administered group and these results are similar with El-Saad (2011), Marouani *et al.* (2017). Also, the subacute oral administration of arsenic produced significant rise in MDA and AOPP levels in the renal tissue of wistar rats. Similar observations were also recorded by Patel and Kalia (2010), Zhang *et al*. (2014), Kayode *et al.* (2015) and Reckziegel *et al.* (2016). Co-exposure of both the toxicants produced more significant increase in lipid and protein oxidation levels as compared to individual administration of either toxicant. These results are in accordance with Baba *et al.* (2016) and Jain *et al.* (2015). Increased MDA and AOPP levels in renal tissue are indicative of renal damage due to excessive production of free radicals during exposure to toxicants. Increased free radicals level may be due to IMI and arsenic metabolism or due to reduced capacity of the cellular antioxidant system to defend the organism against the free radicals.

5.2.2.2 Effect on enzymatic parameters: In the present study, IMI caused a significant reduction in the antioxidant enzymatic parameters (GST, GR, GPx, SOD and CAT) after repeated exposure for 28 days and these results are in consonance with the studies of El-Saad (2011), Achudume (2012), Shirisha *et al.* (2013), Sakr and Albarakai (2014) and Devan *et al.* (2015). Also, repeated oral administration of arsenic produces fall in the antioxidant enzymes activity in the renal tissue of wistar rats. These findings are in consistence with the findings of Wang *et al.* (2006), Patel and Kalia (2010), Lakshmi *et al.* (2015), Morya and Vachchrajani (2014) and Hamadouche and Hadi (2015). Combined administration of both IMI and arsenic to wistar rats produced more significant decrease in antioxidant enzymatic activity. These findings have also been reported by Baba *et al.* (2016) and Jain *et al.* (2015). The activities of antioxidant enzymes in rat kidney were dramatically decreased which may due to induction of oxidative stress which results in inhibition of the activities of antioxidant enzymes. Repeated exposure to IMI or arsenic alone or in combination produces renal damage either due to increased free radical generation, reduced antioxidant status of renal tissue or both. Such alterations were more pronounced in animals exposed concurrently to both the toxicants.

5.2.3 Histopathology alterations in kidney: Microscopic examination of the kidney sections of the IMI administered group revealed slight alterations from the normal renal structure of the normal control rats with no appreciable pathological abnormalities. There was slight shrinkage of glomeruli and very mild degeneration of the epithelial cells in the kidneys. Similarly, Kapoor *et al.* (2010) reported that high doses of IMI (20 mg/kg/day) resulted in mild focal necrosis with swollen cellular nuclei in rat liver and slight degeneration of tubules and glomeruli of kidney of the female rats. Soujanya and Lakshman (2013) reported that kidney sections of male rats treated with 80 mg/kg/day of IMI revealed cystic dilatation of tubules, shrunken glomeruli, vacuolation, inter tubular haemorrhages and hyaline casts. Also, Kammon *et al.* (2010) reported that kidneys of IMI intoxicated chicken showed sub-capsular haemorrhages and coagulative necrosis of the tubular epithelium. In the current study, subacute exposure of different doses of arsenic produces histopathological lesions which become more severe with the increasing doses of the arsenic administered wistar rats in the drinking water. At low dose of arsenic (50 ppb), slight alterations with no appreciable pathological lesions were observed but with the increasing doses, mild to moderate vacuolar degeneration of tubular epithelium becomes more prominent. Such findings are in accordance with Singh and Rana (2007) who reported that the arsenic treated rats demonstrated glomerulonephritis, proximal tubular necrosis, epithelial damage and loss of nuclei. Chowdhury *et al.* (2016) has reported that the arsenic exposure had adverse effects on liver and kidney of wistar rats, revealing moderate to severe histological changes including haemorrhages, infiltration of inflammatory cell, necrosis and desquamation of epithelium. Hemalatha *et al.* (2013) studied the effect of arsenic administration @ 10 mg/kg orally for 4 weeks in wistar rats and histopathological examination of the kidneys of the exposed rats revealed moderate to severe fatty change along with mononuclear cell infiltration in the tubules and reduced glomerular space. Co-administration of IMI and arsenic produces histopathological lesions like degeneration and necrotic changes in tubular epithelial cells, necrosis and infiltration of phagocytic cells, disruption of tubular basement membrane along with edema, haemorrhages and inter-tubular congestion were very prominent which becomes more severe with increasing dose of arsenic in the combination groups.

5.3 Effect on Testes

5.3.1 Effect on antioxidant parameters in testes

5.3.1.1 Effect on non-enzymatic parameters: Free radicals and ROS like hydroxyl ($OH^{\cdot}$), superoxide ($O_2^{\cdot-}$), nitric oxide ($NO^{\cdot}$), hydrogen peroxide (H_2O_2) radicals are the commonly generated by auto-oxidation processes or enzymatic metabolic reactions are responsible for disturbance in antioxidant system of testes. Inability of non-enzymatic components to onslaught these ROS/free radicals produced during metabolism may be responsible for testicular damage as indicated by increased levels of MDA and AOPP in testes on exposure of IMI and arsenic in wistar rats. The increased levels of MDA and AOPP may be due to reduced availability of TTH and free –SH group either due to increased utilization or reduced recycling of these endogenous sulfhydryl compounds. These results are in accordance with the findings of Bal *et al*. (2012), El-Kholy *et al.* (2015), Lonare *et al.* (2015) and Olayinka and Ore (2015). Similarly, subacute oral administration of different doses of arsenic alone leads to fall in TTH levels of the testes of wistar rats and these findings are in agreement with Sudha (2012), Khan *et al*. (2013) and Sumedha and Miltonprabhu (2014). Combined administration of both IMI and arsenic produced more significant fall in TTH levels as compared to administration of either IMI or arsenic in rats. The exposure of rats to IMI and arsenic induces depletion in antioxidants, as indicated with decreased TTH levels in testes because the generation of excessive free radicals/ROS in tissues and sub-cellular compartments is scavenged by the antioxidant defence system (Naziroglu, 2009).

A significant increase in the lipid peroxidation product and protein oxidation product was observed in the IMI alone administered group and these results are similar with El-Kholy *et al.* (2015) and Taib *et al.* (2013) Also, the subacute oral administration of arsenic produced significant rise in MDA and AOPP levels in the testes of wistar rats. Similar observations were also recorded by Muthumani and Miltonprabhu (2012a), Sumedha and Miltonprabhu (2014) and Kumar *et al.* (2013). Co-exposure of both the toxicants produced more significant increase in lipid and protein oxidation levels as compared to individual administration of either toxicant. Since testes are rich in PUFA, they are easily prone to lipid peroxidation (Aitken and Roman, 2008). Peroxidation of membrane lipids can result in disruption of cell structural integrity (Bell *et al*., 1992) and cell damage.

Hence, the increase in MDA and AOPP levels caused by both the toxicants in the rat testes suggests peroxidation of PUFA in testicular cells, which can cause impairment of normal testicular and sperm function (Lenzi *et al*., 2000).

5.3.1.2 Effect on enzymatic antioxidant parameters: In the present study, IMI caused a significant reduction in the antioxidant enzymatic parameters (GST, GR, GPx, SOD and CAT) after repeated exposure for 28 days and these results are in consonance with the studies of Sharma *et al.* (2014), El-Kholy *et al.* (2015), Olayinka and Ore (2015) and Lonare *et al*. (2015). Repeated oral administration of arsenic produces reduction in the antioxidant enzymes activities in the testes of wistar rats. This finding is in consistence with El-Demerdash *et al.* (2009), Muthumani and Miltonprabhu (2012a), Sudha (2012), Khan *et al*. (2013) and Sumedha and Miltonprabhu (2014). Combined administration of both IMI and arsenic to wistar rats produced more significant decrease in antioxidant enzymatic activity than individual administration of either IMI or arsenic. The present findings of decreased antioxidant enzymes may reflect the oxidative damage of cellular membranes of spermatozoa due to free radicals induced by the oral administration of either of the toxicant alone as well as in combination which lead to inhibit their number and increase their abnormality rate (Akunna *et al.*, 2012).

5.3.2 Histopathology alterations in testes: In the present study, the microscopic examination of testes in rats of control group revealed normal mature seminiferous tubules with complete series of spermatogenesis and high spermatozoa concentration in the lumen. However, the individual administration of imidacloprid in rats showed progressive congestion in blood vessels and mild edema in the interstitial space and mild to severe degenerative changes of germ cells in the combination groups of IMI with different doses of arsenic in wistar rats. Present findings are in agreement with studies of Lonare *et al.* (2015) who reported that IMI alone treated rats showed mild edema with reduced or lack of spermatogenesis, depletion of spermatocytes and spermatogenesis varying from weak to arrest in some seminiferous tubules with mild interstitial edema. Also, Zhang *et al.* (2011) reported that the acetamiprid administration produced vacuolization of the seminiferous tubules with reduced number of spermaids and interstitial leydig cells was also evident in the testes of wistar rats. These results are similar with the effects of β-cypermethrin in male mice (Wang *et al*., 2009) while α-

cypermethrin resulted in edema between the seminiferous tubules and vacuolization of the tubules (Manna *et al.*, 2004).

The histopathological examination of testes in arsenic administered rats revealed mild degenerative changes in the seminiferous tubules at low doses of arsenic while higher doses of arsenic administration demonstrate severed degenerative and necrotic changes in the germ cells. These findings are in agreement with Muthumani and Miltonprabhu (2012) who also reported that 5 mg/kg/day administration of arsenic in wistar rats for 4 weeks produced marked disturbances in the normal architecture of the testicular tissues, necrotic spermatogonium, loss of sperm bundles, pyknotic spermatogonia, scanty spermatocytes, disintegrated cyst wall, vacuoles in sertoli cells and giant cell formation. Sumedha and Miltonprabhu (2014) had reported that arsenic administration in rats produced marked disorder of the normal testicular arrangement where seminiferous tubule swell and enlarge with focal haemorrhage and necrosis in the interstitial stromes. Hemalatha *et al.* (2013) reported that the arsenic exposure @ 10 mg/kg produced ruptured follicles and few follicles showed reduction in the number of spermatozoa in the testes of male albino rats. Co-administration of IMI and arsenic showed severe degenerative changes in seminiferous tubules, tubular shrinkage, decreased lumen diameter with generalized necrosis and depletion of germ cells which became more prominent with the increasing concentration of arsenic doses in the combination groups.

CHAPTER VI

SUMMARY AND CONCLUSIONS

It is becoming increasingly evident that environmental exposure to humans and animals are not just limited to single chemicals. Rather, they are exposed concurrently or sequentially to a large number of chemicals from a variety of sources. Thus, the exposure to chemical mixtures is environmental reality. At the present time, issues related to assessing, managing and communicating health risk, associated with exposures to chemical mixtures are becoming steadily more critical from a public health perspective. Pesticides are being used extensively in the field of agriculture, veterinary medicine and public health. Imidacloprid was the first neonicotinoid insecticide introduced and is one of the most widely used pesticide worldwide. Imidacloprid is the world's fastest growing and is considered possible replacement for organochlorine and organophosphorus insecticides in agriculture. Arsenic, a metalloid, found in ground drinking water in many parts of India including Jammu and Kashmir. The largest source of arsenic and other metals is usually drinking ground water and food, of which the main dietary forms are seafood, rice, mushrooms and poultry. In consequence of its widespread use in the microelectronics industry, gallium arsenide is an inorganic arsenic compound which may also impact adversely on the human health.

Therefore, the current study was carried out to determine the alterations in the biochemical and antioxidant indices indicating hepatic, renal and testicular abnormalities along with the histopathological changes in these organs induced by the repeated oral administrations of imidacloprid and arsenic alone as well as in combinations in wistar rats.

Wistar rats of 180-200 g body weight were divided into eight groups comprising six rats in each viz. group I served as control receiving distilled water orally, group II received IMI @ 16.9 mg/kg b.wt, group III, IV and V received arsenic @ 50, 100 and 150 ppb in drinking water orally whereas group VI, VII and VIII received co-exposure of IMI and

arsenic @ 16.9 mg + 50 ppb, 16.9mg + 100 ppb and 16.9 mg+ 150 ppb respectively orally for 28 days of experimental study in wistar rats.

Among the biochemical parameters, the mean values of plasma hepatic biomarker enzymes (SGOT, SGPT, ACP and ALP) were elevated significantly ($P<0.05$) after 28 days of exposure as compared to the control group in all the treatment groups. Similarly oral exposure of both the toxicants alone as well as in combination produced a significant ($P<0.05$) increase in the values of renal biomarkers (BUN and Plasma creatinine) while a significant ($P<0.05$) decline was observed in the total plasma proteins and albumin levels in the higher doses of arsenic alone (100 and 150 ppb) administered groups as well as in co-administered groups as compared to control group.

The findings revealed a significant ($P<0.05$) increase in the levels of malondialdehyde (a lipid peroxidation marker) and AOPP (a protein oxidation marker) while a significant ($P<0.05$) decline in the levels of total thiols (TTH) content was observed in the blood, liver, renal and testicular tissue. There was also significant ($P<0.05$) decrease in the levels of blood and tissues (liver, kidney and testes) antioxidant enzymes viz. blood glutathione (GSH), GST, GR, GPx, SOD and CAT activities in the IMI and arsenic alone as well as in the combination groups.

Histopathological examination of the liver revealed mild degenerative changes in the normal hepatic structure in the IMI administered group while mild to moderate degenerative changes were observed in the arsenic alone treated groups which became more pronounced in a dose dependent manner and also included mild congestion and edema accumulation in the portal triad. In the combination groups, haemorrhages, mild infiltration of inflammatory cells, disruption of hepatic cords and necrosis of hepatocytes was seen which became more severe with the increasing dose of arsenic in the co-administered groups.

Kidneys of the rats exposed to IMI (Group II) and low dose of arsenic (Group III) individually produced no appreciable histological alterations. But higher doses of arsenic alone administered groups had mild vacuolar degeneration of tubular epithelium in the renal parenchyma. In the groups co-administered with IMI and arsenic, degenerative

changes like haemorrhages, moderate inter-tubular congestion (in group VI), cytoplasmic vacuolar degeneration and presence of hyaline casts (in group VII) along with disrupted integrity of basement membrane, severe degeneration and necrosis (in group VIII) was observed.

Microscopic examination of the testes of IMI administered group revealed mild alterations with edematous fluid accumulation in the interstitial spaces while arsenic exposed groups demonstrated degenerative changes in the seminiferous tubules in a dose dependent manner. Co-administration of IMI and arsenic in the rats revealed severe degenerative changes like degeneration of germ cells, tubular shrinkage and generalized necrosis of the germ cells.

Based on the findings of the present study, it is revealed that individual exposure of IMI @ 16.9 mg/kg b.wt/day as well as different doses of arsenic (50, 100 and 150 ppb) along with co-administered groups leads to alterations in the biochemical as well as antioxidant parameters in the wistar rats which contrives to toxicities viz. hepatotoxicity, nephrotoxicity and reproductive abnormalities. Also, IMI, arsenic and their combined administration caused histopathological alterations in the visceral organs viz. liver, kidney and testes most probably due to induction of oxidative stress.

CONCLUSIONS

- Administration of Imidacloprid and/or arsenic (100 and 150 ppb) induced significant biochemical alterations indicative of hepatic and renal damage.
- Exposure to either toxicant resulted in altered antioxidants parameters in blood and different tissues viz. liver, kidney and testes.
- Combined use of toxicants induced more prominent alterations in antioxidant and Histopathological changes in hepatic, renal and testes as compared to individual use of toxicants.
- Metabolism of these toxicants clearly indicates the generation of free radicals as evident by reduced antioxidant enzyme activities resulting in oxidative stress.

- Imidacloprid induced significant alterations in biochemical and antioxidant enzyme indices in blood and tissues. Arsenic was found to have a potentiating effect on such changes.

CHAPTER-VII

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Certificate – IV

Certified that all the necessary corrections as suggested by the external examiners / evaluators and the advisory committee have been duly incorporated in the thesis entitled **"Alterations in biochemical and oxidative biomarkers in subacute oral toxicity of imidacloprid alone and in conjunction with arsenic in wistar rats"** submitted by **Lakshay Mahajan**, Registration No **J-15-MV-433.**

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No.: VPT/FVSJ/2017-18/438
Dated: 22.09.2017

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