SUB-ACUTE TOXICITY OF MANCOZEB ALONE AND IN-COMBINATION WITH ARSENIC IN WISTAR RAT

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(Registration No. J-20-MV-642)

A Thesis submitted to
Faculty of Post Graduate Studies
in partial fulfillment of the requirements
for the degree of

MASTER OF VETERINARY SCIENCE
IN
VETERINARY PHARMACOLOGY AND TOXICOLOGY

Division of Veterinary Pharmacology and Toxicology
Sher-e- Kashmir University of Agricultural Sciences and Technology of Jammu
Main Campus, Chatha, Jammu- 180009

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CERTIFICATE – I

This is to certify that the thesis entitled **"SUB-ACUTE TOXICITY OF MANCOZEB ALONE AND IN-COMBINATION WITH ARSENIC IN WISTAR RAT"** submitted in partial fulfillment of the requirements for the degree of **Master of Veterinary Science** in **Veterinary Pharmacology and Toxicology,** to the **Faculty of Veterinary Sciences and Animal Husbandry,** Sher-e- Kashmir University of Agricultural Sciences and Technology of Jammu, is original work and has similarities with published work not more than minor similarities as per UGC norms of 2018 adopted by the University. Further the level of minor similarities has been declared after checking the manuscript with **URKUND** software provided by the University.

The work has been carried out by **Miss. Rasia Yousuf**, Reg. No **J-20-MV-642** 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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Rasia Yousuf

Rasia Yousuf

Place: R S Pura- Jammu

Date: 19.9.2022

ABSTRACT

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ABSTRACT

The aim of present study was to determine the impact of toxic interaction between Mancozeb (MZ) and arsenic (As) on erythrocytes, brain, liver, kidney and testicular tissue of rats and to compare ameliorative potential of quercetin and catechin against the induced toxicity. Sixty adult male rats were randomly allocated into 10 groups with 6 animals in each. Group I served as control, group II was exposed with MZ (800 mg/kg, b. wt, PO). Group III, IV and V were exposed with drinking water containing sodium arsenite at the rate of 10, 50 and 100 ppb continue for 28 days, respectively. Group VI, VII and VIII were treated with sodium arsenite containing drinking water at 10, 50 and 100 ppb respectively along with MZ for 28 days. Group IX and X were given sodium arsenite in drinking water at 100 ppb and MZ at 800 mg/kg, b. wt. PO along with quercetin and catechin at the same dose (50 mg/kg), respectively. A significant ($p<0.05$) alteration was seen in hepatic, renal biomarkers along with alterations in the tissue antioxidant status in different tissues of MZ treated rats in comparison to control. Exposure to different doses of arsenic (10, 50, 100ppb) also produced a dose dependent effect on these biomarkers. Co-exposure of toxicants potentiated the toxic effects exhibited by biochemical, oxidative and histopathological alterations in different tissues. In contrast, administration of toxicants along with quercetin or catechin markedly attenuated the alterations in biochemical, oxidative biomarkers and histopathological alterations.

Keywords: Arsenic, Mancozeb, Quercetin, Antioxidant, Catechin.

Signature of Major Advisor

Rasia Yousuf

Signature of Student

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ABBREVIATIONS AND SYMBOLS USED

Abbreviations	Meaning
%	per cent
<	Less than
>	Greater than
µl	Microliter
^{0}C	Degree Celsius
AChE	Acetylcholine esterase
AE	Aryl esterase
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
AOPP	Advanced oxidation protein products
As	Arsenic
AST	Aspartate aminotransferase
ASTDR	Agency for toxic substances & disease registry
AST	Aspartate aminotransferase
BUN	Blood urea nitrogen
BW	Body weight
CAT	Catalase
CR	Creatinine
Dl	Deciliter
DTNB	5,5- dithiobis – (2-nitrobenzoic acid)
EBDC	Ethylene bisdithiocrabamate
ETU	Ethylene thiourea
EDTA	Ethylene diamine tetra-acetic acid
EPA	Environmental Protection Agency
GGT	Gamma glutamyl transferase
GLO	Globulin
Gm	Gram(s)
GPx	Glutathione peroxidase
GSH	Reduced blood glutathione
GST	Glutathione-S-transferase
GR	Glutathione reductase
hr	Hour

Hb	Hemoglobin
H & E	Hematoxylin and Eosin
H_2O_2	Hydrogen peroxide
Kg	Kilogram
LDH	Lactate dehydrogenase
MDA	Malondialdehyde
mg	Milligram
min	Minute
ml	Milliliter
MZ	Mancozeb
NADH	Nicotinamide adenine dinucleotide sodium salt reduced
NO	Nitric oxide
PBS	Phosphate buffered saline
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
SOD	Superoxide dismutase
TAS	Total Antioxidant Status
TBA	Thiobarbituric acid
TPP	Total plasma proteins
TTH	Total thiols
UA	Uric acid
ug	Microgram
US	United States
WHO	World Health Organization

Chapter-I

Introduction

Chapter 1

Introduction

Agricultural set-up is changing very fast in modern day agricultural system with a need to produce high quality and quantity of grains per unit area to feed huge world population. For this we need effective management of plant diseases which contribute to 26% of food crop loss. Plant protection strategies have assumed significance in the overall crop production programmes for sustainable agriculture (Goswami *et al*., 2018). Among plant protection strategies, fungicides serve as an important management tool, used widely in disease control and contribute to more than 35% of the pesticide market worldwide. Geographically, Europe is said to be the predominant market of fungicides with major applications on grains, cereals, fruits and vegetables (Zubrod *et al*., 2019). Although they are successful in controlling plant diseases but their excessive, irrational and indiscriminate use causes potential environmental and human health hazards. The major problems include residue accumulation, resistance build up and impact on non-target micro-flora (Goswami *et al*., 2018; Zubrod *et al*., 2019). According to estimate, only around 0.1% of pesticides is able to reach the intended organism, and the rest is believed to reach the environment causing harmful effects (Nayak and Solanki, 2021). As compared to insecticides and herbicides, fungicides have been reported to induce more non-target harmful effects on soil microbial communities (Chen *et al*., 2001; Cycon *et al.,* 2006; Munoz-Leoz *et al*., 2011). Despite the potential of ecotoxicological risks in non-target aquatic systems due to intensive use of fungicides, their environmental fate and effects have received far less attention when compared to insecticides and herbicides. For instance, only 13% of the studies between 1991-2013 focused on fungicides while as 62% and 24% focused mainly on insecticides and herbicides, respectively (Zubrod *et al*., 2019).

Fungicides are biocidal chemical compounds used to kill parasitic fungi or their spores which cause serious damage in agriculture resulting in critical losses of yield (7.0 – 24.0%), quality and profit. Fungicides are used both in agriculture and to fight fungal infections in animals. Prior to 1882, fungal pests were controlled using Bordeaux mix, sulfur and copper and then up to 1934 diseases were controlled using

organo-metallics. It was in 1931 that the first patented fungicide of Ethylene bisdithio carbamate (EBDC) group named thiram gained prominence. Among the various fungicides used globally, EBDCs most important and extensively used group for more than 80 years. They were originally used as vulcanization accelerators in the rubber industry but in the recent years, their applications has become apparent as fungicides (Kanchi *et al*., 2013). According to Word Health Organization (WHO), most of the EBDCs applied as fungicides are classified as hazardous. These chemicals have been reported to be toxic to human beings when consumed orally. The symptoms of poisoning associated with EBDC compounds include irritation of eyes, skin and respiratory tract; chronic skin disease has also been reported in occupationally exposed workers. These are found to be teratogenic, carcinogenic, mutagenic and reproductive toxicant (Preeti *et al.*, 2015). Mancozeb which belongs to EBDC category of fungicide has been one of the most commonly used fungicide in commercial use for several decades (Belpoggi *et al*., 2002).

Mancozeb is [[1,2-ethanediylbis] carbamodithiote] (2-) manganese (maneb) mixture with [1,2-ethanediylbis] carbamodithiote] (2-) zinc (zineb), that is combination of other two ethylene-bis- dithiocarbamate: maneb and zineb, hence the name mancozeb. The other members of this family include thiram, ferbam, nebam, maneb, metiram, propineb and zineb (Gullino *et al.*, 2010). Because of its broad-spectrum activity against fungi, low toxicity in humans and short environmental persistence, mancozeb (MZ) is used globally (Pavlovic *et al*., 2015). In Kashmir Valley, the fungicide has found an important place and is being used for more than 30 years for various fungal diseases of apple like green tip, scab, fruit rot etc. Although the high-risk group include the producers of the compound, agricultural workers, residents living in agricultural areas where the fungicide is sprayed, the general population can also be affected by residues present in food (Rossi *et al.*, 2006). MZ has low solubility in water with field half-life of 1-7 days, but rapidly hydrolyses over a wide pH range into many metabolites. The most important metabolite being ethylene thiourea (ETU) which has high-water solubility, is potentially mobile through the soil, can contaminate the ground water. Residues are being detected in fruits and vegetables on regular basis with significant amount of ETU being produced during cooking of contaminated vegetables (Belpoggi *et al.*, 2002; Corsini *et al.*, 2006). MZ is a contact fungicide having low acute toxicity with LD_{50} value of 8

g/kg/day in Wistar rats (Edward *et al*., 1997). Despite of its low toxicity value in mammals, MZ has been shown to cause adverse health effects in both humans and experimental animals. It has been associated with pathomorphological changes in liver, kidney and brain. The widely used fungicide MZ has been shown to cause hypothyroxinaemia in adult experimental animals. Studies also had shown teratogenic and reproductive effects of MZ in animals. Inhibition of implantation by MZ due to its toxic effects or by hormonal imbalances has been studied (Preeti *et al.*, 2015). The metabolite ethylene thiourea (ETU) is a potential carcinogen that exerts various toxic effects in rats, including thyroid neoplasms (goitrogenic), developmental toxicity and teratogenicity (Axelstad *et al*., 2011; Goswami *et al*., 2018). Moreover, MZ exposure has been reported to reduce growth, survival and immunity with increased malformations, oxidative stress and altered behavior in vertebrates in the range of µg/L (Zubrod *et al*., 2019). MZ has a characteristic of a typical multi-site protectant type action and can be considered a pro-fungicide which on being exposed to water and UV light releases ethylene bisisothiocyanate sulphide (EBIS) and ethylene bisisothiocyanate (EBI) respectively. Both EBIS and EBI are active toxicants and thought to be involved in interfering with enzymes containing sulfhydryl group and thus inhibit different biochemical processes within cytoplasm and mitochondria of fungal cell (Gullino *et al*., 2010). MZ has been found to produce toxicological manifestations in different cells by formation of reactive oxygen species (ROS) which is due to its chemical structure, since it contains transitional metals known to induce ROS production through Fenton reaction (Calviello *et al*., 2006). The production of ROS and subsequent oxidative stress has been found as the most probable mechanism of MZ induced toxicity (Domico *et al*., 2007; Bailey *et al*., 2016). Additionally, the inactivation of sulfhydryl group and production of ROS are responsible for producing toxicities such as reproductive toxicity, hepatotoxicity, neurotoxicity, nephrotoxicity, genotoxicity and carcinogenicity in various laboratory animals.

Arsenic

Arsenic is the 20th most abundant element in earth's crust, 14th in sea water and 12th in our body (Jomova *et al*., 2010). The Greek physicians such as Hippocrates and Galen popularized the use of arsenic as a healing agent. Arsenic compounds became available as solutions, pastes, tablets and in injectable forms. Fowler's solution (1% arsenic trioxide) was used widely in 19th century for leukemia, skin

conditions, stomatitis, angiosarcoma of the liver and nasopharyngeal carcinoma. Arsenic as Arsphenamine was primary treatment for syphilis until World War 2. Arsenic trioxide is now widely used to induce remission in patients with acute promyelocytic leukemia. Arsenic continues to be an essential constituent of many non-western traditional medicine products like some Chinese medications contain realgar (arsenic sulphide). In India, herbal medicinal preparations of arsenic are used in homoeopathy and hematological malignancies. In Korea, herbal medicine containing arsenic are used to treat hemorrhoids. However, arsenic is more often a contaminant rather than being an intended ingredient, with lead and mercury (Ratnaike, 2003).

Arsenic is one of the most hazardous metalloids derived from natural environment and a potential human carcinogen, ranking as first on the US EPA and US Agency for Toxic Substances and Disease Registry (ATSDR) and Priority List of Hazardous Substances since 1997 (Coryell *et al*., 2018). The International Agency for Research on Cancer (IARC) classifies inorganic arsenic (iAs) in Group 1, carcinogenic to humans. The maximum limit set by WHO and EPA for arsenic in drinking water is 10 ppb and for Asian countries like Bangladesh, India the nationally accepted level is 50 ppb (Islam and Islam, 2010; Nurchi *et al.*, 2020). The countries which are at considerable risk include Bangladesh, Taiwan, India, Mexico, China, Chile, Argentina and USA (Singh *et al.*, 2011). However, Bangladesh and India (West Bengal) are the worst affected countries where the groundwater arsenic concentrations are above the recommendations set by WHO. In both the areas, the source of arsenic is geological in origin, contaminating aquifers which provide water for over million tube wells. According to an estimate 59 out of 64 districts of Bangladesh are arsenic contaminated in Bangladesh and WHO called the arsenic disaster of the country as "the largest mass poisoning of a population in history" (Ghosh *et al*., 2012). In West Bengal, the arsenic concentration in some tube wells is as high as 3400 ppb and at least six districts including with 30 million population are affected by this problem (Behari and Prakash, 2006). Exposure depends upon location of the population, their life style and diet. On exposure, skin lesions, cardiovascular diseases, reproductive problems, psychological, neurological and mental health problems have been reported in different countries (Chandravanshi *et al*., 2018).

Arsenic (As) poisoning or arsenicosis, the outcome of groundwater contamination with arsenic, has been increasing in an alarming manner since its detection in 1993 (Khan *et al*., 2003). Toxicity effects may range from acute toxicity which manifest as gastrointestinal symptoms along with encephalopathy and peripheral neuropathy to various levels of long term toxicity effects (Singh *et al*., 2011). The potential of arsenic to act as a carcinogen is of serious concern as it increases risk of various cancers such as those of skin, kidney, urinary bladder, lungs and prostate along with type 2 diabetes and depression (Mazumder, 2008; Sodhi *et al*., 2019). It can cross blood brain barrier and deposit in parts of brain tissue, such as cortex, cerebellum, striatum, hypothalamus and hippocampus resulting in neurobehavioral disorders (Tripathi *et al*., 1997; Peruru *et al.*, 2020 Thakur *et al*., 2021; Li *et al*., 2021a). Arsenic leads to detrimental impacts on physiology and morphology of brain cells. Neural tube defects, still births, spontaneous abortion and neonatal deaths were reported in pregnant women exposed with arsenic contaminated drinking water. Children with chronic exposure have difficulties in cognitive behaviour, memory and verbal intelligence (Ehrenstein *et al.*, 2007; Brinkel *et al*., 2009; Chandravanshi *et al*., 2018). Arsenic toxicity, if diagnosed late or left untreated, can cause high degree of mortality (Susan *et al.*, 2019).

The mechanisms of arsenic toxicity are complex and vary with the chemical form of As. At biochemical level, arsenate substitutes phosphate and result in inhibition of many enzymes and several reactions in the mitochondria, while as arsenite and its methylated form (trivalent organic) react with thiol (-SH) in proteins and inhibit their activity. It also causes inactivation of various cellular enzymes, particularly those involved in cellular energy pathways and DNA replication and repair. Since the trivalent form has higher affinity for thiol groups, it inhibits enzymes like glutathione peroxidase, reductases, thioredoxin and lipoic acids and cysteinyl residues of enzymes (Sodhi *et al*., 2019). Unbound arsenic also exerts toxicity by producing ROS during their redox cycling and metabolic processes that cause lipid peroxidation by oxidative stress (Ratnaike, 2003). The ROS generation and consequent oxidative stress is the most important mechanism of As toxicity particularly in cancer of skin and other organs where it leads to instability of mitochondrial DNA (Modica-Napolitano and Singh, 2002) (Rao and Balachandran, 2002). The generation of ROS disturb cellular signalling pathways and epigenetic modifications or cause direct damage to

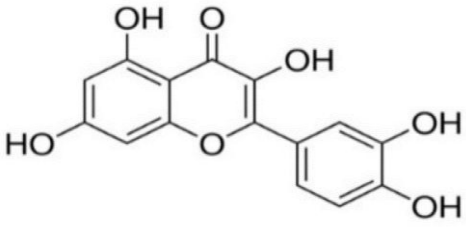
macromolecules (Hu *et al*., 2020). Other mechanisms thought to play role are epigenetic alteration, inflammation and autophagic defects.

Now days, much attention is being paid to the role of naturally occurring dietary substances for the control and management of toxicities. Phenolic compounds *viz*. quercetin and catechin, plant secondary metabolites, are widely distributed in fruits and nuts and used since ancient times for promoting health. Catechin consists of flavan-3-ols, which is included in the chemical family of flavonoids (Wongmekiat *et al*., 2018). These are widely distributed in nature and an important constituent of tea, apples, red wine, cacao with numerous health benefits (Manach *et al*., 1999). They are mainly composed of four kinds of compounds such as epicatechin (EC), epicatechin-3- gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG). Among all, EGCG is the most abundant kind of tea catechins responsible for majority of the biological actions. The versatile structures of catechins endow them with strong antioxidant activity. Because catechins have ortho dihydroxy or ortho trihydroxy in their structure, they could form half quinone free radicals which upon acting on free radicals lead to their inactivation. The antioxidant properties of catechins are also due to their redox properties, which let them act as reducing agents by donating hydrogen and by acting as singlet oxygen quenchers (Grzesik *et al*., 2018). Both clinical and epidemiological studies have found catechin to be effective as antioxidant, anti-inflammatory, anti-bacterial, anti-viral, immuno-stimulatory, cardio-protective, diuretic, anti-carcinogenic, anti-mutagenic, anti-tumorigenic, anti-allergic, anti-diabetic, anti-hypertensive, anti-thrombogenic, and anti-hyperlipidemic (Uzun *et al*., 2010; Bharrhan *et al*., 2011; Chen *et al*., 2016; Wongmekiat *et al.,* 2018; Cosarca *et al*., 2019). Previous studies suggested that catechin possesses neuroprotective properties in neurodegenerative diseases, like Alzheimer's and Parkinson's diseases, because of its transitional metal (iron and copper) chelating property and antioxidant activity. These properties indicate that catechin might also play a neuroprotective role in brain injuries. Pre-treatment with catechin hydrate resulted in neuroprotective effects via nuclear factor-κB-mediated down regulation of the inflammatory response in a rat model of middle cerebral artery occlusion (Jiang *et al*., 2017). Catechins also play pivotal role in maintaining homeostasis and its derivative especially epigallocatechin gallate would inhibit aggregation of platelets. Along with chemotherapy, catechins are synergistically effective in alleviating various forms of

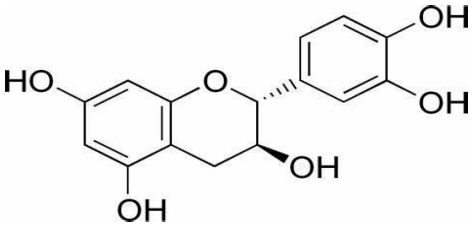
neoplasia (Cheruku *et al*., 2018; Samarghandian *et al.,* 2017; Cosarca *et al*., 2019). Green tea catechins are best studied for its anti-cancer activity and are associated with moderate reduction in risks of cancers like esophageal, stomach, colorectal and prostate (Cheng *et al*., 2020)

Similarly, quercetin (3,5,7,3^0,4^0-pentahydroxyflavone) has a polyphenolic structure and a common flavone nucleus composed of two benzene rings linked via a heterocyclic pyrone ring. It is widely distributed in kingdom plantae and commonly found in apples, teas, citrus fruit, broccoli, mulberry, potato, brassica vegetables and onions and a low level of this flavanol are also found in red wine and tea (Manach *et al*., 1999; Uzun *et al*., 2010: Baltaci *et al*., 2016). Quercetin occurs as a condensation product of p-glycosides and is responsible for more than half of the daily flavanol and flavone consumption. It is usually obtained from the hydrolysis of rutin (quercetin-3-rutinoside) which is naturally occurring flavonoid glycoside, although it can also be obtained synthetically. It has an important place in human nutrition and has been used in traditional medicine as prophylactic or to treat a variety of diseases, including cancer, obesity, chronic inflammation, cardiovascular and nervous disorders (Zhang *et al*., 2011; Satyendra *et al*., 2012). In the previous studies, it has been shown that quercetin has anti-apoptotic, antioxidant, anticancer, antiulcer, antibacterial, antiviral, anti-allergic, anti-inflammatory and cardioprotective activities and also has cataract preventive effects (Uzun *et al*., 2010; Bu *et al*., 2011; Baltaci *et al*., 2016). *In vitro* studies revealed that quercetin may be effective in treatment of various types of cancers and can be combined with other anticancer drugs to reduce their doses (Satyendra *et al*., 2012). Although, its biological properties and underlying mechanism of action are largely unknown. But similar to other flavonoids, it is an important research subject due to its free radical scavenging property and anti-inflammatory effect in various *in-vitro* and *in-vivo* studies. Due to its chelating property and free radical scavenging ability, quercetin can prevent oxidative damage to DNA and also to cell membranes thus stabilizing lipid membranes by preventing lipid peroxidation. Additionally, quercetin is also known to reduce ROS production, increases Mn SOD (manganese superoxide dismutase) activity and glutathione level (Nirankari *et al*., 2016**)**. The anti-inflammatory effect is mediated by several mechanisms such as inhibition of nuclear factor-kappa B (NF-kappaB), suppression of over-expression of the inducible form of nitric oxide synthase (iNOS), activation and

modulation of C-reactive protein (CRP) expression and cyclooxygenase-2 (COX-2) (Zhang *et al.*, 2011).



Catechin ($C_{15}H_{14}O_6$)



Quercetin ($C_{15}H_{10}O_7$)

Chemical structures of polyphenolic compounds *viz.* catechin and quercetin.

Individual toxic effects of mancozeb and sodium arsenite have been well documented, and studies have also highlighted the therapeutic potential of variety of natural agents in protecting against the toxicity induced by either of these toxic agents. There is however, a dearth of information on the protective potential of natural agents against multiple toxic exposure. Therefore, present study was designed to evaluate the ameliorative role of antioxidants like catechin and quercetin against MZ toxicity or arsenic toxicity alone or in combination. We investigated whether the antioxidant and other pharmacological potential of catechin and quercetin can attenuate sub-acute exposure of these toxicants.

The study was thus conducted with the following objectives:

1. To evaluate biochemical, antioxidant and histopathological alterations in visceral organs following sub-acute exposure of mancozeb in Wistar rats.
2. To evaluate biochemical, antioxidant and histopathological alterations in visceral organs following sub-acute exposure of arsenic in Wistar rats.
3. To evaluate biochemical, antioxidant and histopathological alterations in visceral organs following sub-acute exposure of mancozeb and arsenic in Wistar rats.

Chapter-II

Review of Literature

Chapter 2

Review of Literature

Humans and animals are exposed to a wide range of environmental contaminants. The existence of myriad of pollutants in the environment and further addition of such agents with each passing day, exposure to complex mixture of environmental pollutants and toxic agents is the rule rather than the exception. Environmental pollutants including pesticides and heavy metals are commonly known to cause serious health hazards via the induction of wide range of toxicological events and biochemical dysfunctions (Kayode *et al.,* 2019). From last two decades, the use of pesticides has been a substantial increase in the agriculture industry to either improve yield or to secure yields from insects, pests and fungus. Pesticides are also used for the management of external parasites infestation and control vector borne diseases. More than 1000 active ingredients are used in pesticides around globe to ensure food safety and highest amount is spent on herbicides (~45%) followed by insecticides, fungicides, and other pesticides. Pesticide exposures and their residual concentrations have been linked to many human diseases such as Alzheimer, Parkinson, amyotrophic lateral sclerosis, asthma, bronchitis, infertility, birth defects, autism, attention deficit hyperactivity disorder, diabetes, obesity and various types of cancers of visceral organs (Zubrod *et al*., 2019). Dithiocarbamate is a functional group in organic chemistry and an analogue of a carbamate in which oxygen atoms are replaced by sulphur atoms. The MZ which also belongs to EBDC category is being used globally as one of the major fungicides (Gullino *et al*., 2010). Some earlier studies on exposure to MZ and heavy metals have reported various toxicological effects such as immunotoxin, hematologic, hepatic, renal, reproductive and developmental toxicity (Hass *et al*., 2012; Jacobsen *et al*., 2012; Runkle *et al*., 2017; Yahia *et al*., 2019; Dallognol *et al*., 2021). However, there is a paucity of information on the co-exposure of MZ and As together and use of natural agents in attenuating or ameliorating these toxic effects.

2.1. Mancozeb (MZ): Mancozeb (MZ) has been an invaluable tool to the growers around world faced with the challenge of controlling fungal pests in their crops. Since its introduction by Rohm and Haas in 1962, it has been found to be efficient for over

70 crops and 400 different diseases (Gullino *et al*., 2010). However, it is particularly used against the pathogen *Glomerella cingulata* responsible for the rot of ripe grape (da Silva Gundel *et al.*, 2019). It grew very fast and is now the world's second leading fungicide after azoxystrobin. The global production of MZ already exceeds 200,000 tons. At present, India is not only leading producer, but also has the leading consumption market accounting over 28% of the global market. According to national pesticide production data from the Ministry of Chemicals and Fertilizers of India, the country's MZ output in 2018 was 82,000 tons.

Calviello *et al*., 2006 studied effect of MZ on the fibroblasts and peripheral blood mononucleated cells (PBMC) isolated from Wistar rats. Both the cells produced dose-dependent induction of DNA single strand break (SSB) formation, measured via single cell gel electrophoresis (SCGE). There were elevated levels of oxidative markers of DNA oxidation, 8-hydroxy-2'-deoxyguanasine (8-OHdG) and of ROS, indicating pro-oxidant action of MZ. The extent of damage and oxidative insult was more pronounced in fibroblasts than PMBC. Srivastava *et al*., 2012 studied effect of MZ on cultured human lymphocytes (CHLs) and observed significant dose dependent increase in the frequency of chromosomal aberrations (CAs) and micronuclei (MN), suggesting the genotoxic potential of MZ. Simultaneously, pro-oxidant potential of MZ was reported due to increase in the levels of ROS. Further it was observed that MZ causes down-regulation of anti-apoptotic proteins and up-regulate the expression of apoptotic proteins, indicating that MZ act as proapoptotic in CHLs. A study by Lorio *et al*., 2015 of MZ exposure on mouse granulosa cells (GCs) also revealed ROS involvement in causing decreased p53 content, GSH levels, ATP and a depolarized membrane potential in GCs, suggesting low doses of MZ induces a mild oxidative stress. Balaji *et al*., 2014 studied the protective effect of quercetin against oxidative stress induced by MZ in human erythrocytes. Observations revealed quercetin reversed the elevated levels of lipid peroxidation (LPO) and decreased levels of SOD, catalase (CAT), glutathione (GSH) and glutathione peroxidase (GPx) in erythrocytes induced by MZ. Pavlovic *et al*., 2015 studied effect of ascorbic acid on MZ-induced toxicity in rat thymocytes and results revealed MZ treated cells showed concentration-dependent increase of hypodiploid cells and ROS generation with decrease in viability of cells, mitochondrial membrane potential and ATP levels. Application of ascorbic acid reduced these effects in cells particularly in those treated with lower MZ

concentration. Aparna *et al*., 2021 studied the amelioration effects of quercetin against methotrexate induced toxicity in Wistar rats and it was revealed that pre-administration of quercetin improved body weights and haematological parameters.

Brody *et al*., 2013 observed the exposure of MZ disrupted key behaviours in round worm (*Caenorhabditis elegans*), the important being dopamine-mediated swim to crawl locomotory transition behaviour which precede neuronal structural damage, indicating behavioural dysfunction are a sensitive early marker of MZ toxicity. A study by Domico *et al*., 2007 also suggested that MZ exposure caused generation of ROS and free radicals are involved in producing toxicity and the damage to mesencephalic dopamine and GABA cell populations were markedly attenuated when carried out in presence of ascorbate. Tsang and Trombetta, 2007 evaluated the neurotoxic potential of MZ in rat hippocampal astrocytes exposed for 1 hour and protective role of various antioxidants. The cytotoxicity of MZ was found to be dose dependent and among various antioxidants used, butylated hydroxytoluene (BHT) was reported to be most protective against MZ insult. Cheruku *et al*., 2018 showed catechin caused a significant restoration of time-induced memory deficit induced by doxorubicin in Wistar rat model (*in vivo* and *in vitro*). Additionally, catechin lead to significant decrease in oxidative stress, AChE and neuroinflammation in the cerebral cortex and hippocampus in doxorubicin induced toxicity model. Josiah *et al*., 2022 found catechin and quercetin possess potential relevance in treatment of Parkinson's disease through optimization of dopamine metabolism, alleviation of redox stress, modulation of anti-inflammatory and anti-apoptotic pathways in rotenone-induced Parkinsonism in male Wistar rats.

Dallognol *et al*., 2021 and Yahia *et al*., 2019 proposed systemic effects of the MZ and found that it causes various health issues including hepatic, renal and genotoxicity which was demonstrated with increase in indicator enzymes like AST, ALT, urea, creatinine of concerned organs. Additionally, in latter study, histological changes in the colon and liver of Sprague Dawley rats were noticed with marked increase in triglycerides and total cholesterol and decrease in glucose levels, accompanied by DNA damage in liver and colon. Adjrah *et al.,* 2013 proposed a study on toxic potential of MZ treated lettuce (*Lactuca sativa*) on rat liver and found increased levels in plasma transaminases, alkaline phosphatase, and total bilirubin, indicating the vegetable in not fit for human consumption. Simakani *et al*., 2018 studied MZ

exposure and its biochemical effects in *Cyprinus carpio* (common carp) and found increase in plasma glucose, decrease in plasma proteins like albumin and globulin with increased ALT and AST levels. Hashem *et al*., 2018 studied the protective potential of *Nigella sativa* oil (NSO) against carbendazim and MZ induced hematotoxicity, hepatotoxicity and genotoxicity in female Sprague-Dawley rats and found pre-treatment with NSO diminished the elevated liver enzymes, DNA damage, micronucleus incidence and lipid peroxidation and also normalized the structural architecture of hepatic tissue. Sakr, 2007 reported ameliorative effects of ginger (*Zingiber officinale*) on MZ induced hepatic injury on albino rats. According to the study ginger improved the histological picture of MZ treated rats, showing marked decrease in alanine aminotransferases (ALT), aspartate amino transferase (AST) activity and serum malondialdehyde (MDA) level with increased serum level of superoxide dismutase (SOD), proving ameliorative effect of ginger against oxidative damage caused by MZ is mainly mediated by its potent antioxidant potential. Another study by Saber *et al*., 2019 on MZ exposure in presence of curcumin in rats revealed curcumin mitigated the increased activities of liver function markers in serum, pro-inflammatory mediators, lipid peroxidation and DNA damage parameters in hepatocytes, accompanied by improvement in depleted hepatic antioxidant markers, together with protection against histological alterations elicited by MZ treatment. Uzun and Kalender, 2013 found catechin and quercetin significantly decreased chlorpyripos induced hepatotoxicity in rats as revealed from biochemical markers, oxidative paramters and histopathological alterations. A study by Owumi *et al*., 2019 suggested that quercetin suppressed carbendazim mediated increase in tumour necrosis factor α, interleukin-β and caspase-3 significantly in the liver and kidney of the rats. The ameliorative effect was also manifested by histopathological examination by improvement in hepato-renal injury. Izak-Shirian *et al*., 2022 suggested that quercetin has ameliorative effects against diclofenac-induced renal injury in the rat model through mitigation of inflammatory pathway and by modulation of oxidative stress. Quercetin proved a successful candidate in restoring GSH, GPx, SOD and CAT levels of renal tissue.

Varous studies revealed revealed that mixture of five pesticides like epoxiconazole, mancozeb, prochloraz, tebuconazole and procymidone caused adverse developmental toxicity effects and reproductive and neurobehavioral effects respectively even at low

doses in rats, suggesting that there is utmost need to evaluate the risk assessment of multiple exposure of pesticides (Hass *et al*., 2012 and Jacobsen *et al*., 2012). Runkle *et al*., 2017 proposed a review on MZ toxicity and found *in vitro* and *in vivo* studies with support of epidemiological studies suggested that MZ should be considered a developmental as well as reproductive hazard in humans. Bianchi *et al*., 2020 studied association between female reproductive health and MZ through various *in vitro* and mammalian models and results revealed MZ as a powerful threat to female reproductive competence from a cellular to a molecular level. Paro *et al*., 2012 studied the effect of MZ on the mouse and human ovarian somatic granulosa cells and reported both the types of cells showed dose-dependent morphological changes and reduced p53 expression levels with no change in apoptosis. Mahadevaswami *et al*., 2000 and Baligar *et al*., 2001 studied the effects of MZ on ovary of Wistar rat and results revealed decrease in both number of oestrous cycles and duration in all stages except that of dioestrus. There was increase in number of atretic follicles and decrease in healthy follicles at higher doses of MZ. Kackar *et al*., 1997a studied chronic effects of orally administered MZ on gonads of male rats and reported noticeable increase in testes weight and decrease in epididymis weight. Changes like degeneration in seminiferous tubules and epididymal tubules were observed with loss of sperms, corroborated with decrease in gonadal acid phosphatase (ACP), succinic dehydrogenase (SDH) and increase in alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and elevated serum cholesterol. Ksheerasagar *et al*., 2003 observed the effects of MZ on testes and accessory reproductive organs administered orally at 800 mg/kg body weight to male Swiss albino mice. There was significant decline in weight of testes and prostate gland in groups treated for 20 and 30 days, while the weight in case of cowper's gland decreased only in the group treated for 30 days with decrease in number and diameter of spermatogenic cells. Joshi *et al*., 2005 evaluated the impacts of MZ on testis of Wistar male albino rats and observed significant decline in weight of testis, epididymis, seminal vesicle and ventral prostate with marked reduction in weight of epididymis and testis. There was noticeable decline in glycogen and sialic acid of testis with increase in protein and cholesterol content. MZ exposure also increased ACP and declined ALP and testosterone levels. Girish *et al*., 2018 found that forskolin showed protective effect against MZ-induced reproductive toxicity in adult male Wistar rats with significant decrease in sperm

production, sperm count and reduced levels of dehydrogenases (3β and 17β-hydroxysteroid dehydrogenases) and testosterone in MZ-treated rats. Additionally, there was significant decrease in GSH, SOD, CAT and increase in LPO in testes and epididymis and all the negative changes were mitigated by co-administration of forskolin. Sardoo *et al*., 2018 studied sub-chronic effects of MZ on testis of male albino mice administered orally at different doses and protective role of N-acetylcysteine. MZ caused dose dependent decrease in sperm motility and count with increase in lipid peroxidation, reduced antioxidant enzymes activities like GSH content, indicating MZ induced oxidative damage in testes causing apoptosis. N-acetylcysteine showed ameliorative role in reducing oxidative stress by regulating enzymes at molecular level. Aziz *et al*., 2018 found ameliorative potential of quercetin and L-carnitine against atrazine-induced reproductive toxicity in male Albino rats. Co-administration with low dose of quercetin and high doses of L-Carnitine counteracted the negative effects of atrazine on serum oxidative stress indicators. Akinmoladun *et al*., 2020 found ameliorative effects of catechin, quercetin, and taxifolin against rotenone-induced toxicity and revealed improvement in testicular weight, spleenic weight and oxidative damage in rats. Taxifolin and quercetin proved better than catechin against rotenone toxicity.

2.2. Other reported Toxicities: Kackar *et al*., 1997b studied chronic oral administration of MZ caused significant increase in thyroid/body weight ratio and dose-dependent pathomorphological changes in the thyroid gland in rats. There was a decline in levels of thyroid radioiodine uptake, serum protein bound iodine, thyroxine and thyroid peroxidase, suggesting MZ produces significant structural and functional alterations in thyroid of rats. Belpoggi *et al.*, 2002 studied long term effects of MZ on Sprague-Dawley rats and concluded that MZ caused an increase in the total number of malignant tumours including malignant mammary tumours, malignant tumours of pancreas and thyroid, zymbal gland and ear duct carcinomas, osteosarcomas of the bones of head and hemolymphoreticular neoplasias, indicating multi-potent carcinogenic activity of MZ. Nordby *et al*., 2005 proposed a study on farmer's family which revealed that there is a moderate association between MZ exposure and neural tube defects but no association between thyroid cancer and MZ exposure. Kumar *et al*., 2019 studied that MZ caused ROS generation which induced significant apoptosis in human gastric carcinoma cells and pre-treatment with N-acetylcysteine resulted in

attenuation. Asparch *et al*., 2020 evaluated toxicity effects of MZ at different concentrations on the early development of South American common toad *Rinella arenarum* and found MZ has marked teratogenic potential and was 5 times more toxic to embryo than larva with serious lethal and sub-lethal effects. The teratogenic index and estimated risk quotient for embryo of studied species was higher compared to the value of concern, suggesting potential threat of MZ to the species. Vieira *et al*., 2020; Gurol *et al*., 2020 and Leandro *et al*., 2021 studied effects of MZ at different concentrations in Zebra fish, *Danio rerio*. Viera *et al*., 2020 reported that embryos exposed showed a marked decline in the hatching rate and malformations like spinal curvatures and cardiac and yolk sac oedema with behavioural disorders were observed. A study by Gurol *et al*., 2020 revealed alterations such as spermatogenic cell degeneration, disorganizations of seminiferous tubules, haemorrhage, oedema, vacuolization, fibrosis, hypertrophy of spermatocytes, reduced spermatogenic cell clusters and sperm concentration with pyknotic and karyolitic nuclei. Another study by Leandro *et al*., 2021 suggested that at all exposed time periods, MZ altered spontaneous movement, swimming ability, escape response and exploratory behaviour with changes in ROS levels and alterations in antioxidant enzymes found after behavioural changes.

2.3. Arsenic: Mukherjee *et al*., 2003 studied about neuropathy on 451 patients of arsenicosis from groundwater contamination in West Bengal, India and found that all had skin lesions, positive biomarkers and identified source of As contaminated drinking water with peripheral neuropathy as the predominant neurological complication in 187 human patients. Panaullah *et al*., 2009 studied about As toxicity in *Oryza sativa*, rice in Bangladesh and it was concluded that rice cultivated on As contaminated soil resulted not only drastic decrease in yield from 7-9 to 2-3 / hectare with 16% as the average decrease, but also caused accumulation in straw and grain in As-affected regions, compromising both food quality and food security. Upadhyay *et al*., 2018 proposed that arsenic from various sources is responsible for accumulation of arsenic in wide variety of crops (rice, wheat, maize, pulses), vegetables (potatoes, leafy vegetables), mushrooms, foods of animal origin (meat, meat products, egg, milk, fish, seafood, dairy based products). However, rice and rice-based food products constitute the major source for human exposure, even the infants and toddlers are not

spared from As contaminated rice-based products, indicating As in different food items influence the associated As toxicity.

Coryell *et al*., 2018 studied role of gut microbiome for protection against acute arsenic toxicity in mouse model and results revealed that disruption of microbiome affects arsenic excretion and specific gut microbiome members are necessary to get protection against acute arsenic toxicity and mortality. Santra *et al*., 2000 studied hepatic damage caused by chronic As toxicity in mice model administered via drinking water at 3.2 mg/l for 3, 6, 9, 12 and 15 months. Results suggest initial biochemical evidence was observed in 6 months due to decline in GSH and antioxidant enzymes. Fatty liver, increased liver weight with elevated levels of serum ALT and AST were found at 12 months and hepatic fibrosis at 15 months. Souza *et al*., 2017 studied effects of As compounds like sodium arsenite and sodium arsenate on micro-minerals content and antioxidant enzyme activities in male Wistar rat liver and results revealed that animals exposed to arsenite showed increase in proportion of Zn^{+2} and Cu^{+2} with reduction in Mg^{+2}, Na^{+} and SOD levels, while as group treated with sodium arsenate caused changes in Cu^{+2} and Mg^{+2} with reduced levels of CAT and total protein content, indicating that oral exposure to arsenic compounds cause disturbance in balance of trace elements in the liver of rat. Souza *et al*., 2018 studied effects of As in diabetic rats and results revealed that arsenate exposure declined the antioxidant activity and increased the MDA and carbonyl protein levels with signs of hepatic inflammation and increased production of TNF-α and mast cells, while as, the increase in these parameters further intensified in diabetes induced group treated with arsenic. Susan *et al*., 2019 suggested the role of plant-based interventions to ameliorate arsenic toxicity and to combat arsenic-mediated toxicity without any side-effects. These phytochemicals were found to have prophylactic effect with antioxidant and anti-inflammatory properties responsible for countering arsenic-mediated toxicity. Further their role in the elimination of arsenic from the body can prove more effective than conventional therapeutic agents in ameliorating arsenic toxicity. Afolabi *et al*., 2016 found that catechin protected against arsenic induced dyslipidaemia and hepato-renal damage in male Wistar rats when administered concurrently with arsenic and improved AST, ALT and ALP levels in liver. Additionally, creatinine and urea levels were also restored in renal tissue. Another study by Khatun *et al*., 2020 on *Spirulina platensis*, spirulina and vitamin E in presence of As in quail suggested that the tested

substances caused marked improvement in body weight, haematological, histopathological parameters compared to groups treated with arsenic only. In the study by Ishaq *et al*., 2021, turmeric-extracted curcumin effectively restored liver functions as well as attenuated the oxidative injury that occurred after the intoxication of sodium arsenate in male rats. A study by Maji *et al*., 2020 showed ameliorative effect of turmeric and *Paederi afoetida* powder on experimentally induced arsenic toxicity in sheep and found that administration of both the test drugs not only increased elimination of arsenic from faeces, urine and wool but also improved haematological (Haemoglobin and Total erythrocyte count), biochemical (AST, ALT, blood urea nitrogen and serum creatinine) and antioxidant (SOD and CAT) parameters, compared to group treated with arsenic only. Bahrami *et al*., 2020 studied role of curcumin in counteracting As toxicity and found protective role in various conditions induced by As such as genotoxicity, hepatotoxicity, nephrotoxicity, angiogenesis, neurotoxicity, skin diseases, reproductive toxicity and immunotoxicity by exerting various anti-oxidant functions, suggesting that co-treatment with curcumin and arsenate chelator may prove advantageous in a synergistic manner to combat adverse effects of oxidative stress induced by As. Kayode *et al*., 2019 showed that the combination of As and Deltamethrin caused marked alterations in parameters like GSH, TAS, MDA, AOPP, SOD, CAT and GPx in liver, kidney and testis of Wistar rats but supplementation with catechin attenuated these effects. Mosolin *et al*., 2022 found that concurrent treatment with green tea and quercetin attenuated Cd induced oxidative damage, serum biochemical changes and histopathological alterations related to hepato-renal damage in rats. Arslan *et al*., 2022 found ameliorative effects of quercetin against Pb induced oxidative stress, biochemical changes and apoptosis in laying Japanese quails. Blood biochemical markers like ALT, AST, ALP, total protein, albumin, globulin and BUN were improved and oxidative markers like CAT, GPx, GSH and MDA in liver, kidney and heart tissue were also corrected towards control.

Bjorklund *et al*., 2017 studied arsenic toxicity beyond epigenetic modifications and it was revealed that epigenetic alteration in the patterns of DNA methylation, histone posttranslational modifications and micro RNAs after iAs exposure has been evident in many experimental and human population studies. These changes can disrupt cellular homeostasis and modulate key pathways in the As-induced carcinogenesis.

Hu *et al.*, 2020; Maji *et al*., 2020 and Khatun *et al*., 2020 studied the role of ROS in arsenic toxicity. The results revealed As can alter signalling pathways via ROS alteration and the main pathways affected by arsenic mediated ROS include Nrf2-antioxidant response element (ARE) signalling pathway, mitophagy pathway, mitogen-activated protein kinase (MAPKs), microRNAs (miRNAs), tyrosine phosphorylation system, nuclear factor κB (NF-κB) and activator protein-1. In addition, various antioxidants showed protective role in combating ROS-induced toxicity by acting on different pathways. Sharma *et al*., 2013 studied As toxicity induced endothelial dysfunction and dementia, using sodium arsenite and role of sodium butyrate, a histone deacetylase inhibitor and aminoguanidine, a selective inducible nitric oxide synthase inhibitor on rats via drinking water. Results revealed both inhibitors convalesce arsenic induced impairment of endothelial function, learning, memory and reduced oxidative stress parameters and serum nitric oxide (nitrite/nitrate) levels. Nageshwar *et al*., 2019 showed that quercetin reversed the sodium arsenate induced oxidative damage, behavioural and histological alterations in brain tissue of rat. Another study by Metwally *et al*., 2020 proposed neuroprotective potential of chlorogenic acid against sodium arsenite induced toxicity and showed the substance has protective efficacy by altering antioxidant parameters like SOD, CAT, GPx, GR and MDA and NO. Peruru *et al*., 2020 studied neuroprotective efficacy of devil's claw (*Harpagophytum procumbens*) against arsenic induced neurotoxicity in female rats and revealed motor in-coordination related behavioural changes and significant change in antioxidant biomarkers like SOD, CAT, MDA and NO, which were improved by treatment with devil's claw.

Kim *et al*., 2015 studied role of As toxicity in male reproduction and development and found iAs exposure causes reproductive dysfunctions in males such as reduced weight in testis, accessory sex organs and epididymal sperm count and alterations in spermatogenesis and steroidogenesis with decline in levels of testosterone and gonadotrophins. In animal and some epidemiological studies outcomes like fetal malformations, growth retardation, premature delivery, spontaneous abortion, stillbirth and death have been reported. Mahajan *et al*., 2018a found that simultaneous exposure of arsenic and imidacloprid declined TTH, SOD, CAT, GPx, GR, and GST activities, increased AOPP and MDA levels along with severe histopathological changes in the testes of Wistar rats. Sharma *et al*., 2021a studied the effects of

coenzyme Q_{10} and vitamin E on As induced testicular oxidative stress and DNA damage in Swiss Albino mice for 30 days and found that body weight and testis weight gain, GSH, total thiol, SOD and total protein levels were lower in the arsenic treated group compared to control group and the group treated with antioxidants (vitamin E & coenzyme Q_{10} combination). The combination reduced the DNA damage, observed in As treated mice having lower level of head DNA percentage and higher level of tail DNA percentage, length and moment.

Chapter-III

Materials and Methods

Chapter 3

Materials and Methods

3.1. Chemicals/ drugs/ diets: All the chemicals used in the study were of analytical grade procured from Sigma Chemical Corp or Hi-Media, Mumbai or SD-Fine Chemicals Mumbai or Central Drug House, New Delhi. Quercetin and catechin were also procured from Sigma Chemical Corp. Mancozeb as 75 % wettable powder was procured from Indofil Industries Limited, Mumbai, India.

3.2. Experimental design: The animal experiments were conducted on healthy male Wistar rats procured from the Indian Institute of Integrative Medicine (IIIM), Council of Scientific & Industrial Research (CSIR, Lab), Jammu. The animals were acclimatized for a week on pelleted feed with access to *ad-libitum* tap water under standard conditions (25±2°C temp, 50±15% relative humidity) and normal photoperiod (12h light and dark). All the experimental animals were kept under continuous observation for any toxicological signs and symptoms during the entire study period. The use of rat model for the study was approved by ethical committee of the institution (3/IAEC/2021) and experiment was conducted as per standard guidelines.

The sub-acute toxicity was induced by using MZ at $1/10^{th}$ of median lethal dose (LD_{50} - 8000 mg/kg, b. wt) alone and along with different doses of arsenic i.e., 10 ppb, 50 ppb and 100 ppb. As per world health organization (WHO) the recommended level of arsenic in drinking water is 10 ppb and in some Asian countries like Bangladesh, the nationally accepted levels are 50 ppb.

The toxicological study was conducted on sixty adult male Wistar rats, weighing between 160-200 gm. The animals were randomly allocated into 10 groups, with 6 animals in each. Group I served as control, group II was administered MZ [800 mg/kg, b. wt, oral gavage (PO), for 28 days]. Groups III, IV and V received sodium arsenite (10 ppb, 50 ppb and 100 ppb) containing drinking water continuously for 28 days respectively. Group VI, VII and VIII were provided with sodium arsenite in drinking water at different dose levels 10 ppb, 50 ppb and 100 ppb respectively, along with MZ (800 mg/kg, b. wt, PO) for 28 days. Group IX and X were given sodium arsenite orally with drinking water at 100 ppb and MZ at 800 mg/kg, b. wt, PO along

with quercetin and catechin at 50 mg/kg, b. wt, PO respectively for 28 days. The treatment groups administered with different toxicants can be presented as follows:

Groups	Treatment	Dosage	Route & mode of administration (28 days)
I	Control (drinking water)	1 ml /day/rat	Orally
II	Mancozeb (MZ)	$1/10^{th}$ LD_{50} (800 mg/kg)	Oral gavage
III	Arsenic	10 ppb	Daily with drinking water
IV	Arsenic	50 ppb	Daily with drinking water
V	Arsenic	100 ppb	Daily with drinking water
VI	MZ+ Arsenic	$1/10^{th}$ LD_{50} +10 ppb	PO (MZ) + As in drinking water
VII	MZ + Arsenic	$1/10^{th}$ LD_{50} + 50 ppb	PO (MZ) + drinking water (As)
VIII	MZ + Arsenic	$1/10^{th}$ LD_{50} + 100 ppb	PO (MZ) + drinking water (As)
IX	MZ+Arsenic + Quercetin	$1/10^{th}$ LD_{50} + 100 ppb + 50 mg/kg	PO (MZ) + drinking water (As) + PO (Quercetin)
X	MZ+ Arsenic +Catechin	$1/100^{th}$ LD_{50}+ 100 ppb + 50mg/kg	PO (MZ) + drinking water (As) + PO (Catechin)

3.3. Body Weight and water intake*:* The body weights of all experimental animals were recorded weekly till the completion of experiment for accordingly dose of the toxicants were calculated. Similarly, water intake was recorded daily for 24 hours for each respective group.

3.4. Collection of blood and tissues samples: Blood and then tissue samples were collected for oxidative, biochemical and histopathological studies. From each animal approximately 4-5 ml of blood was collected directly from heart in heparinized tubes and animals were sacrificed by cervical dislocation on the 29^{th} day under light diethyl ether anaesthesia. The organs *viz.* testes, kidney, liver, brain were removed and 1gm of respective tissue was collected in 10ml ice-cold 0.5M phosphate buffer (pH-7.4) for antioxidant studies. Additionally, a portion of tissue samples were also collected in formal saline solution (10%) for histopathological studies. 10 % tissue homogenate

was prepared for the antioxidant enzyme analysis using tissue homogenizer (1000 rpm for 5-7 minutes at room temperature). The whole blood was used to determine haemoglobin (Hb) and blood reduced glutathione (GSH). The remaining blood samples for each animal from group was centrifuged for 15 min at 3000 rpm and the plasma was removed by using 1ml pipette in clean sterilized test tubes. Harvested plasma was stored immediately at 4°C for biochemical and antioxidant biomarkers analysis. The remaining erythrocyte sediment after separation of plasma was diluted with normal saline solution (NSS) of 1:1 ratio (on v/v basis) which was gently and thoroughly mixed with the sediment and then centrifuged at 6000 rpm for 10 minutes. After centrifugation the supernatant along with the buffy coat was discarded and again NSS was added in equal amount to the erythrocytes on v/v basis and this process was repeated three times. Ultimately, 1% hemolysate was prepared by adding 9.9 ml of 0.1M PBS (pH 7.4) to 100µl washed erythrocyte which was then used for determination of various antioxidant parameters.

3.5. Antioxidant biomarkers: Different antioxidative biomarkers *viz*. total antioxidant status (TAS), total thiols (TTH), malondialdehyde (MDA), advanced oxidation protein products (AOPP), enzymatic activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) were determined in tissue samples as well as in RBC lysate. The activities of arylesterase (AE), acetylcholinesterase (AChE) was determined in RBC lysate and brain tissue homogenate. Additionally, TAS, TTH and nitric oxide (NO) levels were determined in plasma along with estimation of NO in brain tissue. The procedure for estimation of different antioxidant and other parameters is discussed briefly as follows:

3.5.1. Haemoglobin: The haemoglobin content was estimated in the blood by mixing 20 µl of blood in 5.0 ml of Drabkin solution as per the method mentioned by Dacie and Lewis, 1968. After incubation for 5 minutes absorbance was measured at 540 nm and the value was multiplied by the factor 36.7 to get the haemoglobin content.

3.5.2. Blood Glutathione (GSH): The blood GSH was estimated as per the method described by Beutler, 1975. Chemicals required:

- Phosphate solution (0.3M Na_2HPO_4): Dissolve 13.35g disodium hydrogen phosphate dihydrate in 250ml distilled water.

- Precipitating solution: 16.7 g glacial metaphosphoric acid + 2 g disodium salt of EDTA + 300 g NaCl in 1L distilled water. The solution is stable for at least 3 weeks at 4 °C.
- DTNB: Weigh 40 mg of 5-5'-dithiobis-2- nitrobenzoic acid in 100 ml of 1% trisodium citrate solution and the reagent is stable approximately for 3 months at 4 °C.
- Standard GSH solution: Dissolve 60 mg in 100 ml distilled water and the solution can be used for 4 weeks if stored at 4 °C.

Procedure:

Test	Blank
1. Take 0.2ml whole blood+ 1.8 ml distilled water 2. 3ml precipitating solution & then wait for 5 minutes 3. Centrifuge at 3000 rpm for 15 min. 4. Take 1 ml supernatant and add 4ml of phosphate solution 5. Add 0.5 ml DTNB just prior to recording 6. Take O.D. at 412 nm	1. Take 2 ml distilled water 2. 3ml precipitating solution, then wait for 5 minutes 3. Centrifuge at 3000 rpm for 15 mins 4. Take 1 ml supernatant and add 4 ml of phosphate solution 5. Add 0.5 ml DTNB just prior to recording 6. Take O.D. at 412 nm to set zero base

Note: A standard curve was prepared by using glutathione at different concentrations in distilled water.

3.5.3. Total Thiols status (TTH)**:** Total thiols in tissues and plasma were estimated as per the standard method using reduced glutathione (GSH) as a standard (Motchnik *et al*., 1994).

Reagents required:

- 0.2 M disodium hydrogen phosphate (0.2 M = 17.79 g / 500 ml)
- 2mM EDTA (ethylene diamine tetra acetic acid) 2mM = 74.448 mg/ 100ml buffer
- DTNB (5-5'-dithiobis,2-nitrobenzoic acid) 10mM = 3963.6 mg/L buffer

Procedure: Briefly, reaction mixture containing 900µl of EDTA (2mM in 0.2 M Na_2HPO_4), 20µl of DTNB (10mM in 0.2 M Na_2HPO_4) and 100µl of fresh plasma sample and tissue homogenate was taken. The reaction mixture was incubated for 5 min at room temperature and the absorbance was taken at 412nm using UV visible

spectrophotometer. Similarly, a reagent blank without sample and sample blank without DTNB were prepared accordingly. Concentrations of total thiols (mM) were estimated by using reduced glutathione as a standard (Motchnik *et al*., 1994).

Reagents	**Reagent blank**	**Sample blank**	**Test**
EDTA 2 mM	900uL	900uL	900uL
DTNB 10 mM	20uL	-	20uL
Buffer 0.2 M	100uL	20uL	-
Sample	-	100uL	100uL
The absorbance was read at 412 nm			

3.5.4. Determination of total antioxidant status (TAS): The TAS was estimated as per standard methods by Re *et al*., 1999. Total antioxidant activity was estimated spectrophotometrically as per the ability of plasma/tissue samples to scavenge the 2,2′-azinobis (3-ethylbenzothiazoline 6-sulphonate) (ABTS) radical cation, $ABTS^{+}$, inhibiting its absorption at 734 nm. ABTS radical cations were prepared by reacting ABTS solution (7mM) with 2.45mM ammonium persulphate. The reaction mixture was allowed to stand in the dark for 12-16 hours at room temperature before its use. The ABTS solution formed was diluted with phosphate buffer (pH=7.4) to give an absorbance reading of 0.70 ± 0.05 at 734 nm before using it in the sample. Radical scavenging analysis was performed by mixing 10µl of tissue homogenate in 1.0ml of ABTS solution. In case of plasma sample, 50µl of the plasma was mixed with 950µl of $ABTS^{\bullet+}$ solution. Finally, the absorbance was read at 734 nm after 3 minutes. Similarly, a blank solution was prepared by using 50µl phosphate buffer in 950µl of $ABTS^{\bullet+}$ solution to set zero base. Calibrations were performed by using the ascorbic acid instead of sample as antioxidant dissolved in PBS.

3.5.5. Arylesterase (AE) activity: The activity of AE was measured by using phenyl acetate as a substrate (Burlina *et al*. 1977; Furlong *et al*., 1988). Phenyl acetate (12 mmol/L) was prepared freshly in a mixture containing distilled water and ethanol in the ratio of 1:1. The reaction mixture contained 750µl of 0.1 mol/L Tris-HCL (pH 8.5) containing 1 mmol/L $CaCl_2$, 125µl of 12 mmol/L phenyl acetate and 125µl plasma with 1:10 dilution with distilled water. The reaction was initiated by the addition of the diluted plasma and the increase in aborbance was meaured at 270 nm. The aborbance was costantly monitored up to 60 sec having lag time as 20 sec. Blank was included to correct the spontaneous hydrolysis of phenyl acetate. A molar extinction coefficient (EC) of 1310 was used to calculate enzyme activity which was

expressed in unit per ml (U/ml), where one unit corresponds to µmol phenol formed per min.

3.5.6. Acetylcholinesterase (AChE) activity in Blood and Brain: The cholinesterase activity in erythrocytes and brain tissue was estimated as per the standard method by Voss and Sachsse, 1970.

Reagents:

a. 0.0067 M Sorensen phosphate buffer, pH 8.0
b. DTNB [5,5′-Dithiobis-(2-nitrobenzoic acid),0.01%]in phosphate buffer saline (pH – 7.4)
c. Acetylthiocholine iodide (0.001 M)
d. Eserine salicylate (0.1%)

Procedure: A sample of 300 ul diluted erythrocyte (1:10) or brain homogenate was taken in a test tube and add 3 ml of DTNB solution to it. 0.9 ml of acetylthiocholine iodide solution was added as a substrate for AChE and the mixture was thoroughly shaken after each addition. The mixture was allowed to incubate for 20 min at 37 °C. At the end of the incubation, 300 µL of eserine salicylate solution was added to stop enzymatic reaction. The absorbance was read at 405 nm on a UV spectrophotometer. AChE activity was calculated by using different concentrations of reduced glutathione from a standard curve.

Preparation of standard curve: For standard curve, different concentrations *viz.* 976, 448, 244, 122 and 61 nmol/ml of GSH was prepared. Six tubes of 15 ml capacity were marked as blank (B), standard **(**S1, S2, S3, S4 and S5) and 8 ml of 0.8M phosphate solution (Na_2HPO_4 $2H_2O$) pH 8 was added in each tube. Then 2 ml reduced glutathione solution of varying concentrations was added to all tubes except in blank in which distilled water was added. The reaction was ended by adding 1 ml DTNB reagent (40 mg DTNB in 100 ml of 1% of sodium citrate). The optical density of different standards was read at 412 nm against blank and the results were recorded.

3.5.6. Assay of Catalase activity: The enzymatic parameter catalase (CAT) in erythrocyte lysate and tissue homogenate was determined as per the methods of Aebi, 1983.

Reagents: 50 mM Phosphate buffer (pH 7.0), hydrogen peroxide, (30 mM): Dilute the 0.34 ml of 30% H_2O_2 with buffer until its optical density is around 1.5 at 240. Then use the solution in the test.

Procedure:

1. Set zero base using catalase buffer.
2. Take 1ml of phosphate buffer.
3. Add 10 ul of sample.
4. Add 0.5 ml of catalase buffer solution.
5. Read optical density at 240 nm after 60 seconds interval.

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

1. **Reagents Required:**0.6 mM Pyrogallol (freshly prepared): Dissolve 76 mg of pyrogallol in 100 ml distilled water and store in amber bottle; 6 mM EDTA: Dissolve 223 mg disodium EDTA salt in 100 ml distilled water; 100 mM Tris HCl buffer: Dissolve 1.21 g Tris in 80 ml distilled water. Adjust pH to 8.2 using 10 mM HCL and make volume to 100 ml.
2. 6 mM EDTA

   Dissolve 223 mg Disodium EDTA in 100 ml distilled water
3. Pyrogallol (fresh)

   Dissolve 76 mg of Pyrogallol in 100 ml distilled water and store in amber bottle

Procedure:

Reagents	**Control**	**Test**	**Blank**
Tris HCl	1.5 ml	1.5 ml	1.5 ml
EDTA	0.5 ml	0.5 ml	0.5 ml
Pyrogallol	1 ml	1 ml	-
Enzyme preparation (lysate)	-	20 µl	-

Read optical density at 420 nm every 30 seconds up to 4 min. The unit of enzyme activity is the amount of enzyme causing 50% inhibition of auto-oxidation of pyrogallol observed in control.

3.5.9. Determination of Glutathione peroxidase (GPx) activity: The assay of glutathione peroxidase (GPx) in erythrocyte lysate and tissue homogenate was performed as per the methods of Hafeman *et al*., 1974.

Reagents required:

a. 20 mM reduced glutathione: 9.2 mg GSH dissolved in 15 ml of distilled water.
b. 0.4 M sodium phosphate buffer (pH – 7) containing 0.4 mM EDTA.

c. Sodium azide: 65 mg sodium azide in 100 ml of distilled water.

d. H_2O_2 (30%)

e. 0.4 M disodium hydrogen phosphate (Na_2HPO_4): 7.12 g in 100 ml of distilled water.

f. Precipitating solution: 16.7 g glacial metaphosphoric acid + 2 g EDTA + 300 g NaCl in 1L of distilled water.

g. DTNB: 40 mg of 5-5'-dithiobis (2- nitrobenzoic acid) in 100 ml of 1% trisodium citrate solution.

Procedure:

TEST	CONTROL
1. Take 0.1 ml lysate.	1. Take 0.1 ml lysate.
2. Add 1 ml GSH (reduced).	2. Add 1 ml GSH (reduced).
3. Add 1 ml phosphate buffer.	3. Add 1 ml phosphate buffer.
4. Add 0.5 ml sodium azide.	4. Add 0.5 ml sodium azide.
5. Add 1.4 ml distilled water to make total volume to 4 ml.	5. Add 1.4 ml distilled water to make total volume 4 ml.
6. Incubate for 5 minutes.	6. Incubate for 5 minutes.
7. Add 1 ml hydrogen peroxide.	7. Add 1 ml distilled water.
8. Mix well & remove 1 ml from the mixture in separate centrifuge tube after 1 minute.	8. Mix well & remove 1 ml from the mixture in separate centrifuge tube after 1 minute.
9. Add 4 ml precipitating solution and centrifuge at 3000 rpm for 15 min.	9. Add 4 ml precipitating solution and centrifuge at 3000 rpm for 15 min.
10. Take 2 ml supernatant in a separate tube.	10. Take 2 ml supernatant in a separate tube.
11. Add 2 ml disodium hydrogen phosphate.	11. Add 2 ml disodium hydrogen phosphate.
12. Add 1 ml DTNB.	12. Add 1 ml DTNB.
13. Read optical density at 412 nm.	13. Read optical density at 412 nm.

Activity of GPx = 10 log C_o/C (Where C_o =concentration of GSH at zero time and C= concentration of GSH after one min incubation)

3.5.10. Determination of Glutathione reductase (GR) activity: The activity of glutathione reductase (GR) was determined as per the method described by (Carlberg and Mannervik,1975)

Reagents required: 0.2 M potassium phosphate buffer (pH-7) containing 2 Mm EDTA; 2 mM NADPH in 10 mM Tris HCL and 20 mM oxidized glutathione.

Procedure:

Test	Blank
1. Take 2.6 ml phosphate buffer	1. 2.6 ml phosphate buffer
2. Add 0.15 ml of NADPH solution	2. Set auto zero base
3. Add 0.15 ml of oxidized glutathione	
4. Add 0.1 ml lysate	
5. Read absorbance at 340 nm at every 30 seconds interval for 4 min.	

3.5.11. Lipid Peroxidation levels: The malondialdehyde (MDA) activity indicating cell membrane oxidative damage in erythrocytes or in tissues was determined using standard methods (Shafiq-Ur-Rehman, 1984).

Reagents required: 10 % trichloro acetic acid (TCA) and 0.67 % thiobarbituric acid (TBA).

Procedure:

Test	Blank
1. Take 1 ml 1% erythrocyte lysate or 10% tissue homogenate. 2. Add 1 ml 10% TCA. 3. Vortex it and then centrifuge at 3000 rpm for 10 minutes. 4. Collect 1 ml supernatant and add 1 ml 0.67 TBA & boil for 10 minutes in hot water bath. 5. Cool down the mix and dilute with 1 ml distilled water. 6. Measure absorbance at 535 nm.	1. Take 1 ml of distilled water. 2. Add 1 ml 10% TCA. 3. Vortex it and then centrifuge at 3000 rpm for 10 minutes. 4. Collect 1 ml supernatant and add 1 ml 0.67 TBA & boil for 10 minutes in hot water bath. 5. Cool down the mix and dilute with 1 ml distilled water. 6. Measure absorbance at 535 nm.

3.5.12. Advanced oxidation protein product (AOPP): The AOPP levels in erythrocyte lysate and tissue homogenate were determined using standard methods (Witko-sarsat *et al*., 1996). Reagents: Glacial acetic acid; PBS (pH -7.4) and 1.16 M potassium iodide (KI).

Procedure: Briefly, 160 µl of sample and 640 ul of PBS was taken. Then 40µl of potassium iodide and 80 µl of glacial acetic acid was added. The mixture was incubated for 5 minutes at room temperature and absorbance was measured at 340 nm. A reagent blank without sample was also prepared in a similar manner. Concentration of advanced oxidation protein product (µM) was estimated using chloramine-T as a standard.

3.5.13. Assay of Nitric acid (NO) in plasma and brain tissue: Levels of NO were assayed in plasma and brain tissue of all the groups by spectrophotometric method using copper-cadmium alloy (Sastry *et al*., 2002). The principle of the assay is the reduction of nitrate to nitrite by copper-cadmium alloy, followed by colour development on addition of Griess reagent (sulphanilamide and N- naphthyl ethylenediamine) in acidic medium, measured spectrophotometrically at 545 nm.

Reagents: 0.1N HCL: 2 ml of 35% HCL in 198 ml of distilled water; 0.5 N HCL: 10 ml of 35% HCL in 190 ml of distilled water; 0.35 M sodium hydroxide; 1.44 g of NaOH in 100 ml of distilled water; 120mM zinc sulphate; 0.344 of zinc sulphate in 10 ml of distilled water; 1% sulphanilamide; 1g of sulphanilamide in 100ml of 3N HCL

(3N HCl=26.13 ml HCl in 73.87 ml of distilled water); 0.1 % naphthyl ethylene diamine dihydrochloride (NEDD), 50 mMcarbonate buffer using 0.5 M sodium bicarbonate (4.2g in 100 ml distilled water) and 0.05 M sodium carbonate (0.53 g in 100ml distilled of water). Now 50 mM was prepared by taking equal volume of bicarbonate and carbonate buffer and using 5 ml of it in 22.5 ml of distilled water with a pH of 9.

Preparation of copper-cadmium alloy: The copper-cadmium alloy procured was powdered using a metal file. The powdered alloy was washed twice with distilled water in a beaker. The supernatant was discarded and alloy was washed twice with 0.5 N HCL to remove hydroxide of cadmium. The activated filings were ultimately washed twice with 0.1N HCL and stored at 2-8 °C in 0.1N HCL or filtered using Whatman filter paper 1 to use it in the sample.

Preparation of standard: 1mM potassium nitrate (KNO_3) was prepared by addition of 10.1 mg KNO_3 in 100 ml of distilled water. Aliquots were prepared in five test tubes in decreasing order of their concentrations. Five test tubes were labelled as 1000µM, 800µM, 600µM, 400µM and 200µM. To these test tubes, volumes of 1000 µM KNO_3 were added in the order of 1000µl, 800µl, 600µl, 400µl and 200µl followed by adding distilled water in volumes of 200µl, 400µl, 600µl and 800µl in second, third, fourth and fifth test tube, respectively. Now different standards were prepared using these concentrations of KNO_3. From 1000µM, 10µl was taken in a fresh test tube followed by addition of 90ul distilled water to achieve final concentration as 100µM. Likewise, 10µl of 800µM, 600µM, 400µM and 200µM was taken in four separate test tubes with addition of 90µl of distilled water to each test tube, so that final concentrations of 80µM, 60µM, 40µM and 20µM are achieved respectively. Now prepare the standard curve using these standards.

Procedure: The 1 gm brain tissue was weighed and homogenized in ice-cold 1.M phosphate buffer saline with a pH of 7.4. The homogenate was centrifuged at 4000 rpm for 10 min at 4 °C. The supernatant was collected in a fresh test tube and 100µl of supernatant along with 100 µl of plasma sample separated from heparinized blood were taken in eppendorf tubes and 400µl of 50mM of carbonate buffer was added to them. Activated and dried copper-cadmium filings of approximate weight of 150mg was added in each tube and the mixture was allowed to incubate for 1 hour at 37 °C with thorough shaking. The process of incubation causes full reduction of nitrate.

100ul of 0.35M sodium hydroxide was added followed by addition of 400ul of 120mM zinc sulphate on a vortex mixture. Sodium hydroxide was added to halt the reaction while as zinc sulphate leads to deproteinization. The tubes were left undisturbed for 10 min and then centrifuged at 4000g for 10 min at room temperature. Then aliquots of 500µl containing clear supernatant were transferred to fresh eppendorf tubes and 250µl of 1% sulphanilamide and 250µl of 0.1% NEDD was added with proper shaking. After 10 min the absorbance was measured at 545 nm against a blank containing same concentration of ingredients except sample was replaced by distilled water.

3.6. Biochemical parameters: Different biochemical parameters such as lactate dehydrogenase (LDH), aspartate and alanine aminotransferases (AST & ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine (CR), uric acid (UA), total protein (TP), albumin (ALB) were estimated in plasma by standard kits (Transasia Bio-Medicals Ltd, India) with the help of Chemistry Analyzer (CHEM-7, ERBA, Manheim).

3.7. Histopathological studies: The histopathological alterations in different tissues were carried out according to standard protocol (Drury and Wallington, 1980). Briefly, a small piece of tissue (liver, kidney, testes and brain) from each animal of respective group was immediately fixed in 10% formalin. The formalin fixed tissues were embedded in paraffin, sectioned, stained with haematoxylin and eosin (H&E) and examined under a light microscope for histopathological observations.

3.8. Statistical analysis: The values are presented in mean ± standard error. The results were analysed by analysis of variance (ANOVA) at 5% level of significance ($p<0.05$) using the Duncan Multiple Range test (SPSS 21.0).

Chapter-IV

Results

Chapter 4

Results

Biochemical alterations, oxidative stress and histopathological alterations induced by sub-acute toxicity of mancozeb alone and in-combination with different doses of arsenic (As) was studied in Wistar rats. Sixty Wistar rats having body weight 180-200 gm were randomly allocated in 10 groups with six animals in each group. Group I served as the control receiving only tap water orally whereas group II received MZ at the dose rate of $1/10^{th}$ of LD_{50} alone and group III, IV and V were exposed to drinking water containing arsenic at the dose rate of 10 ppb, 50 ppb and 100 ppb, respectively. Group VI, VII and VIII were exposed with MZ and different doses of arsenic. Group IX and X were treated with these toxicants along with quercetin and catechin respectively for 28 days. On day 29^{th}, blood and tissue samples were collected and analysed for the biochemical and oxidative biomarkers and histopathology in different tissues of control and exposed rats to accesses the damage induced by toxicants and their attenuation by flavonoids (quercetin and catechin).

4.1. Effect on Blood Antioxidant System:

4.1.1. Activities of Superoxide Dismutase (SOD) and Catalase (CAT): Mean values of SOD and CAT in control and treatment groups are presented in table 4.1 and their graphical representation is shown in figure 4.2. A non-significant reduction was observed in SOD and CAT activity of group II as compared to control. Group III, IV and V treated with sodium arsenite alone represented a non-significant decline in CAT activity and a significant curtailment was seen in group IV and V in SOD activity in a dose dependent manner as compared to control. In the interactive groups VI, VII and VIII, a significant reduction in SOD and CAT activities was seen in a dose dependent manner as compared to control. Group VIII revealed a 71.3% and 49.7% decrease in SOD and CAT activity, respectively, as compared to control. Simultaneous administration of quercetin along with toxicants in group IX increased the SOD and CAT values as compared to group VIII but values were still reduced to 54.6% and 29.7% as compared to control. Group X also showed an increase in SOD and CAT activity when compared to group VIII but the levels were significantly lower than control animals as 55.3% and 42.7%. Data clearly indicate protective role of quercetin both for SOD as well as CAT activity in co-exposed group.

4.1.2. Activities of Glutathione Peroxidase (GPx) and Glutathione Reductase (GR): In Group II, a significant ($p<0.05$) reduction was observed in GR activity and non-significant decline in GPx activity was noticed as compared to control. Group III, IV and V represented a non-significant decline in GR and GPx values in a dose dependent manner as compared to control. The groups VI, VII, VIII treated with the combinations of toxicants, witnessed a significant decrease in GR and GPx levels in a dose dependent manner in contrast to control animals. When compared with control, group VIII witnessed a 54.2% and 80.4% decrease in GPx and GR respectively. Group IX increased the GPx and GR values as compared to group VIII but the values were still at fall to 53.3% and 42.9% respectively as compared to control. Group X also showed a increase in GPx and a slight decrease in GR activity as compared to group VIII and when compared to control, they are decreased to 50.5% and 80.8% respectively. This clearly indicates that group IX treated animals restored GR values significantly when compared to group VIII. Mean values ± standard error of GPx and GR activities are presented in table 4.1 and their graphical representation is shown in figure 4.2 and 4.3 respectively.

4.1.3. Activities of Acetylcholinesterase (AChE) and Aryl esterase (AE): The activities of AChE and AE witnessed a significant ($p<0.05$) fall in group II. Group III, IV and V witnessed a decreasing trend in AChE and AE activity in a dose dependent manner, compared to control and groups IV and V represented a significant decline in both the enzymes. The toxicant interactive groups including VI, VII and VIII showed a significant curtailment in both the enzymes compared to control as well as alone treated toxicants. In group VIII, the decreased values of AChE and AE can be depicted as 41.4% and 54.9% compared to control. Groups IX increased the AChE and AE activity significantly compared to group VIII, in spite of that the values are reduced to 23.6% and 39.2% respectively than the control group. Groups X increased the AChE and AE activity significantly compared to group VIII, nevertheless, the values are lower 27.6% and 23.8% respectively as compared to the control group.This leads to conclusion that group IX improved AChE activity and X improved AE activity, when to control group. Mean values were present in table 4.1 and corresponding graphical representation shown in figure 4.3.

4.1.4. Reduced Blood Glutathione (GSH): A significant ($p<0.05$) reduction was observed in GSH activity of group II treated with MZ as compared to control. In

group III, IV and V treated with sodium arsenite alone a significant decline in GSH values was seen in a dose dependent manner as compared to control. The groups VI, VII, VIII treated with the combinations of toxins, a significant decrease in GSH levels was noticed when compared to control as well with sodium arsenite alone treated groups. Meanwhile, 57.6% decrease in GSH value was observed in group VIII as compared to control. Groups IX and X treated with quercetin and catechin along with MZ and sodium arsenite at highest dose restored the levels of GSH activity as only 38.9% and 43.9% decline was seen compared to control and is shown in table 4.2 and figure 4.1. The restored values of reduced glutathione by quercetin indicate better protection during intoxication.

4.1.5. Total antioxidant status (TAS) and total thiols (TTH): Mean values of TAS and TTH are presented in table 4.2 and their graphical representation is shown in figure 4.1. A significant ($p<0.05$) fall in activity of TAS and TTH was noticed in group II in comparison to control. Group III, IV and V depicted a deduction in TAS and TTH levels in a dose dependent manner as compared to control. The groups VI, VII, VIII treated with the combinations of toxins, a significant decrease in TAS and TTH levels was observed, compared to control as well as from groups (III, IV and V) treated with sodium arsenite alone. In group VIII, the decreased values of TAS and TTH are as low as 82.0% and 84.9% as compared to control. Groups IX increased the TAS and TTH activity compared to group VIII, in spite of that the values declined to 71.1% and 53.5% respectively than the control group. Groups X increased the TAS and TTH levels compared to group VIII, nevertheless, the values are lowered to 75.2% and 75.6% respectively as compared to the control group. This leads to conclusion that group IX (quercetin) improved the levels of both TAS and TTH more efficiently than group X.

4.1.6. Plasma levels of Nitric oxide (NO) and Total antioxidant status (TAS): Mean values of TAS and NO are given in table 4.2 and their graphical representation is shown in figure 4.5. Group II showed a non-significant decline in TAS levels compared to control. Group III, IV and V treated with sodium arsenite alone revealed a reducing trend in plasma TAS level. Plasma TAS values depicted a non-significant deduction in group VI and significant fall in group VII and VIII (36.6%) as compared to the control in a dose dependent manner. Plasma NO levels showed a significant increase in group II as contrast to control. Group III represented a non-significant rise

and IV and V treated animals witnessed a significant rise in NO levels in a dose dependent way as compared to control. The interactive groups (VI, VII and VIII) represented further increment in NO levels in a dose dependent manner as compared to control as well as alone treated toxicant groups. Group IX (498.7%) restored NO levels significantly when compared to group VIII (731.8%) but the values are significantly higher than the control. Group X (800.1%) showed a slight increase in NO levels as compared to group VIII. Thus, for decreasing elevated NO levels, quercetin proved to have a better potential. Meanwhile, there was a decline in plasma TAS levels in group IX (25.9%) and X (29.5%) as compared to control but the values are still less reduced compared to group VIII. Thus, the TAS values in group IX are non-significant with the control group indicating protection of quercetin against the combined toxicity.

4.1.7. Levels of malondialdehyde (MDA) and Advanced Oxidation Protein Product (AOPP): A non-significant rise in activity of MDA and AOPP was noticed in group II in comparison to control. Group III, IV and V depicted an elevating trend in MDA and AOPP levels in a dose dependent manner as compared to control. Mean while, only the MDA values of group IV and V showed a significant ($p<0.05$) elevation when compared with control. The toxicant interactive groups including VI, VII and VIII showed a increment in MDA and AOPP values which was significant in group VII, VIII when compared to control as well as alone treated toxicants. In group VIII, the raised values of MDA and AOPP were at high 2585.7%% and 25.2% as compared to control. Groups IX increased the MDA (2692.5%) and decreased the AOPP levels to 7.4% compared to control. Group X also showed an increase in MDA (1303.2%) and decrease in AOPP (6.6%) values when compared to control. So, for group IX, AOPP values were lowered significantly compared to group VIII and non-significantly lower than control. Group X revealed decrease in both MDA and AOPP levels significantly compared to group VIII and the AOPP levels were non-significantly lowered than control group. This indicates improvement in AOPP levels in group IX and restoration of MDA as well as AOPP levels in group X. Mean values of MDA and AOPP are presented in table 4.2 and their graphical representation is shown in figure 4.4.

Table 4.1: **Alterations in antioxidant biomarkers in erythrocyte lysate following repeated administration of mancozeb (MZ) alone or in-combination with arsenic (As) and its amelioration with quercetin and catechin.**

Treatment given	Blood antioxidant parameters					
	SOD	CAT	GPx	GR	AE	AChE
Control	$48.45^{d}\pm3.31$	$36.88^{d}\pm3.06$	$2.14^{d}\pm0.305$	$2.24^{f}\pm0.138$	$2.73^{f}\pm0.089$	$6060.63^{f}\pm107.64$
MZ (800mg/kg)	$39.75^{cd}\pm5.96$	$27.73^{abcd}\pm1.53$	$1.69^{bcd}\pm0.196$	$0.185^{a}\pm0.058$	$0.96^{a}\pm0.053$	$5269.38^{e}\pm76.89$
Arsenic (10 ppb)	$44.89^{d}\pm1.78$	$35.07^{cd}\pm2.56$	$1.90^{cd}\pm0.083$	$2.19^{ef}\pm0.244$	$2.56^{f}\pm0.061$	$6006.50^{f}\pm328.27$
Arsenic(50 ppb)	$27.75^{bc}\pm2.18$	$30.88^{bcd}\pm3.26$	$1.60^{abcd}\pm0.141$	$1.81^{ef}\pm0.163$	$1.81^{de}\pm0.048$	$5031.88^{de}\pm130.83$
Arsenic (100ppb)	$19.61^{ab}\pm1.60$	$26.58^{abcd}\pm2.02$	$1.30^{abc}\pm0.082$	$1.69^{de}\pm0.038$	$1.49^{bcd}\pm0.091$	$4622.25^{cde}\pm234.28$
MZ + As (10ppb)	$21.44^{ab}\pm1.92$	$25.37^{abc}\pm2.85$	$1.57^{abcd}\pm0.079$	$1.02^{bc}\pm0.045$	$1.52^{bcd}\pm0.067$	$4022.25^{abc}\pm93.73$
MZ + As (50ppb)	$16.41^{ab}\pm1.77$	$20.71^{ab}\pm1.95$	$1.28^{abc}\pm0.090$	$0.61^{ab}\pm0.034$	$1.42^{bc}\pm0.043$	$3809.75^{ab}\pm36.08$
MZ +As (100 ppb)	$13.89^{a}\pm1.68$	$18.57^{a}\pm2.94$	$0.98^{a}\pm0.087$	$0.44^{a}\pm0.074$	$1.23^{ab}\pm0.073$	$3550.25^{a}\pm95.97$
MZ+ As (100ppb) + Quercetin (50 mg/kg)	$22.01^{ab}\pm2.68$	$25.94^{abcd}\pm1.07$	$1.00^{a}\pm0.056$	$1.28^{cd}\pm0.034$	$1.66^{cd}\pm0.080$	$4627.75^{cde}\pm79.94$
MZ+ As (100ppb) + Catechin (50mg/kg)	$21.68^{ab}\pm2.06$	$21.15^{ab}\pm2.14$	$1.06^{ab}\pm0.101$	$0.43^{a}\pm0.037$	$2.08^{e}\pm0.061$	$4390.25^{bcd}\pm104.00$

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

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

Values of SOD (Superoxide dismutase) are expressed in Unit/ g of tissue

Values of CAT (Catalase) are expressed in µmol H_2O_2 decomposed/ min/ g of tissue

GPx (glutathione peroxidase) are expressed in Unit/ g of tissue

Values of GR (glutathione reductase) are expressed nmol of NADPH/min

Activities of arylesterase (AE) expressed in U/ml

Acetylcholinesterase (AChE) activity expressed in nmole of thiols produced/min/mg of tissue

Table 4.2: Antioxidant biomarkers in blood and plasma following repeated administration of mancozeb (MZ) alone or in-combination with arsenic (As) and its amelioration with quercetin and catechin.

Treatment given	Blood antioxidant parameters					Plasma	
	TAS	TTH	GSH	MDA	AOPP	TAS	NO
Control	$15.34^{d}\pm1.30$	$0.430^{b}\pm0.050$	$4.62^{g}\pm0.214$	$2.79^{a}\pm0.36$	$0.707^{ab}\pm0.025$	$13.65^{d}\pm0.617$	$51.87^{a}\pm9.17$
MZ (800mg/kg)	$6.33^{bc}\pm0.605$	$0.100^{a}\pm0.029$	$3.45^{ef}\pm0.076$	$10.96^{a}\pm0.26$	$0.792^{bcd}\pm0.021$	$13.33^{cd}\pm1.14$	$281.23^{c}\pm11.41$
Arsenic (10 ppb)	$13.25^{d}\pm0.533$	$0.400^{b}\pm0.024$	$3.98^{f}\pm0.231$	$3.11^{a}\pm0.33$	$0.744^{ab}\pm0.011$	$13.33^{cd}\pm0.548$	$61.22^{a}\pm6.39$
Arsenic (50 ppb)	$7.89^{c}\pm0.797$	$0.193^{a}\pm0.020$	$3.30^{de}\pm0.023$	$50.73^{c}\pm2.59$	$0.762^{abc}\pm0.033$	$12.38^{bcd}\pm0.955$	$136.88^{b}\pm3.19$
Arsenic (100ppb)	$5.34^{abc}\pm0.561$	$0.143^{a}\pm0.042$	$2.88^{cd}\pm0.061$	$65.47^{d}\pm0.88$	$0.815^{bcd}\pm0.032$	$11.44^{abcd}\pm0.798$	$171.15^{b}\pm4.39$
MZ + As (10ppb)	$3.51^{ab}\pm0.283$	$0.113^{a}\pm0.031$	$3.16^{de}\pm0.112$	$37.70^{b}\pm1.63$	$0.805^{bcd}\pm0.015$	$10.46^{abcd}\pm0.537$	$298.42^{c}\pm6.07$
MZ + As (50ppb)	$2.97^{a}\pm0.273$	$0.082^{a}\pm0.012$	$2.23^{ab}\pm0.073$	$61.66^{d}\pm1.51$	$0.862^{cd}\pm0.035$	$9.92^{abc}\pm0.455$	$371.20^{d}\pm3.68$
MZ +As (100 ppb)	$2.76^{a}\pm0.227$	$0.065^{a}\pm0.016$	$1.96^{a}\pm0.048$	$74.93^{de}\pm3.33$	$0.885^{d}\pm0.027$	$8.62^{a}\pm0.628$	$431.45^{e}\pm8.90$
MZ+ As (100ppb) + Quercetin (50 mg/kg)	$4.44^{ab}\pm0.569$	$0.200^{a}\pm0.050$	$2.82^{cd}\pm0.046$	$77.91^{e}\pm1.76$	$0.655^{a}\pm0.026$	$10.12^{abcd}\pm0.723$	$310.52^{c}\pm12.77$
MZ+ As (100ppb) + Catechin (50mg/kg)	$3.81^{ab}\pm0.550$	$0.105^{a}\pm0.007$	$2.59^{bc}\pm0.038$	$39.15^{b}\pm1.52$	$0.660^{a}\pm0.010$	$9.63^{ab}\pm1.08$	$466.90^{e}\pm6.25$

Values are given as mean ± SE of 6 animals unless otherwise stated
Values having different superscripts (a,b,c,d, e,f,g) in a column are statistically different from one another at 5% level of significance
Values of TAS (Total antioxidant status), expressed in mM
Values of TTH (Total thiols)expressed in µM
Values of advance oxidation protein product (AOPP) are expressed in µM of Chloramine-T
Values of malondialdehyde (MDA) are expressed in nmole of MDA formed/gm/hr
Values of nitric oxide (NO) are expressed in µM

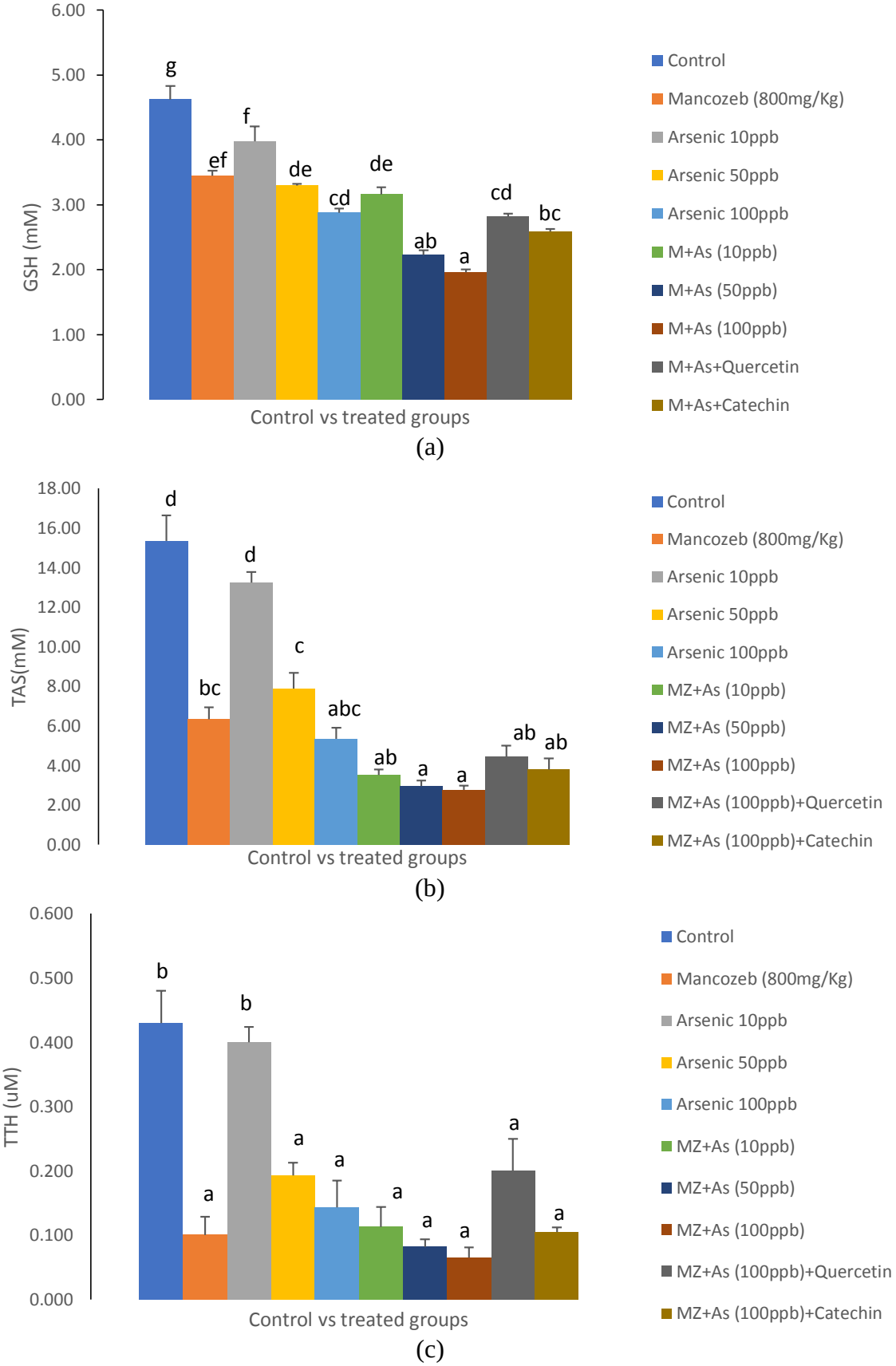


Figure 4.1: **Graphical representation of antioxidant biomarkers *viz.* GSH (a), TAS (b) and TTH (c) in erythrocyte lysate following subacute exposure of mancozeb alone or in-combination with arsenic and its attenuation with quercetin and catechin.**

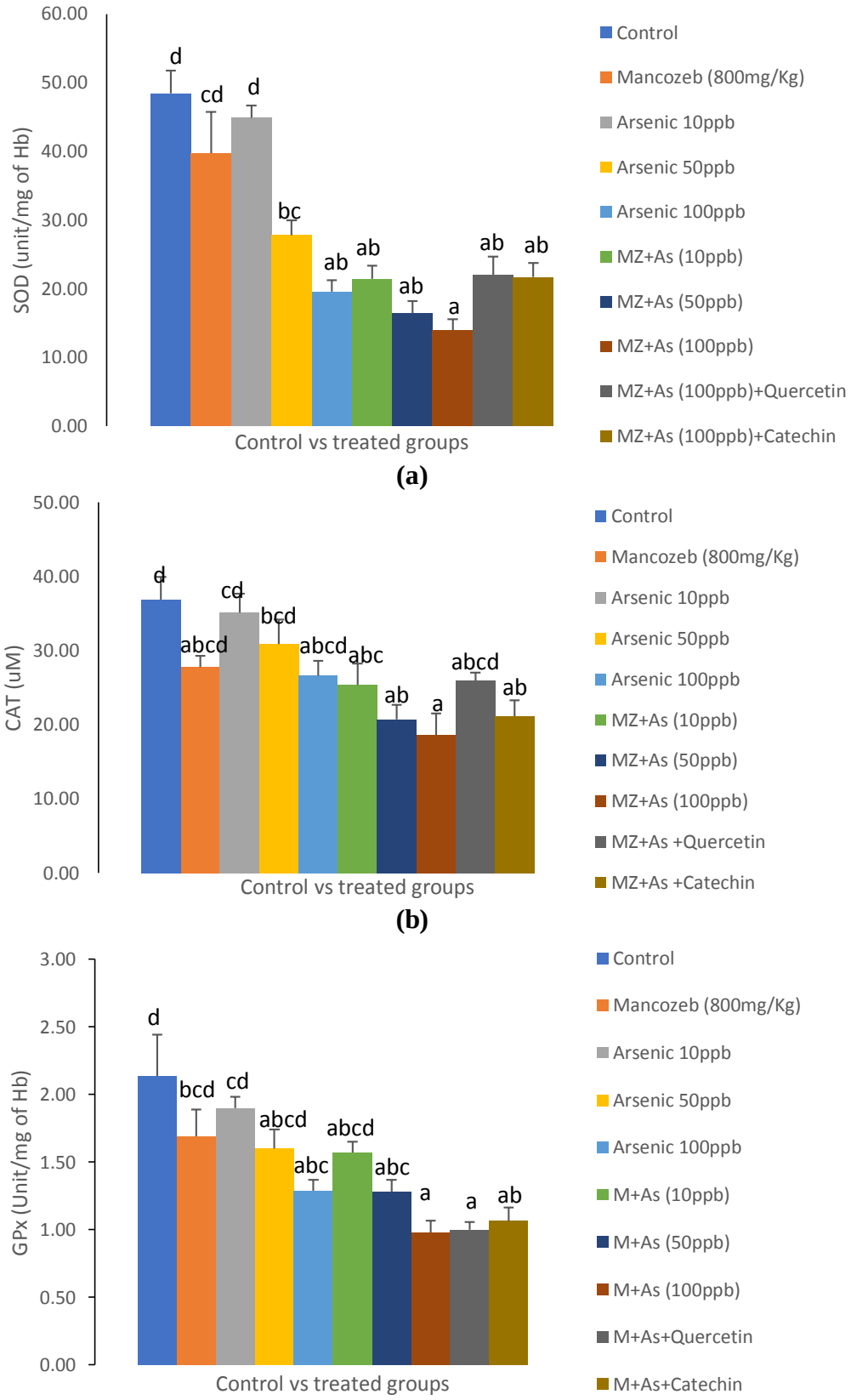


Figure 4.2: **Graphical representation of antioxidant biomarkers *viz.* SOD (a), CAT (b) and GPx (c) in erythrocyte lysate following subacute exposure of mancozeb alone or in-combination with arsenic and its attenuation with quercetin and catechin.**

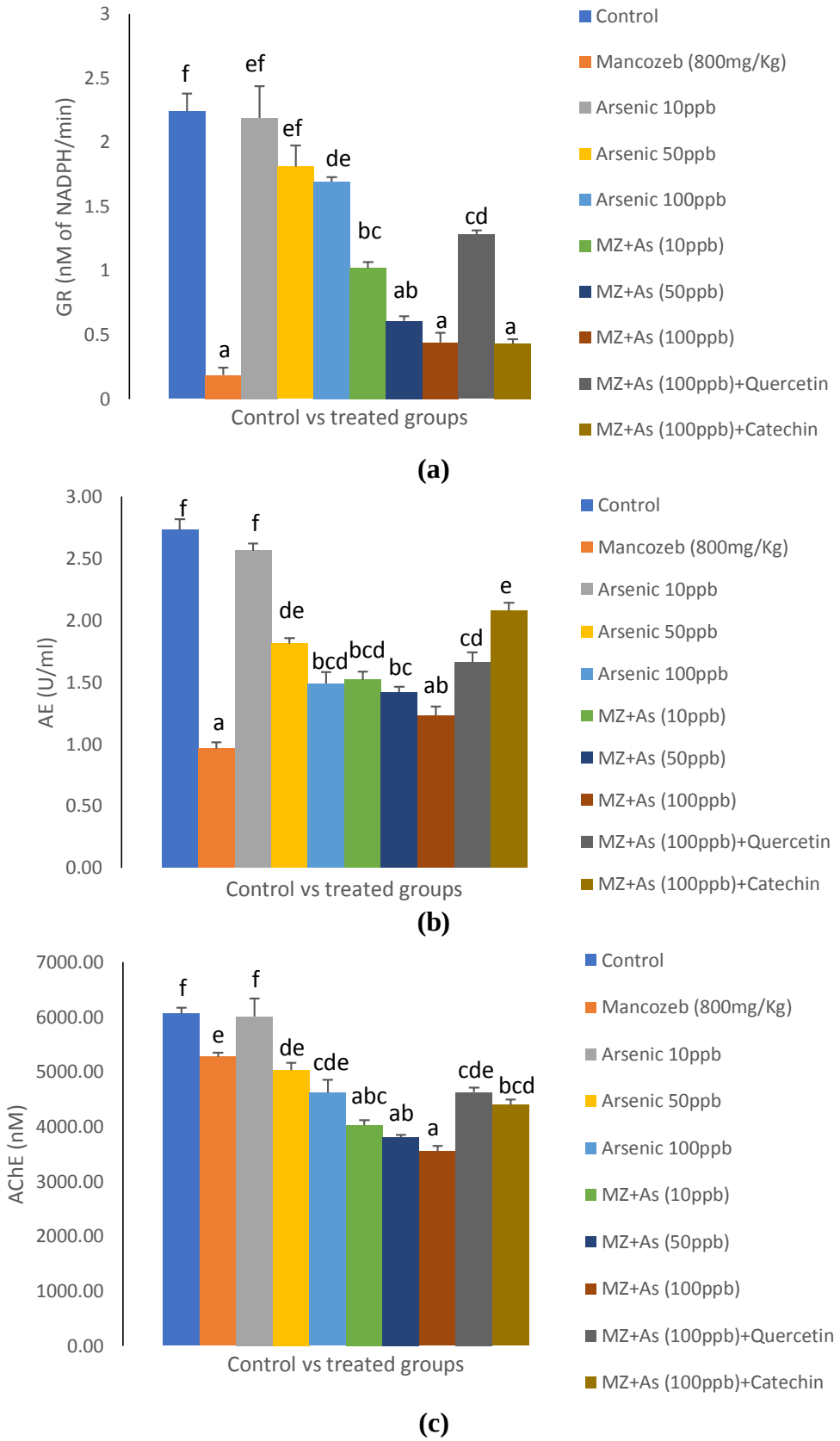
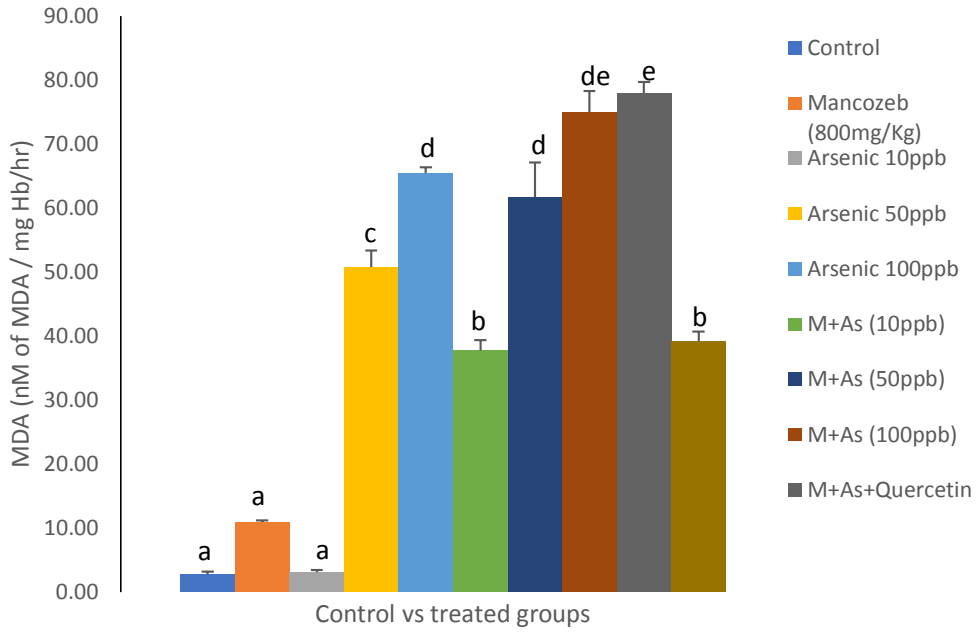
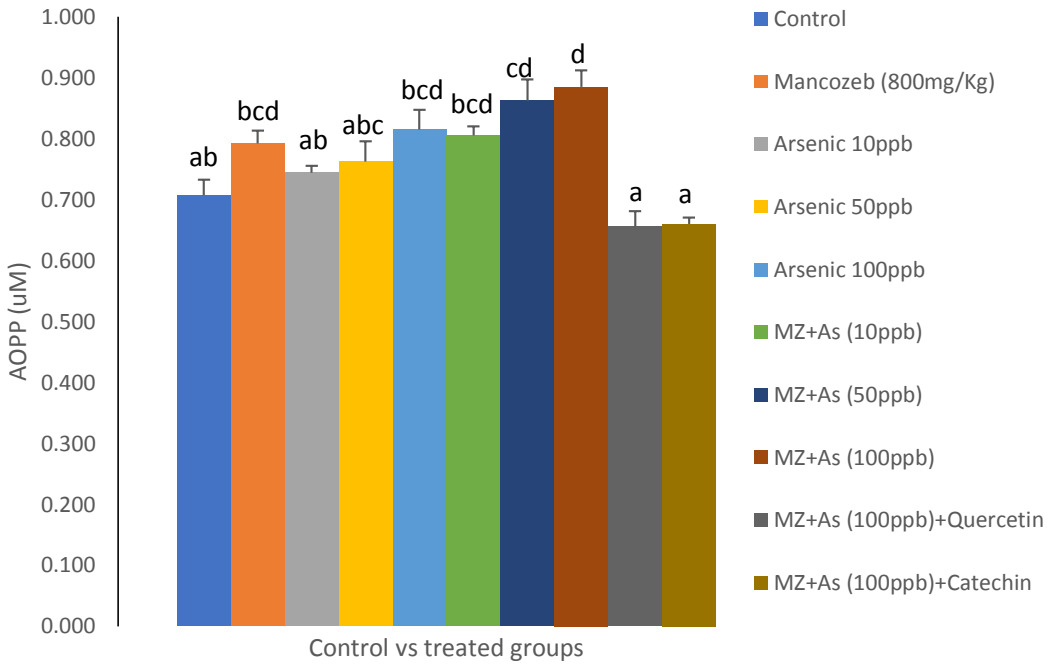


Figure 4.3: **Graphical representation of antioxidant biomarkers *viz.* GR (a), AE (b) and AChE (c) in erythrocyte lysate following subacute exposure of mancozeb alone or in-combination with arsenic and its attenuation with quercetin and catechin.**

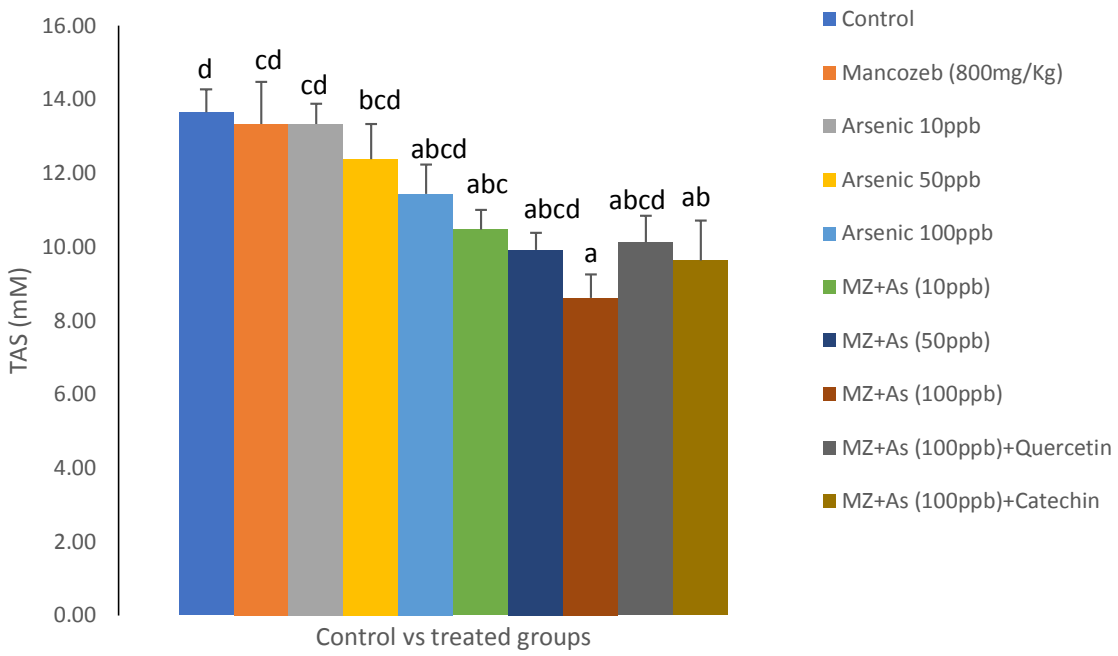


(a)

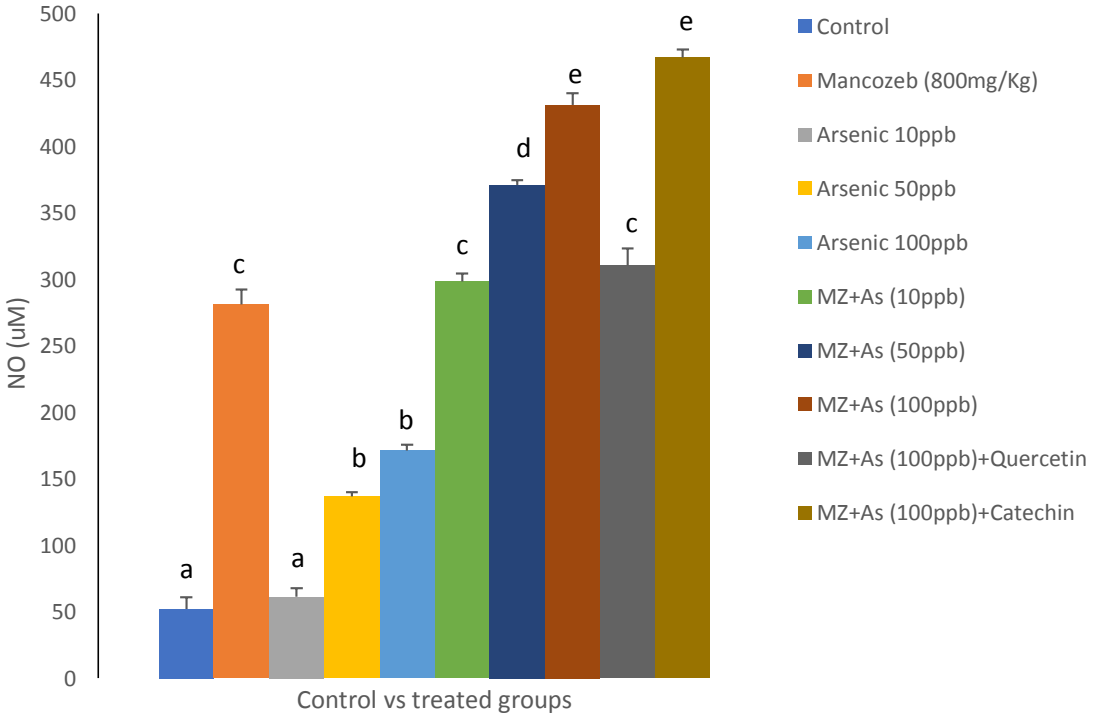


(b)

Figure 4.4: **Graphical representation of cellular damage indicators *viz.* MDA (a) and AOPP (b) in erythrocyte lysate following subacute exposure of mancozeb alone or in-combination with arsenic and its attenuation with quercetin and catechin.**



(a)



(b)

Figure 4.5: Graphical representation of plasma TAS (a) and NO (b) levels in rats following subacute exposure of mancozeb alone or in-combination with arsenic and its amelioration with quercetin and catechin.

4.2. Effect on Brain Antioxidant System:

4.2.1. Total antioxidant status (TAS) and total thiols (TTH): Mean values of TAS and TTH are given in table 4.4 and their graphical representation is depicted in figure 4.8. A significant ($p<0.05$) fall in TAS activity was noticed in group II while it was non-significant fall for TTH levels in comparison to control. Group III, IV and V showed a significant reduction in TAS levels in a dose dependent manner as compared to control. Meanwhile, a significant reduction was seen in group V for TTH levels as compared to control. The TAS values in groups VI, VII, VIII (54.7% decrease) treated with the combinations of toxins revealed a significant decrease and TTH values for group VII and VIII (42.1%) also showed a significant decline when compared to control. Group IX showed 47.5% decrease compared to control in the TAS values but the values were non-significantly improved compared to group VIII. Groups X treated with catechin restored the levels of TAS significantly as compared to group VIII, but the values are still significantly lower (36.1%) than control group. Group IX and X showed only 26.7% and 22.1% decrease respectively in TTH levels as compared to control. This indicates catechin in group X alleviates the toxicity more effectively than quercetin in group IX.

4.2.2. Determination of malondialdehyde (MDA) and protein peroxidation end products (AOPP): Mean values of MDA and AOPP are presented in table 4.4 and their graphical representation is portrayed in figure 4.8 and 4.9 respectively. The activity of malondialdehyde (MDA) and AOPP represented a significant ($p<0.05$) and a non-significant increase respectively, in group II as compared to control animals. MDA and AOPP values in group III, IV and V showed a rising trend in a dose dependent manner as compared to control. The MDA values of group IV increased significantly and group V showed significant increment in MDA as well in AOPP values, compared to control in a dose dependent manner. The toxicant interactive groups including VI represented a significant increase in MDA levels while as group VII and VIII showed a significant increment in MDA as well in AOPP values compared to control as well as alone treated toxicants. Group VIII showed a 79.1% and 21.01 % increment in MDA and AOPP activity respectively as compared to control. While as group IX increased the MDA and AOPP values to 35.4% & 13.1% and X elevated the levels to 90.9% & 15.9% as compared to control. This indicates

quercetin in group IX alleviates the toxicity more effectively than catechin in group X.

4.2.3. Plasma Nitric oxide (NO): Mean values of NO are depicted in table 4.4 and their graphical representation is illustrated in figure 4.9. Plasma NO levels showed a significant increase in group II as contrast to control. Group III, IV and V witnessed a significant rise in NO levels in a dose dependent manner as compared to control. The interactive groups such as VI, VII and VIII represented further increment in NO levels in a dose dependent manner as compared to control as well as alone treated toxicant groups. Group VIII showed a 228.2% increment in NO levels as compared to control while as group IX and X showed only 115.5% and 119.5% increase in NO levels as compared to control. This indicates quercetin in group IX lowers the toxicity more effectively than catechin in group X.

4.2.4. Superoxide dismutase (SOD) and Catalase (CAT): Mean values of SOD and CAT are manifested in table 4.3 and their graphical representation is portrayed in figure 4.6. A significant ($p<0.05$) reduction was observed in CAT activity of group II as compared to control. Group III witnessed a non-significant reduction while as group IV and V represented a significant decline in SOD as well as CAT activity in a dose dependent manner as compared to control. In the interactive groups VI, VII and VIII, a significant curtailment in SOD and CAT levels was seen in a dose dependent manner as compared to control. Group VIII showed a 53.8% and 44.9 % fall in SOD and CAT activity respectively as compared to control. While as group IX lowered the SOD and CAT values to 28.2% & 32.7% and X declined the levels to 20.9% and 44.9% as compared to control. This indicates group IX alleviates the toxicity as per CAT activity is concerned and group X improved the SOD activity.

4.2.5. Glutathione peroxidase (GPx) and glutathione reductase (GR): Mean values of GPx and GR are given in table 4.3 and their graphical representation is depicted in figure 4.7 and 4.6 respectively. In Group II, a significant ($p<0.05$) reduction was observed in GPx activity as compared to control. Group III presented a non-significant fall and group IV and V represented a significant decline in GPx values in a dose dependent manner as compared to control. The group VI witnessed a significant decline in GPx activity while as group VII, VIII represented a significant decrease in GPx as well as in GR levels in a dose dependent manner in contrast to control. Group VIII showed a 42.7% and 47.7% reduction in GPx and GR activity

respectively as compared to control. While as group IX lowered the GPx and GR values to 43.4% & 37.3% and in group X declined the levels to 43.3% and 21.2% as compared to control. This indicates group X improved the GR activity prominently, while as GPx activity was restored equally by both the groups.

4.2.6. Activities of AChE and AE: Alterations in the activities of AChE and AE in control and toxicants treated groups are depicted in table 4.3 and their graphical representation is manifested in figure 4.7. The activity of AChE witnessed a significant ($p<0.05$) decline in group II as compared to control. Group III, IV and V presented a declining trend in AChE and AE in a dose dependent manner, compared to control. The toxicant interactive groups including VI, VII and VIII showed a significant curtailment in AChE and AE activity compared to control as well as alone treated toxicants (group III, IV and V). Group VIII represented a 41.3% and 52.1% reduction in AChE and AE activity, respectively, as compared to control. While as group IX lowered the AChE and AE values to 23.5% & 47.1% and X declined the levels to 28.3% and 22.1% as compared to control. This indicates group IX improves the AChE activity slightly more than group X while as AE activity was restored prominently by group X.

4.2.7. Histopathological alteration in the brain:

4.2.7.1. Cerebrum: Cerebrum in control rats didn't show any abnormal features. The neurons and neuropil in cerebral cortex as well as white matter were healthy (Plate 1a). Cerebrum from group III rats also didn't manifest any pathological changes (Plate 1c). Cerebrum in group II rats showed consequences of toxic insult such as spongiosis in neuropil as well as white matter along with multifocal aggregates of glial cells (Plate 1b). Congestion and perivascular as well as perineuronal edema besides neuronal degeneration and satellitosis were also appreciated. In group IV (Plate 1d) brain sections showed only mild congestion and neuronal degeneration. Also, there was perivascular edema. In group V perivascular edema, spongiosis along neuronal degeneration was appreciated (Plate 1e). In Group VI, more severe spongiosis and neuronal degeneration was appreciated than the respected brain sections from group III rats (Plate 1f). Likewise, group VII had congestion, neuronal degeneration, perivascular edema occasionally causing small foci of encephalomalacia in their vicinity (Plate 2a). Cerebral cortex from group VIII exhibited the most severe lesions. There was edematous fluid in meninges with

lymphocytic infiltration extending unto outer cortical areas (Plate 2b & 2c). Additionally, perivascular edema, neuronal degeneration, necrosis (Plate 2i) besides presence of multifocal wide areas of cavitation consistent with encephalomalacia (Plate 2d) was presented. In group IX, cerebral lesions were significantly muted with congestion, neuronal degeneration, perivascular edema and spongiosis being of less severe intensity as compared to those seen in group VIII (Plate 2e). In group X too, alterations in cerebrum were suppressed in comparison to those observed in group VIII. Congestion and spongiosis were mild. In the same manner, neuronal degeneration was less although necrotic neurons surrounded by glial cells were rarely occasionally sighted (Plate 2f).

4.2.7.2. Cerebellum: The histopathological studies of cerebellum in control animals (Plate 3a) and group III rats (Plate 3c) did not reveal any deviation from the normal histology of brain. The plate showed normal appearance of granule cell neurons, Purkinje cells and neuronal fibers. In group II, the cerebellar cortex showed perineural oedema, loss of Purkinje cells, spongiosis. Also, meningeal congestion and edema were seen (Plate 3b). Additionally in Group IV, spongiosis with degeneration of Purkinje cells and granule cells was seen (Plate 3d). In group V perivascular edema, spongiosis (Plate 3e) were seen in addition to congestion, meningeal edema and infiltration with lymphoid cells was seen. Cerebellar sections from Group VI, VII, VIII had progression in severity of lesions in a dose dependent manner. In Group VI, congestion and perivascular edema was severe in cerebellar white matter (Plate 3f). However, in group 7 these changes were more severe causing encephalomalacias' foci (Plate 4a). Spongiosis leading to cavitation in cortex as well as white matter was most severe in G8 (Plate 4b and 4d). Purkinje cell degeneration was extremely severe in group VIII and their number was drastically reduced (Plate 4d). Further, there was severe oedema in meninges and presence of inflammatory cells which is encroached outer molecular layer (Plate 3c). Aggregates of glial cells forming nodules were multifocally and randomly distributed in cerebellar white matter which also manifested severe spongiosis. In group IX, the cerebellar lesions persisted and congestion, perivascular edema and Purkinje cell degeneration could still be noted in all animals (Plate 4e). Foci of liquefaction in molecular layer infiltrated with lymphocytes were seen in two animals. In comparison to the changes seen in group IX, milder congestion and spongiosis was seen in group X (Plate 4f). Purkinje cell

degeneration and loss was present in all animals but spongiosis was significantly less severe than in group IX. Notably no focal areas of cavitation were encountered in any animal.

Table 4.3: Antioxidant biomarkers in brain tissue following repeated administration of mancozeb (MZ) alone or in-combination with arsenic (As) and its amelioration with quercetin and catechin.

Treatment group	Brain antioxidant parameters					
	SOD	CAT	GPx	GR	AE	AChE
Control	$891.77^{e}\pm33.21$	$3850.38^{e}\pm50.38$	$248.11^{e}\pm5.75$	$52.61^{d}\pm5.62$	$2.36^{f}\pm0.202$	$21102.00^{c}\pm305.73$
MZ (800mg/kg)	$828.43^{de}\pm38.99$	$3072.39^{d}\pm32.01$	$213.06^{cd}\pm11.63$	$45.50^{bcd}\pm4.33$	$2.13^{ef}\pm0.021$	$15376.27^{b}\pm107.14$
Arsenic (10 ppb)	$877.64^{e}\pm22.24$	$3757.47^{e}\pm73.86$	$227.57^{de}\pm7.97$	$49.99^{cd}\pm2.32$	$2.33^{f}\pm0.110$	$21056.88^{c}\pm720.13$
Arsenic (50 ppb)	$666.16^{bc}\pm31.53$	$2925.38^{cd}\pm43.60$	$190.66^{bc}\pm5.86$	$44.59^{bcd}\pm1.17$	$1.98^{def}\pm0.045$	$20749.38^{c}\pm453.71$
Arsenic (100ppb)	$536.11^{ab}\pm50.42$	$2723.88^{bc}\pm42.68$	$163.54^{ab}\pm6.37$	$39.22^{abcd}\pm3.84$	$1.66^{bcd}\pm0.075$	$19665.00^{c}\pm85.44$
MZ + As (10ppb)	$482.55^{a}\pm19.55$	$2588.44^{b}\pm76.04$	$165.77^{ab}\pm9.91$	$40.30^{abcd}\pm3.47$	$1.66^{bcd}\pm0.091$	$16151.63^{b}\pm304.88$
MZ + As (50ppb)	$397.84^{a}\pm19.62$	$2235.45^{a}\pm66.21$	$151.34^{a}\pm2.51$	$35.40^{abc}\pm2.59$	$1.50^{abc}\pm0.082$	$15152.13^{b}\pm59.38$
MZ +As (100 ppb)	$412.46^{a}\pm14.34$	$2118.29^{a}\pm68.53$	$142.29^{a}\pm4.34$	$27.53^{a}\pm2.78$	$1.13^{a}\pm0.059$	$12391.88^{a}\pm149.96$
MZ+ As (100ppb) + Quercetin (50 mg/kg)	$639.97^{bc}\pm22.93$	$2591.43^{b}\pm62.91$	$140.32^{a}\pm3.06$	$32.98^{ab}\pm1.58$	$1.25^{ab}\pm0.109$	$16153.63^{b}\pm95.33$
MZ+ As (100ppb) + Catechin (50mg/kg)	$704.62^{cd}\pm24.77$	$2121.27^{a}\pm41.70$	$140.68^{a}\pm3.49$	$41.48^{abcd}\pm2.27$	$1.84^{cde}\pm0.040$	$15136.63^{b}\pm78.68$

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

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

Values of SOD (Superoxide dismutase) are expressed in Unit/ g of tissue

Values of CAT (Catalase) are expressed in µmol H_2O_2 decomposed/ min/ g of tissue

GPx (glutathione peroxidase) are expressed in Unit/ g of tissue

Values of GR (glutathione reductase) are expressed nmol of NADPH/min

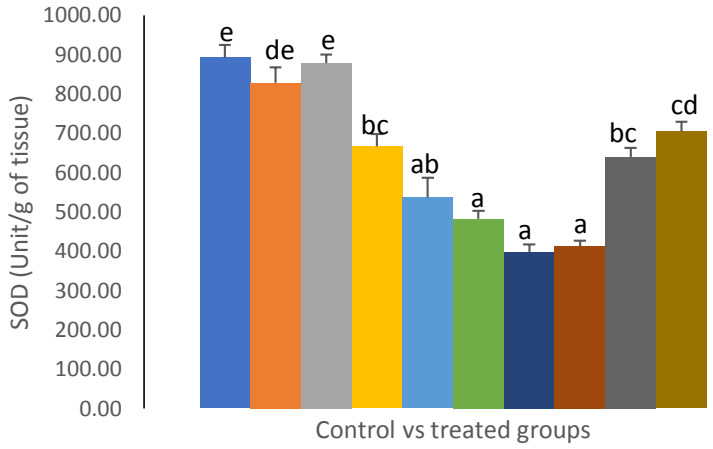
Activities of arylesterase (AE) expressed in U/ml

Acetylcholinesterase (AChE) activity expressed in nmole of thiols produced/min/mg of tissue

Table 4.4: Antioxidant biomarkers in brain tissue following repeated administration of mancozeb (MZ) alone or in-combination with arsenic (As) and its amelioration with quercetin and catechin.

Treatment group	Brain antioxidant parameters				
	TAS	TTH	MDA	AOPP	NO
Control	$20.99^{f}\pm0.284$	$2.40^{b}\pm0.092$	$46.01^{a}\pm2.01$	$1.38^{a}\pm0.034$	$165.38^{a}\pm5.25$
MZ (800mg/kg)	$11.78^{abc}\pm0.865$	$2.32^{b}\pm0.280$	$59.40^{bc}\pm2.62$	$1.48^{abc}\pm0.059$	$281.07^{b}\pm10.72$
Arsenic (10 ppb)	$17.92^{e}\pm0.329$	$2.35^{b}\pm0.124$	$48.21^{ab}\pm2.11$	$1.42^{ab}\pm0.009$	$250.03^{b}\pm8.99$
Arsenic (50 ppb)	$15.28^{de}\pm1.05$	$1.91^{b}\pm0.226$	$59.25^{bc}\pm1.30$	$1.51^{abcd}\pm0.027$	$390.12^{cd}\pm9.77$
Arsenic (100ppb)	$14.20^{cd}\pm0.492$	$1.59^{a}\pm0.289$	$67.31^{cd}\pm1.99$	$1.61^{cd}\pm0.010$	$421.33^{d}\pm6.51$
MZ + As (10ppb)	$11.84^{abc}\pm0.199$	$1.68^{ab}\pm0.266$	$72.45^{de}\pm0.68$	$1.51^{abcd}\pm0.036$	$366.33^{c}\pm4.36$
MZ + As (50ppb)	$10.67^{a}\pm0.367$	$1.48^{a}\pm0.320$	$82.41^{ef}\pm2.05$	$1.67^{cd}\pm0.065$	$415.73^{d}\pm6.64$
MZ +As (100 ppb)	$9.50^{a}\pm0.612$	$1.39^{a}\pm0.217$	$93.52^{f}\pm0.87$	$1.83^{e}\pm0.015$	$542.70^{e}\pm9.68$
MZ+ As (100ppb) + Quercetin (50 mg/kg)	$11.02^{ab}\pm0.268$	$1.76^{ab}\pm0.124$	$62.32^{cd}\pm5.65$	$1.56^{bcd}\pm0.015$	$356.43^{c}\pm2.13$
MZ+ As (100ppb) + Catechin (50mg/kg)	$13.42^{bcd}\pm0.565$	$1.87^{ab}\pm0.216$	$87.87^{f}\pm3.79$	$1.60^{cd}\pm0.011$	$363.07^{c}\pm9.21$

Values are given as mean ± SE of 6 animals unless otherwise stated
Values having different superscripts (a,b,c,d, e,f) in a column are statistically different from one another at 5% level of significance
Values of TAS (Total antioxidant status), expressed in mM
Values of TTH (Total thiols) expressed in µM
Values of advance oxidation protein product (AOPP) are expressed in µM of Chloramine-T
Values of malondialdehyde (MDA) are expressed in nmole of MDA formed/gm/hr
Values of nitric oxide (NO) are expressed in µM



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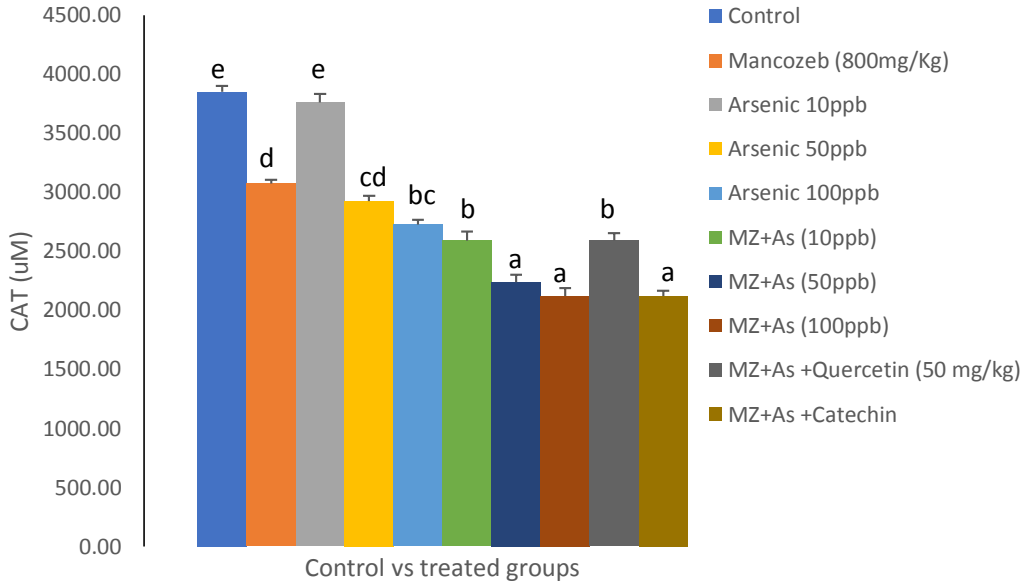

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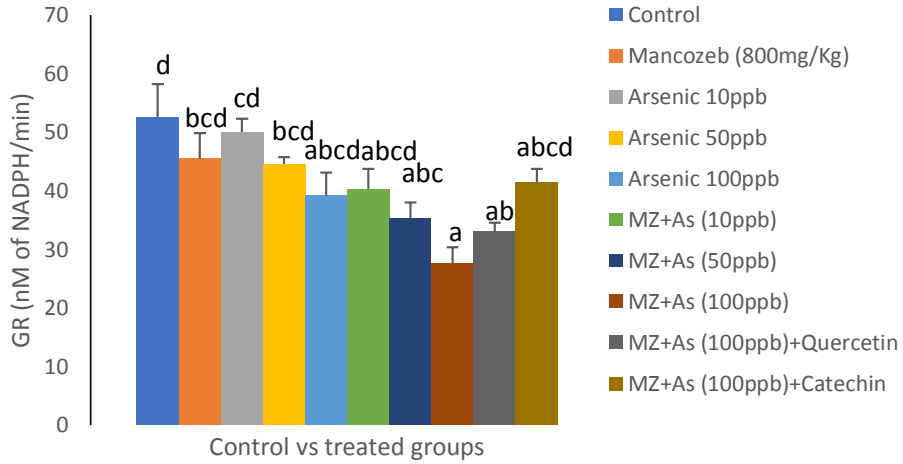
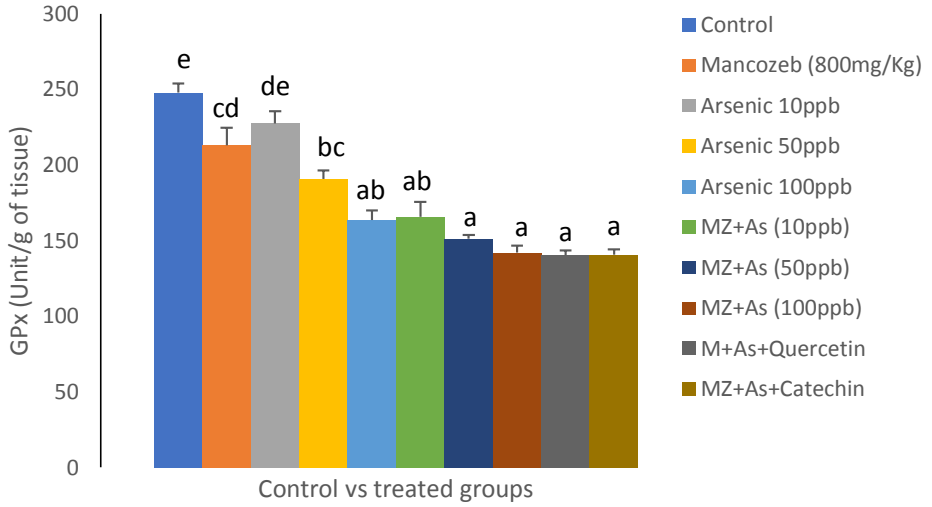


(c)

Figure 4.6: **Graphical representation of antioxidant biomarkers *viz.*SOD (a), CAT(b) and GR (c) in brain tissue following subacute exposure of mancozeb alone or in-combination with arsenic and its attenuation with quercetin and catechin.**



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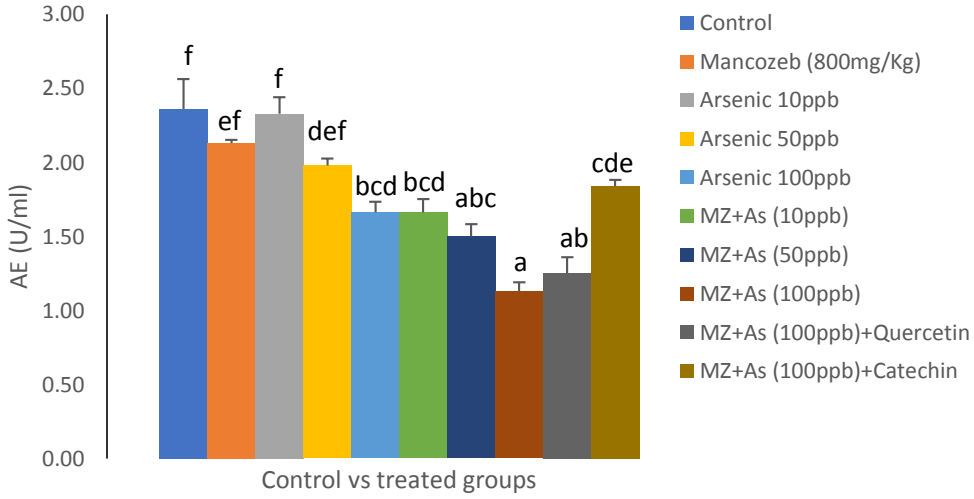

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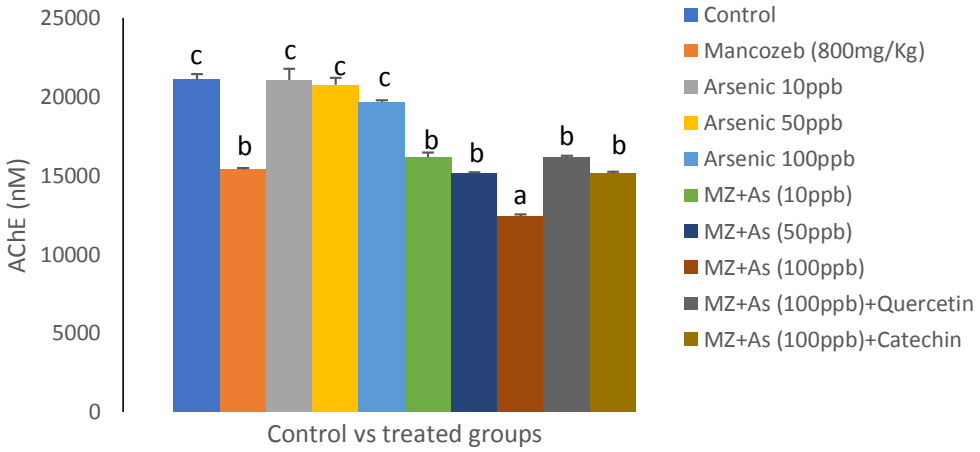
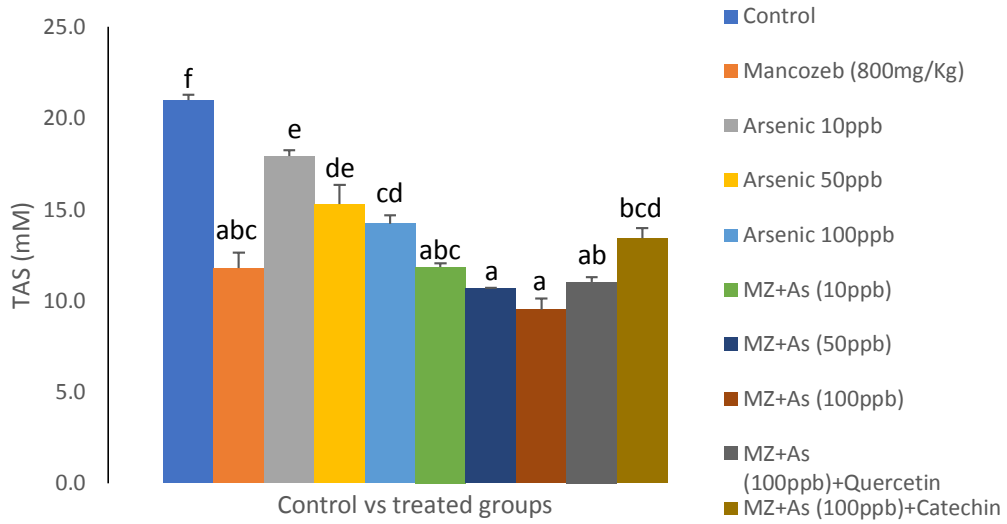
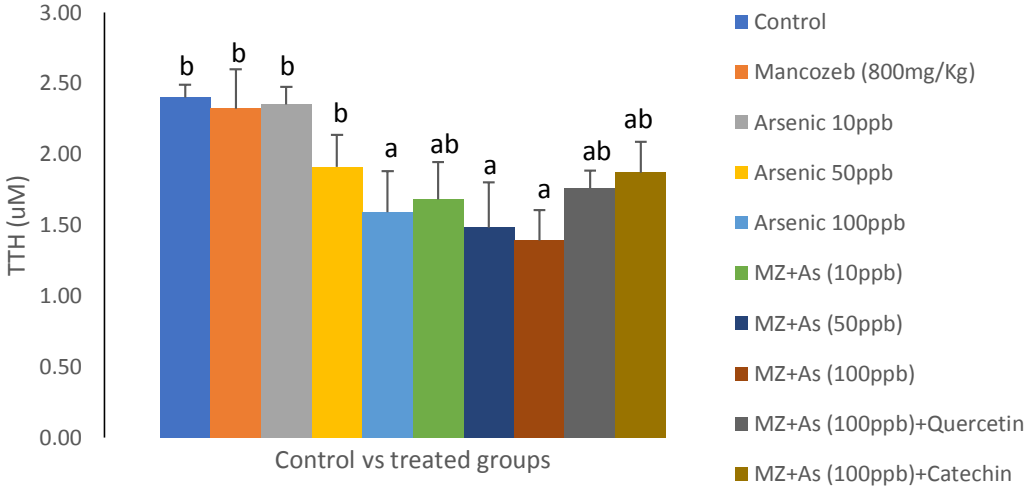


(c)

Figure 4.7: **Graphical representation of antioxidant biomarkers *viz.* GPx (a), AE (b) and AChE (c) in brain tissue following subacute exposure of mancozeb alone or in-combination with arsenic and its attenuation with quercetin and catechin.**



(a)



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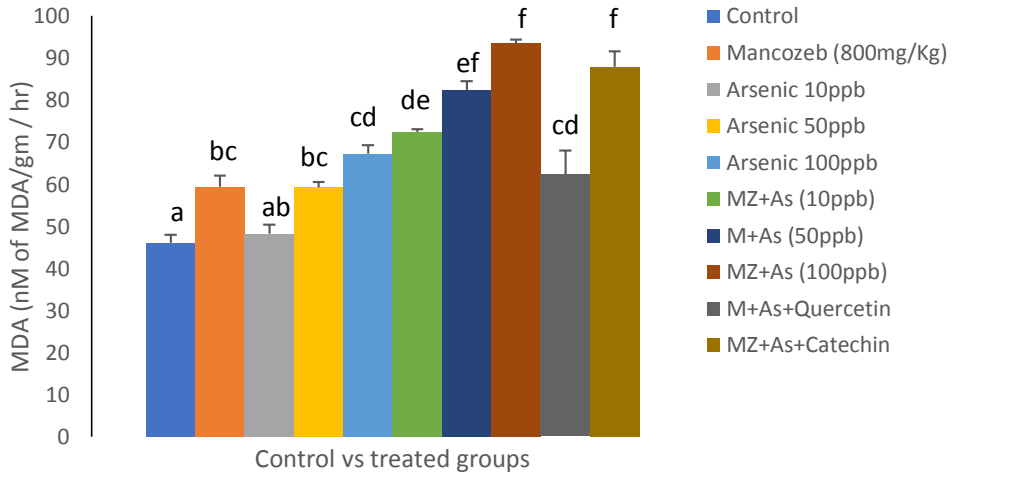
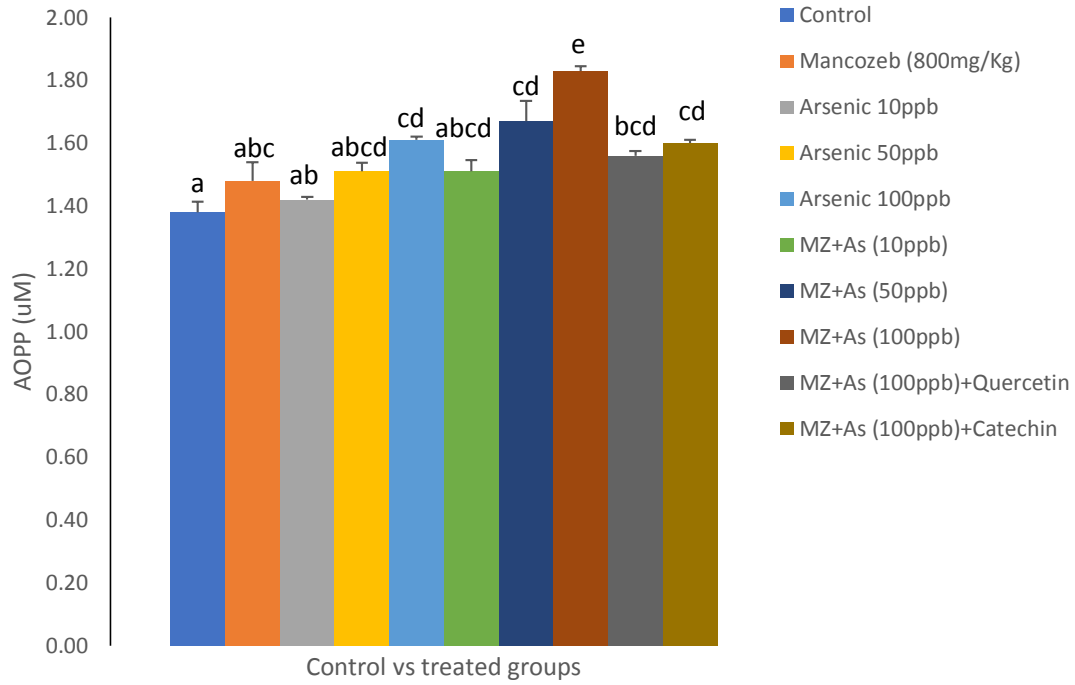
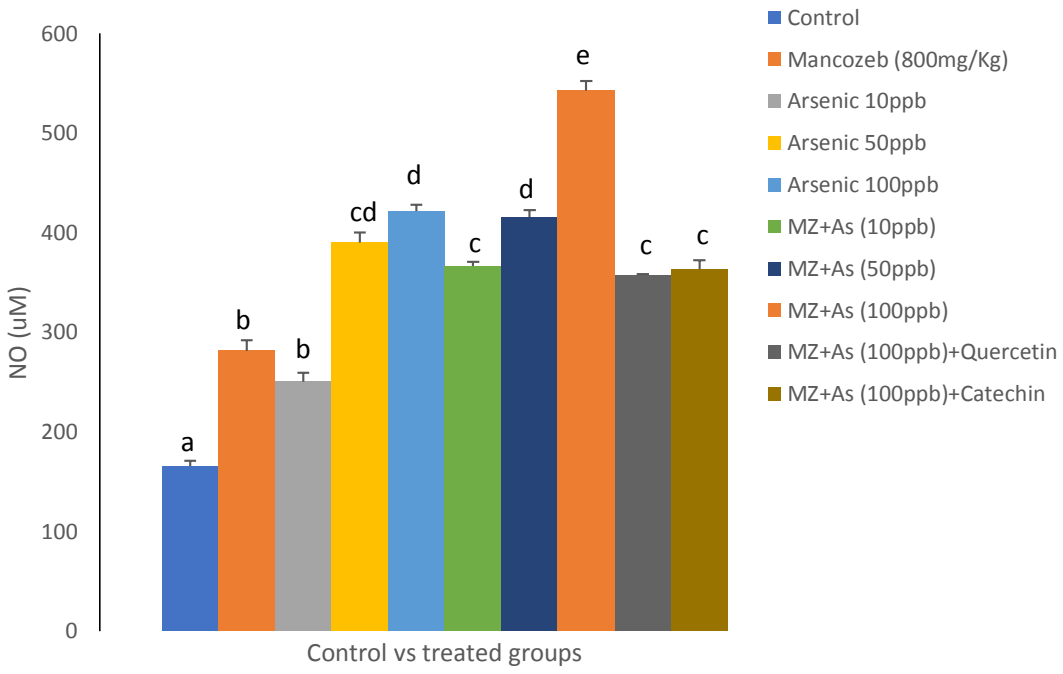


(c)

Figure 4.8: **Graphical representation of antioxidant biomarkers *viz.* TAS (b), TTH (c) and MDA (c) in brain tissue following subacute exposure of mancozeb alone or in-combination with arsenic and its attenuation with quercetin and catechin.**





(b)

Figure 4.9: Graphical representation of AOPP (a) and NO (b) levels in brain tissue following subacute exposure of mancozeb alone or in-combination with arsenic and its attenuation with quercetin and catechin.

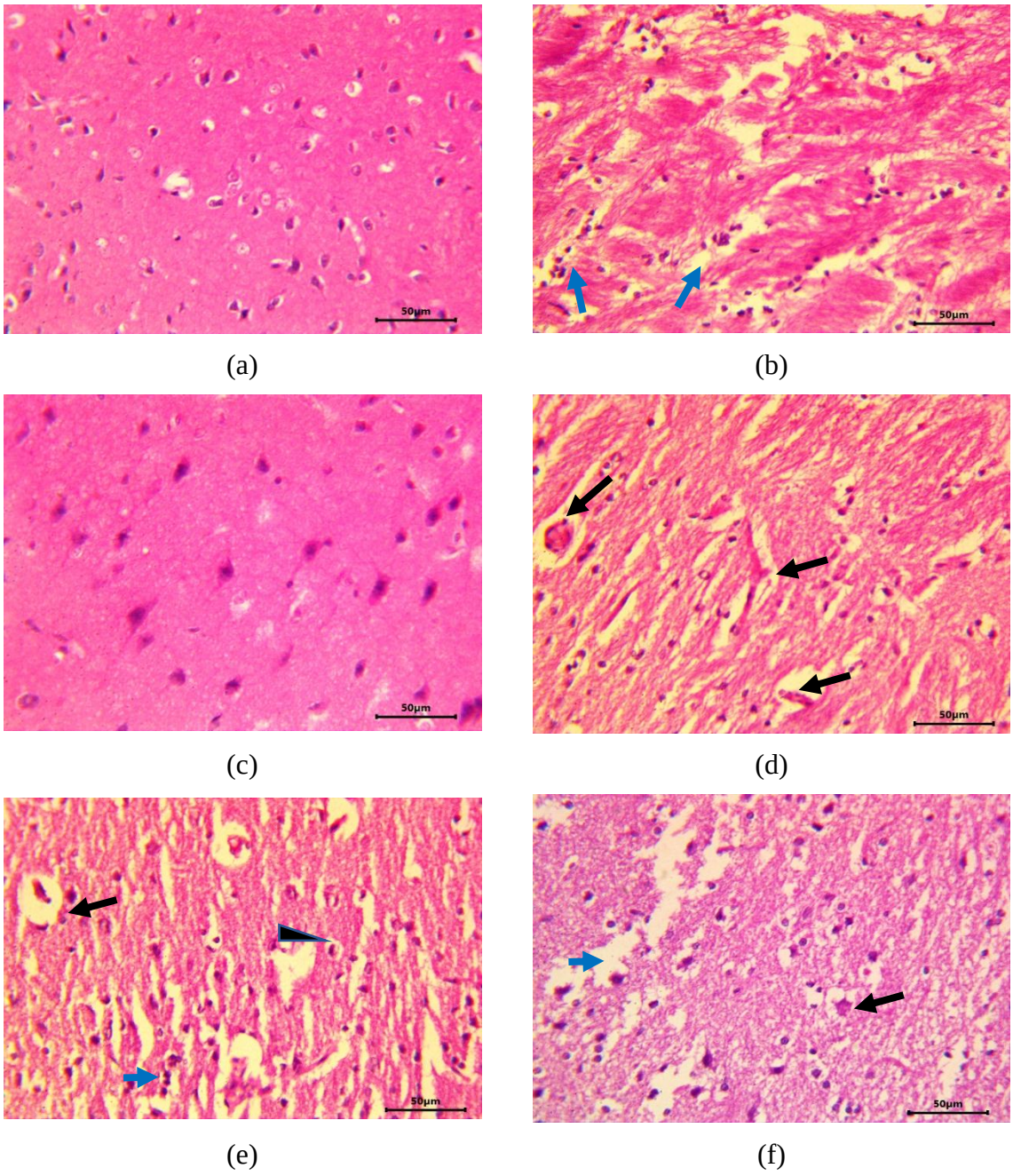
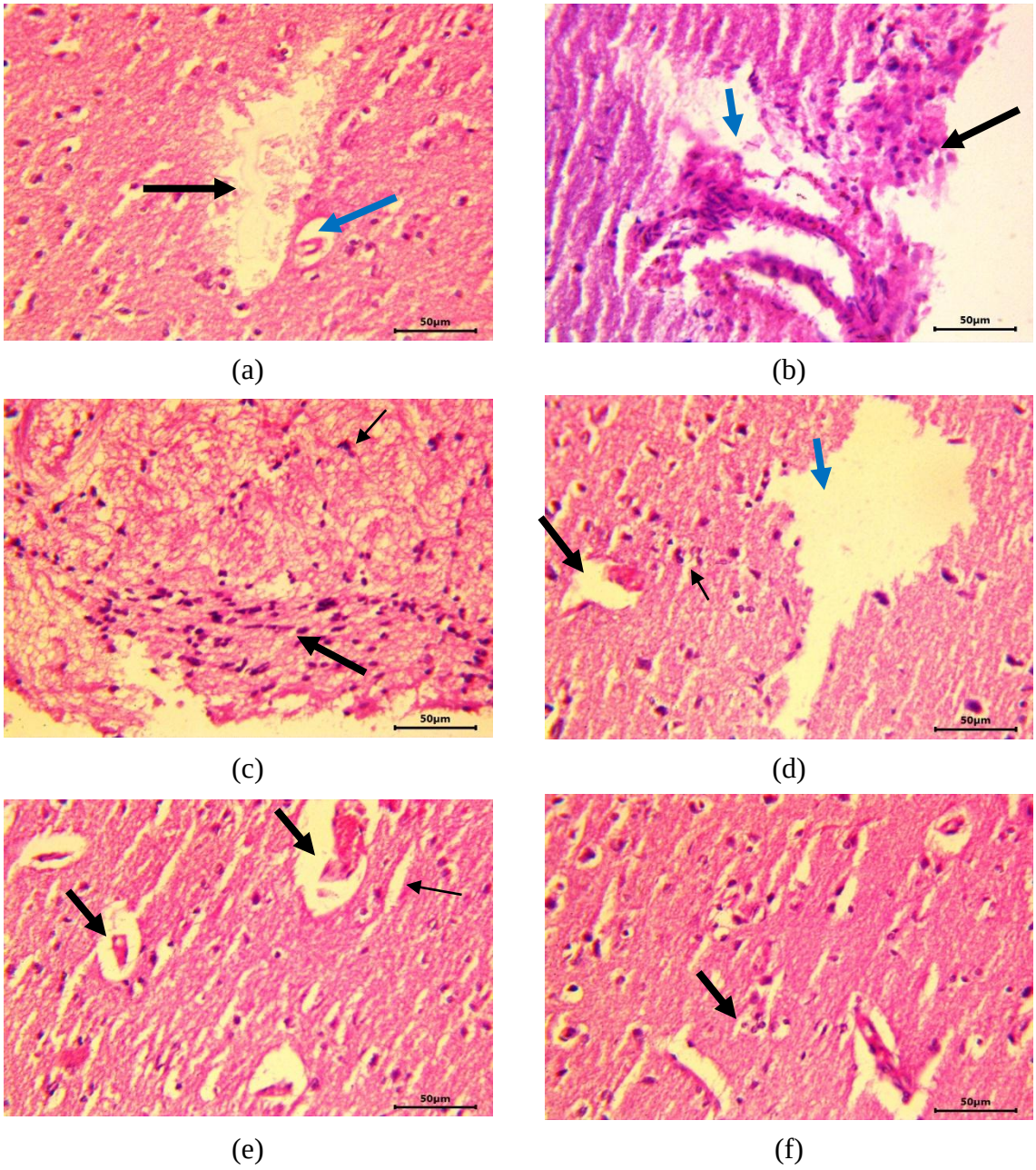


Plate 1: a. Control: Cerebral cortex in G1 rats with normal neurons and neuropil; b. Group II rats: Cerebrum with multifocal aggregation of glial cells (blue arrow) and spongiosis (arrow); c. Group III: No appreciable abnormalities in cerebrum with polygonal neurons and central vesicular nucleus; d. Congestion and perivascular oedema and spongiosis in G IV; e. Moderately severe perivascular oedema (black arrow) gliosis (blue arrow) and spongiosis (arrow head) in G V; f. Neuronal degeneration (black arrow), spongiosis (blue arrow) and gliosis in G VI.

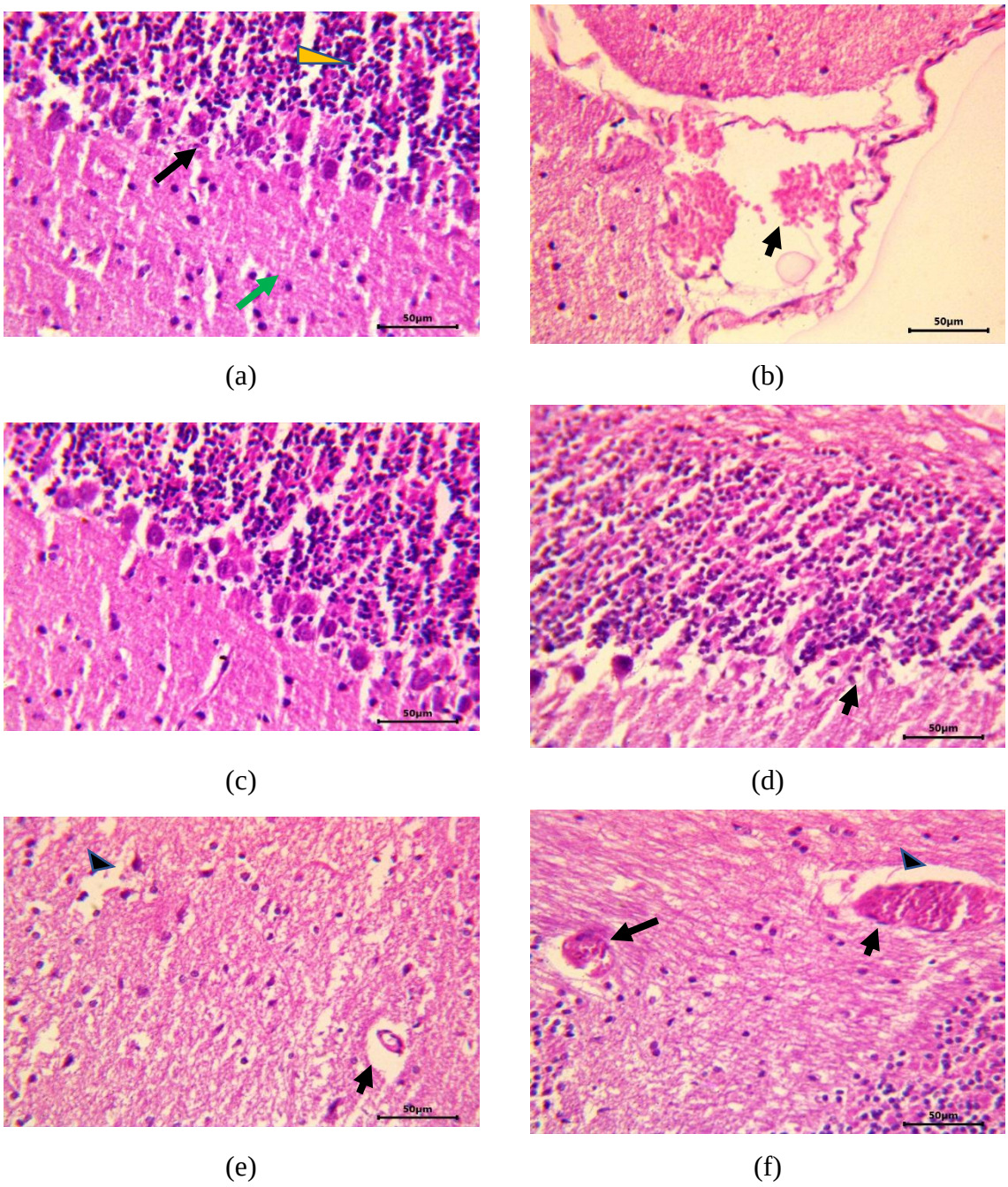


(a) (b)

(c) (d)

(e) (f)

Plate 2: a. Group VII rats showing perivascular oedema (blue arrow) neuronal degeneration and a focal area of encephalomalacia (black arrow); b. Lymphocytic infiltration in meninges (black arrow) encroaching cerebral cortex as well as degeneration of blood vessel walls with perivascular oedema & spongiosis (blue arrow) in G VIII rats; c. Lymphocytic infiltrate permeating cerebral cortex (arrow) from meninges with neuronal degeneration in G VIII rats; d. Perivascular oedema (black arrow), neuronophagia in surrounding neurons (thin arrow) and a large focal area of liquefaction (blue arrow); e. congestion, perivascular oedema (arrow) and spongiosis (thin arrow) in G IX rats; f. Mild perivascular oedema, neuronal degeneration and neuronophagia (arrow) in G X rats.

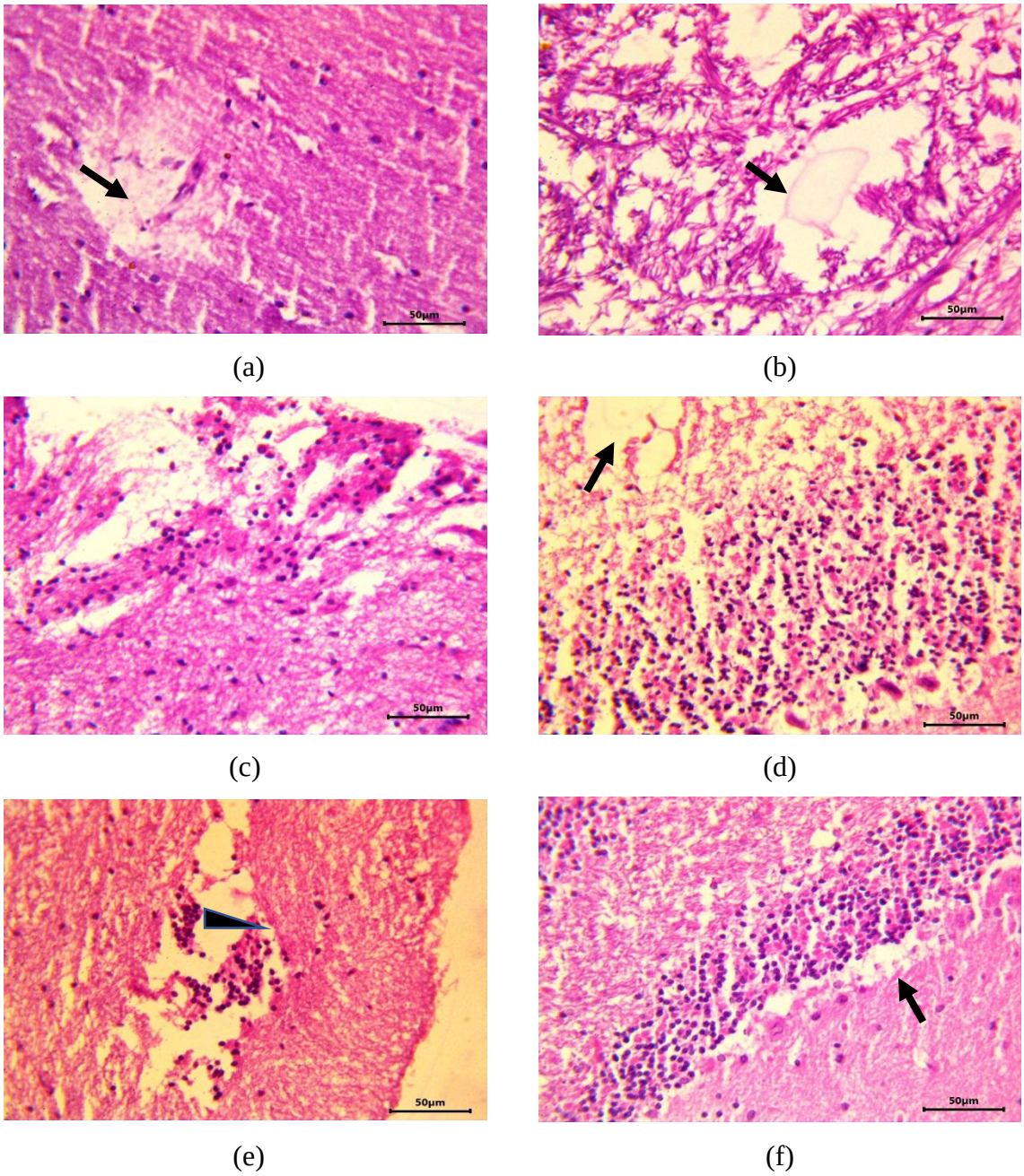


(a) (b)

(c) (d)

(e) (f)

Plate 3: a. Cerebellar cortex in G1 rats with healthy molecular (green arrow), Purkinje cell (arrow) and granule cell layers (arrow head); b. Meningeal oedema, congestion and haemorrhage (arrow) in G II rat cerebellum; c. Mild congestion in cerebellum and Purkinje cell degeneration in GIII; d. Purkinje cell degeneration and satellitosis (arrow) in G IV cerebellum; e. Mild perivascular oedema (arrow) and spongiosis (arrow head) in cerebellar medulla in G V rats; f. congestion (arrow) and perivascular oedema (arrow head) in cerebellar medulla in G VI rats.



(a) (b)

(c) (d)

(e) (f)

Plate 4: a. Group VII rats showing perivascular oedema and degeneration of nerve fibres (arrow) in cerebellar medulla; b. Severe spongiosis (arrow) in cerebellum in G VIII; c. Lymphocytic infiltrates in meninges encroaching cerebellar cortex along with spongiosis in G VIII rats; d. Severe degeneration (arrow head) of neurons and oedema in Purkinje cell (thin arrow) and granule cell layer and white matter spongiosis (arrow) in G VIII rats; e. Focal area of malacia with lymphocytic infiltration (arrow head) in cerebellum G IX rats; f. G X rats showing Purkinje cell degeneration and mild spongiosis (arrow).

4.3. Effect on Biochemical markers of Liver:

4.3.1. Activities of aminotransferases: Alterations in mean values of aminotransferases (ALT and AST) following treatment of toxicants are presented in table 4.5 and their graphical representation is shown in figure 4.10. A significant ($p<0.05$) rise was observed in ALT and AST activity of group II as compared to control. Group III, IV and V treated with sodium arsenite alone represented an increment in ALT and AST activity in a dose dependent manner as compared to control. In the interactive groups (group VI, VII and VII), a significant high was observed in ALT and AST levels in a dose dependent manner as compared to control. In group VIII, the levels of ALT and AST are elevated to 170.0% and 67.6% as compared to control. Group IX increased the ALT and AST activity to 147.9% and 35.3% and group X increased the ALT and AST activity to 176.5% and 28.3% compared to control. This leads to conclusion that group IX (quercetin) improved the levels of ALT and group X alleviated the AST activity. Mean values of GGT are presented in table 4.5 and their corresponding graphical representation is manifested in figure 4.10. A significant ($p<0.05$) rise was observed in GGT activity of group II as compared to control. Group II, IV and V represented an increment in GGT activity as compared to control. The interactive groups such as VI, VII and VIII showed a declining trend in GGT activity, while in group VII and VIII a significant increase in GGT levels was observed in a dose dependent manner as compared to control. In group VIII, the levels of GGT are increased to 64.76% as compared to control. Group IX decreased the GGT activity to 4.6% and X increased the GGT activity to 7.3% as compared to control.

4.3.2. LDH and ALP activities: Mean values of LDH and ALP are shown in table 4.5 and their graphical representation is in figure 4.11. A non-significant rise was observed in LDH activity of group II as compared to control. Group III, IV and V presented an increasing trend in LDH values in a dose dependent manner compared to control and in group V, a significant ($p<0.05$) rise was observed in LDH activity as compared to control. In the interactive groups VI, VII and VII, a significant increment was observed in LDH levels in a dose dependent manner as compared to control. In group VIII, the levels of LDH are elevated to 63.5% as compared to control. Group IX increased the LDH activity to 30.9% and X increased activity to 35.2% as compared to control. A significant ($p<0.05$) rise was observed in ALP activity of

group V as compared to control, whereas in rest groups a non-significant change in ALP activity was seen. Group IX increased the ALP activity to 23.1% and X increased the activity to 4.8% as compared to control.

4.3.3. Protein profile: Mean values of TPP, albumin and globulin are presented in table 4.5 and their graphical representation is manifested in figure 4.12. A significant ($p<0.05$) decline was observed in TPP activity in group V, VII and VIII as compared to control. In group VIII, the levels of TPP are declined to 26.47%% as compared to control. Group IX decreased the TPP levels to 12.4% and X decreased the TPP activity to 15.1% as compared to control. Similarly, there was a non-significant decrease in albumin level in group III and VII and a non-significant increase in group II, IV, V and VIII as compared to control. In group VIII, the levels of albumin are increased to 1.5% as compared to control. Group IX increased the albumin activity to 9.3% and X increased the activity to 7.8% as compared to control. In globulin level, significant ($p>0.05$) decline was observed in group V and VIII as compared to control. In group VIII, the levels of globulin are decreased to 65.9% as compared to control. Group IX decreased the globulin activity to 42.9% and X decreased the globulin activity to 47.5% as compared to control.

4.3.4. Effect on Liver Antioxidant System:

4.3.4.1. Superoxide dismutase (SOD) and Catalase (CAT): Mean values of SOD and CAT are presented in table 4.6 and their graphical representation is illustrated in figure 4.13. A significant ($p<0.05$) reduction was observed in SOD and CAT activity of group II as compared to control. Group III revealed a significant decrease in SOD levels in contrast to control. Group III, IV and V treated with sodium arsenite alone represented a fall in SOD and CAT activity in a dose dependent manner as compared to control. In the interactive groups VI, VII and VII, a significant curtailment in SOD and CAT levels was seen in a dose dependent manner as compared to control. Group VIII showed a 79.5% and 40.8% fall in SOD and CAT activity respectively as compared to control. While as group IX lowered the SOD and CAT values to 64.0% & 31.3% and X declined the levels to 68.4% and 42.2% as compared to control. This indicates group IX treated with quercetin increased the SOD and CAT values significantly compared to group VIII.

4.3.4.2. Glutathione peroxidase (GPx) and glutathione reductase (GR): In Group II, a significant ($P<0.05$) reduction was observed in GR activity and GPx activity as

compared to control. Group III, IV and V represented a decline in GPx values in a dose dependent manner as compared to control. The groups VI, VII, VIII treated with the combinations of toxins, witnessed a significant decrease in GPx levels in a dose dependent manner in contrast to control. Also, group VII and VIII showed a significant reduction in GR activity compared to control. Group VIII showed a 63.8% and 48.2% decline in GPx and GR activity respectively as compared to control. While as group IX lowered the GPx and GR levels to 62.8% &35.9% and X declined the levels to 64.9% and 55.3% respectively as compared to control. Mean values of GPx and GR are given in table 4.6 and their graphical representation is depicted in figure 4.13 and 4.14 respectively.

4.3.4.3. Total antioxidant status (TAS) and total thiols (TTH): Mean values of TAS and TTH are presented in table 4.7 and their graphical representation is portrayed in figure 4.14 and 4.15, respectively. A non-significant deduction was observed in TAS content of group II, while it was a significant decline for TTH levels as compared to control. A decreasing trend in activity of TAS and TTH was noticed in group III, IV and V in a dose dependent pattern in comparison to control. The groups VI, VII, VIII treated with the combinations of toxicants, a significant decrease in TAS as well as TTH levels was observed, compared to control. Group VIII showed a 20.7% and 57.3% decline in TAS and TTH activity, respectively, as compared to control. While as group IX lowered the TAS and TTH levels to 4.4% & 31.5% and X declined the levels to 20.1% and 14.1% as compared to control. The observations indicate that group IX improved TAS activity and group X restored the TTH activity.

4.3.4.4. Effects on MDA & AOPP: Mean values of MDA and AOPP are given in table 4.7 and their graphical representation is illustrated in figure 4.15. A non-significant deduction was observed in mean values of MDA and AOPP of group II as compared to control. An increasing trend in activity of MDA and AOPP was noticed in group III, IV and V in a dose dependent way as compared to control. The activity of MDA represented a significant ($p<0.05$) increase in group V and a significant rise in AOPP levels in group IV and V as compared to control. The MDA values of group IV and V and AOPP values of group V showed a significant increase, compared to control in a dose dependent manner. The toxicant interactive groups (VI, VII and VIII) showed a significant increment in MDA values compared to control as well as alone treated toxicants. Group VIII represented a significant rise (28.7%) in AOPP

while MDA showed a 112.2% increment in MDA levels as compared to control. The group IX elevated the MDA and AOPP values to 41.3% and 11.8% and X declined the MDA levels to 53.4% and negligible increase in AOPP levels was noticed as compared to control.

4.3.4.5. Effects on AE: Mean values AE are given in table 4.6 and their graphical representation is manifested in figure 4.14. The activity of AE witnessed a significant ($p<0.05$) decrease in group II. The sodium arsenite treated groups (II, IV and V) presented a declining trend in AE activity in a dose dependent manner, compared to control. The toxicant interactive groups including VI, VII and VIII showed a significant curtailment in the AE activity compared to control as well as alone treated toxicants respectively. Group VIII showed a 59.9% fall in AE activity as compared to control. While as group IX and X lowered the AE levels to 51.6% and 36.7% respectively as compared to control. Hence, group X increased AE activity significantly compared to group VIII but the improvement is significantly lower than the control.

4.3.5. Histopathological alterations in liver: The histopathological sections of liver in control group exhibited normal lobular architecture (Plate 5a). Chords of hepatocytes ran radially from central vein separated by sinusoids lined by endothelium (Plate 5a). Portal triads were separated from surrounding periportal hepatocytes by a limiting membrane which contained hepatic artery, vein and bile ducts lined by simple cuboidal epithelium. In group II, multifocally were present random areas of hepatocellular necrosis which were infiltrated by inflammatory cells consisting of lymphoid cells and few macrophages. Additionally, congestion, haemorrhages, oedema and necrosis of hepatocytes in centrilobular areas were noticed (Plate 5b). In group III mild congestion was seen in portal triad areas but rest of the liver didn't reveal any change (Plate 5c). Hepatocytes in Group IV exhibited mild degeneration and occasional foci of necrosis infiltrated by lymphocytes (Plate 5d). In GV, degenerative changes were seen in hepatocytes particularly those surrounding periportal areas and random focal areas of necrosis infiltrated by lymphocytes was seen (Plate 5e). Group VI livers had abnormally dilated sinusoids and hepatocytes were atrophied. Large areas of hepatocellular necrosis and inflammation were observed (Plate 5f). Group VII showed marked congestion as revealed by dilated central veins and portal veins. Random focal areas containing necrotic hepatocytes

which were infiltrated by phagocytic cells. Perivenular hepatocytic degeneration and focal necrosis was striking (Plate 6a). Group VIII revealed most severe pathological alterations consisting of severe congestion and hepatocellular vacuolar degeneration throughout liver parenchyma (Plate 6b). Multiple focal areas of necrosis and lymphocytic infiltration causing loss of liver parenchyma were found (Plate 6c). Limiting membrane was often disrupted and periportal hepatocytes in its vicinity were necrotic (Plate 6d). In group IX changes consisted of congestion, widespread hepatic degeneration and few random foci of hepatocellular necrosis, lymphocytic inflammation (Plate 6e) but these alterations were subtle as compared to those seen in group VIII. Group X showed severe vacuolar degeneration, haemorrhage and focal lymphocytic infiltration (Plate 6f).

Table 4.5: **Alterations in plasma hepatic biomarkers following repeated administration of mancozeb (MZ) alone or in-combination with arsenic (As) and its amelioration with quercetin and catechin.**

Treatment group	Liver biochemical biomarkers							
	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	LDH (IU/L)	ALP (IU/L)	TP (gm/dl)	ALB (gm/dl)	GLO (gm/dl)
Control	$38.62^{a}\pm1.18$	$126.70^{a}\pm4.06$	$1.93^{a}\pm0.136$	$381.18^{a}\pm24.55$	$226.07^{abc}\pm10.27$	$6.80^{b}\pm0.574$	$3.98^{a}\pm0.166$	$2.82^{b}\pm0.516$
MZ (800mg/kg)	$100.88^{d}\pm3.23$	$156.60^{bc}\pm2.46$	$2.98^{c}\pm0.209$	$451.98^{abc}\pm32.88$	$194.28^{ab}\pm6.97$	$5.50^{ab}\pm0.302$	$4.17^{a}\pm0.218$	$1.33^{ab}\pm0.359$
Arsenic (10 ppb)	$50.07^{ab}\pm1.36$	$131.28^{ab}\pm2.09$	$1.66^{a}\pm0.149$	$396.03^{ab}\pm13.57$	$241.13^{abc}\pm5.87$	$6.05^{ab}\pm0.139$	$3.68^{a}\pm0.084$	$2.38^{ab}\pm0.133$
Arsenic (50 ppb)	$67.49^{bc}\pm2.15$	$167.07^{cd}\pm11.73$	$2.75^{bc}\pm0.265$	$465.47^{abcd}\pm31.67$	$306.63^{cd}\pm12.27$	$6.01^{ab}\pm0.209$	$4.43^{a}\pm0.121$	$1.58^{ab}\pm0.275$
Arsenic (100ppb)	$72.03^{c}\pm3.99$	$176.03^{cd}\pm1.60$	$1.99^{ab}\pm0.105$	$566.57^{cde}\pm10.85$	$343.17^{d}\pm10.82$	$5.30^{a}\pm0.393$	$4.06^{a}\pm0.073$	$1.24^{a}\pm0.412$
MZ + As (10ppb)	$72.79^{c}\pm10.32$	$160.17^{c}\pm2.76$	$2.17^{ab}\pm0.059$	$580.08^{de}\pm30.67$	$169.62^{a}\pm19.85$	$5.79^{ab}\pm0.415$	$4.16^{a}\pm0.129$	$1.64^{ab}\pm0.340$
MZ + As (50ppb)	$97.18^{d}\pm5.97$	$191.88^{de}\pm5.14$	$3.07^{c}\pm0.205$	$611.18^{e}\pm41.48$	$194.40^{ab}\pm22.33$	$5.32^{a}\pm0.140$	$3.80^{a}\pm0.126$	$1.51^{ab}\pm0.137$
MZ +As (100 ppb)	$104.29^{d}\pm2.83$	$212.30^{e}\pm6.18$	$3.18^{c}\pm0.195$	$623.23^{e}\pm15.14$	$228.10^{abc}\pm14.48$	$5.00^{a}\pm0.222$	$4.04^{a}\pm0.109$	$0.96^{a}\pm0.276$
MZ+ As (100ppb) + Quercetin (50 mg/kg)	$95.74^{d}\pm3.80$	$171.40^{cd}\pm3.99$	$1.84^{a}\pm0.122$	$498.80^{abcde}\pm23.18$	$278.22^{bcd}\pm18.06$	$5.96^{ab}\pm0.078$	$4.35^{a}\pm0.209$	$1.61^{ab}\pm0.269$
MZ+ As (100ppb) + Catechin (50mg/kg)	$106.79^{d}\pm4.56$	$162.50^{c}\pm7.82$	$2.07^{ab}\pm0.083$	$515.37^{bcde}\pm25.80$	$237.01^{abc}\pm23.99$	$5.77^{ab}\pm0.214$	$4.29^{a}\pm0.329$	$1.48^{ab}\pm0.327$

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

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

MZ-Mancozeb; As-arsenic; ALT-Aspartate aminotransferase; AST; Alanine aminotransferase; GGT-gamma glutamyl transferase; LDH-Lactate dehydrogenase; ALP-Alkaline phosphatase; TP-Total protein; ALB-Albumin; GLO-globulin

Table 4.6: **Alterations in antioxidant biomarkers in liver tissue following repeated administrations of mancozeb (MZ) alone or in-combination with arsenic (As) and its amelioration with quercetin and catechin.**

Treatment group	Liver antioxidant parameters				
	SOD	CAT	GPx	GR	AE
Control	$927.79^{f}\pm20.39$	$4992.92^{d}\pm66.91$	$348.64^{c}\pm11.39$	$58.75^{e}\pm3.15$	$3.22^{d}\pm0.147$
MZ (800mg/kg)	$537.41^{d}\pm8.28$	$3535.45^{c}\pm44.91$	$187.61^{b}\pm8.92$	$43.39^{bcd}\pm2.16$	$2.30^{c}\pm0.061$
Arsenic (10 ppb)	$804.02^{e}\pm12.88$	$4652.25^{d}\pm56.41$	$320.62^{c}\pm4.64$	$58.25^{e}\pm3.45$	$2.96^{d}\pm0.160$
Arsenic (50 ppb)	$510.60^{d}\pm31.08$	$3395.90^{bc}\pm20.15$	$200.91^{b}\pm5.98$	$54.22^{de}\pm2.15$	$2.44^{c}\pm0.060$
Arsenic (100ppb)	$436.75^{cd}\pm43.20$	$3023.89^{a}\pm71.70$	$151.31^{a}\pm10.98$	$48.38^{cde}\pm4.09$	$2.04^{bc}\pm0.060$
MZ + As (10ppb)	$352.86^{bc}\pm22.20$	$3391.42^{bc}\pm56.90$	$130.12^{a}\pm5.34$	$55.08^{de}\pm3.91$	$1.62^{ab}\pm0.070$
MZ + As (50ppb)	$258.98^{ab}\pm16.66$	$3136.95^{ab}\pm53.41$	$128.70^{a}\pm6.82$	$31.11^{ab}\pm3.02$	$1.51^{a}\pm0.056$
MZ +As (100 ppb)	$190.57^{a}\pm11.81$	$2955.98^{a}\pm85.63$	$126.18^{a}\pm5.46$	$30.46^{ab}\pm3.59$	$1.29^{a}\pm0.174$
MZ+ As (100ppb) + Quercetin (50 mg/kg)	$333.95^{bc}\pm15.61$	$3429.11^{c}\pm39.29$	$130.09^{a}\pm3.67$	$37.63^{abc}\pm1.66$	$1.56^{ab}\pm0.072$
MZ+ As (100ppb) + Catechin (50mg/kg)	$293.54^{ab}\pm20.66$	$2886.20^{a}\pm46.66$	$122.27^{a}\pm3.55$	$26.26^{a}\pm2.10$	$2.04^{bc}\pm0.069$

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

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

Values of SOD (Superoxide dismutase) are expressed in Unit/ g of tissue

Values of CAT (Catalase) are expressed in µmol H_2O_2 decomposed/ min/ g of tissue

GPx (glutathione peroxidase) are expressed in Unit/ g of tissue

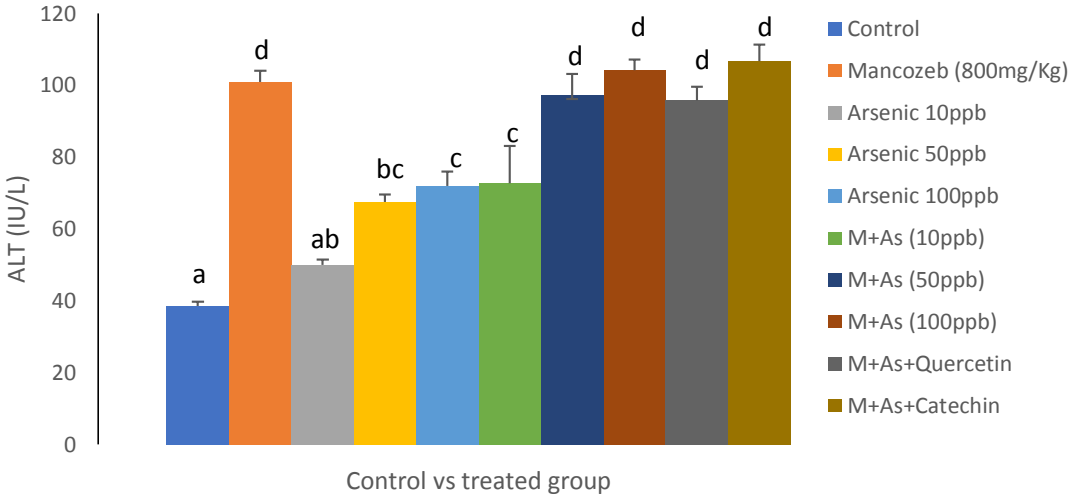
Values of GR (glutathione reductase) are expressed nmol of NADPH/min

Activities of arylesterase (AE) expressed in U/ml

Table 4.7: **Antioxidant biomarkers in liver tissue following repeated administration of mancozeb (MZ) alone or in-combination with arsenic (As) and its amelioration with quercetin and catechin in Wistar rats.**

Treatment group	Liver antioxidant parameters			
	TAS	TTH	MDA	AOPP
Control	22.25^{d}±0.232	2.13^{b}±0.317	39.70^{a}±3.63	1.36^{a}±0.099
MZ (800mg/kg)	21.83^{d}±0.283	1.74^{a}±0.222	55.36^{abc}±5.46	1.47^{abc}±0.044
Arsenic (10 ppb)	20.09^{bcd}±0.487	2.13^{b}±0.299	41.04^{ab}±2.15	1.40^{ab}±0.019
Arsenic (50 ppb)	19.85^{abcd}±0.567	2.01^{b}±0.283	53.94^{abc}±5.87	1.65^{bcd}±0.069
Arsenic (100ppb)	18.91^{abc}±0.687	1.81^{b}±0.249	67.65^{cd}±2.64	1.72^{cd}±0.115
MZ + As (10ppb)	19.04^{abc}±0.839	1.46^{a}±0.144	55.70^{bc}±3.14	1.51^{abcd}±0.012
MZ + As (50ppb)	18.94^{abc}±0.550	1.26^{a}±0.168	74.16^{de}±1.69	1.64^{abcd}±0.008
MZ +As (100 ppb)	17.64^{a}±0.429	0.91^{a}±0.245	84.25^{e}±2.62	1.75^{d}±0.073
MZ+ As (100ppb) + Quercetin (50 mg/kg)	21.27^{cd}±0.355	1.46^{ab}±0.250	56.09^{bc}±2.70	1.52^{abcd}±0.018
MZ+ As (100ppb) + Catechin (50mg/kg)	17.78^{ab}±0.425	1.83^{b}±0.232	60.90^{cd}±4.92	1.37^{ab}±0.031

Values are given as mean ± SE of 6 animals unless otherwise stated
Values having different superscripts (a,b,c,d,e) in a column are statistically different from one another at 5% level of significance
Values of TAS (Total antioxidant status), expressed in mM
Values of TTH (Total thiols)expressed in µM
Values of advance oxidation protein product (AOPP) are expressed in µM of Chloramine-T
Values of malondialdehyde (MDA) are expressed in nmole of MDA formed/gm/hr



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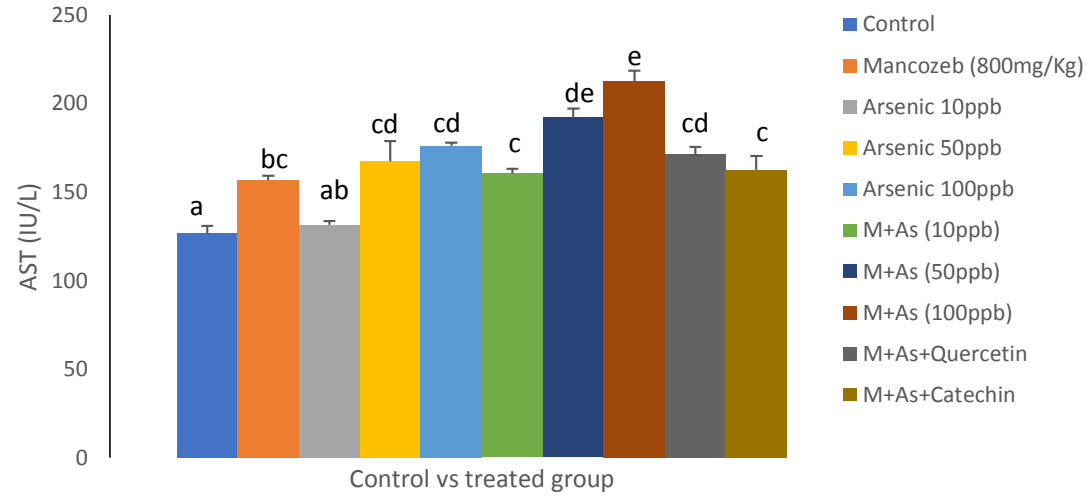

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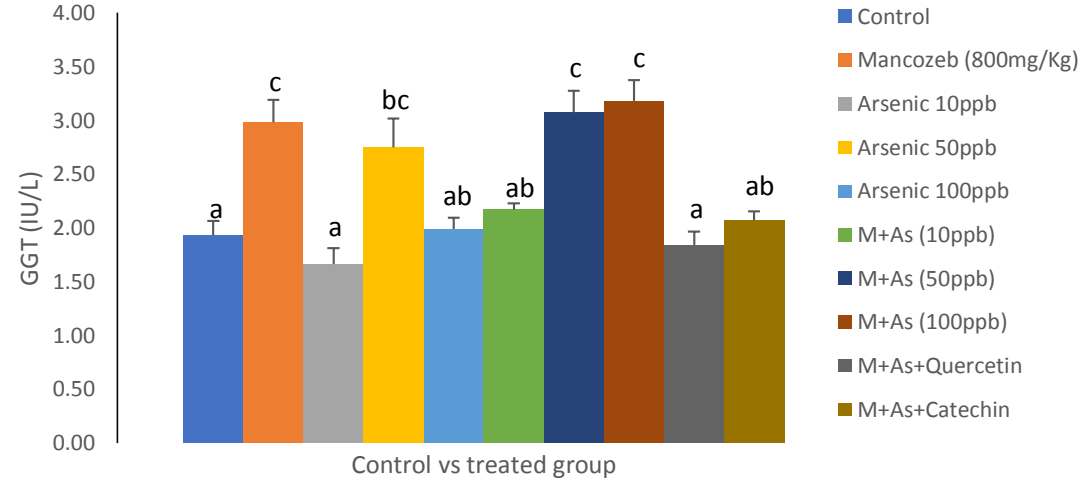
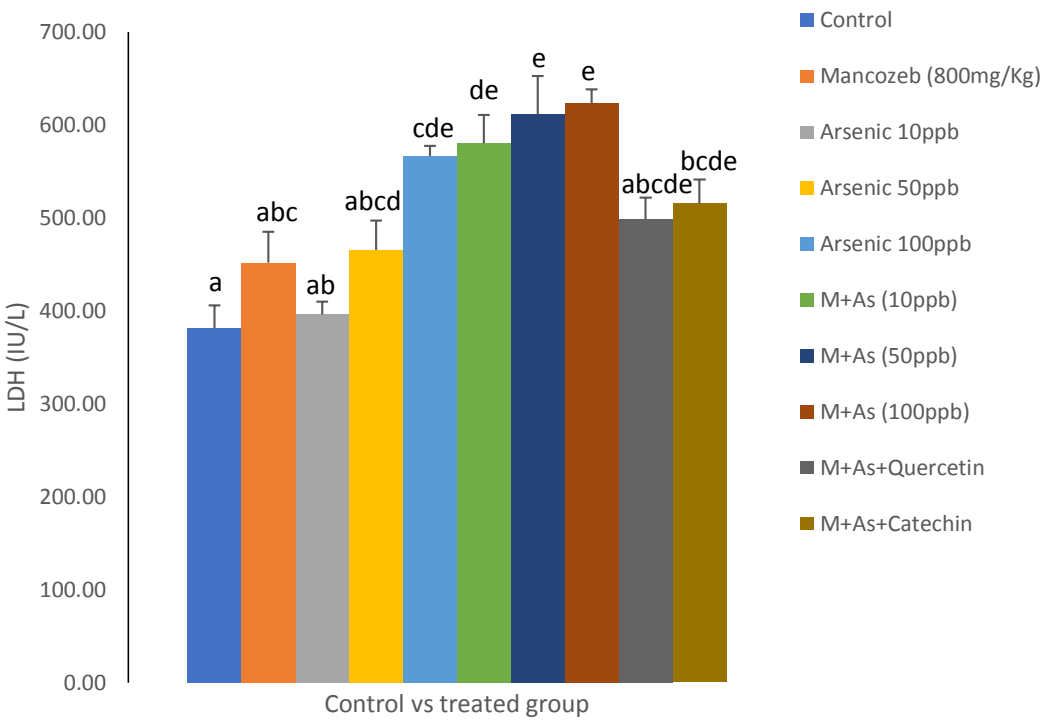


(c)

Figure 4.10: **Graphical representation of plasma biomarkers *viz.*ALT (a), AST (b) and GGT (c) in hepatic tissue of rats following subacute exposure of mancozeb alone or in-combination with arsenic and its attenuation with quercetin and catechin.**



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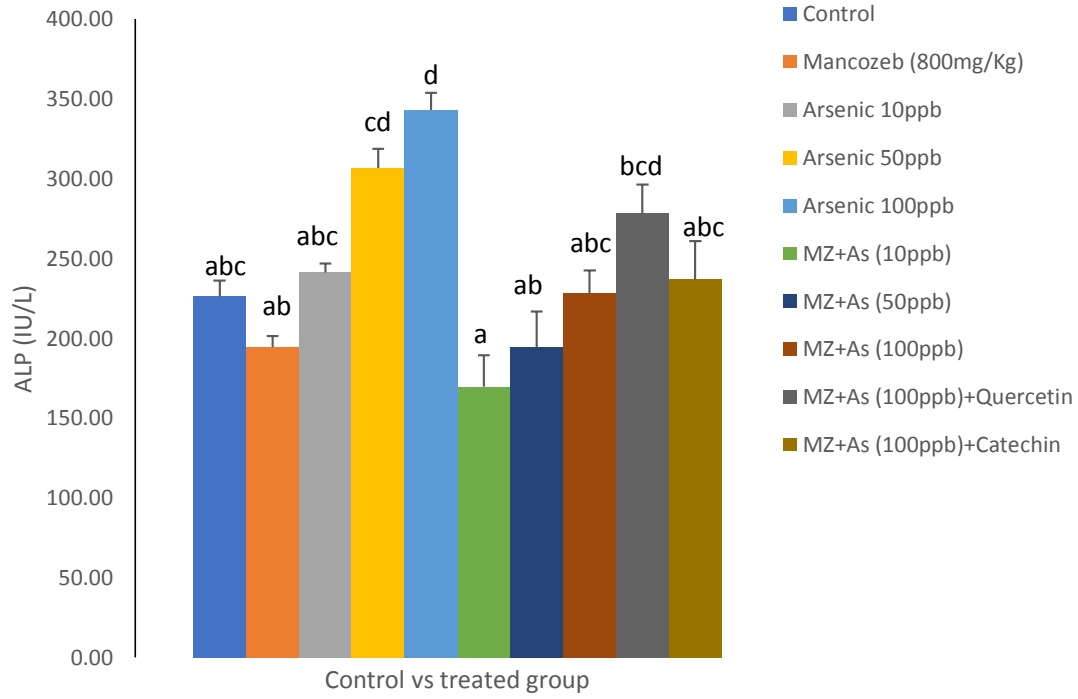
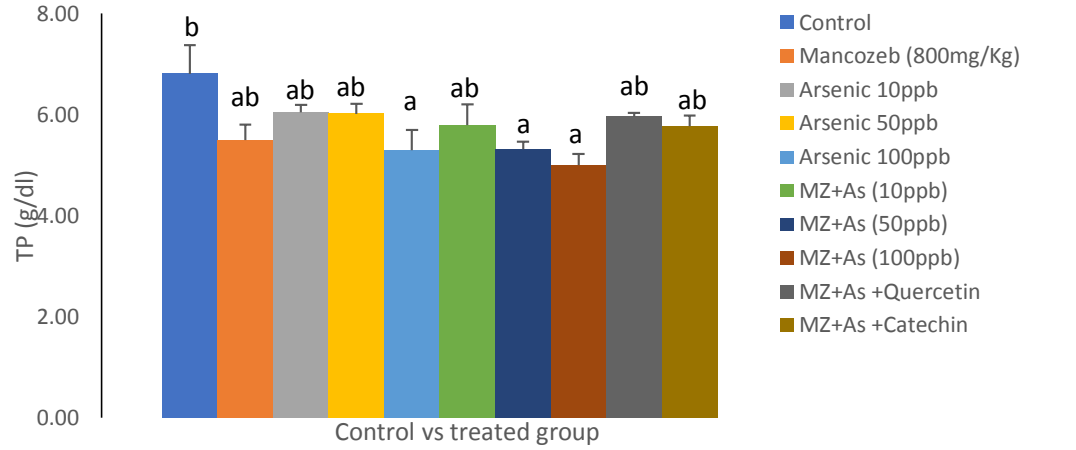
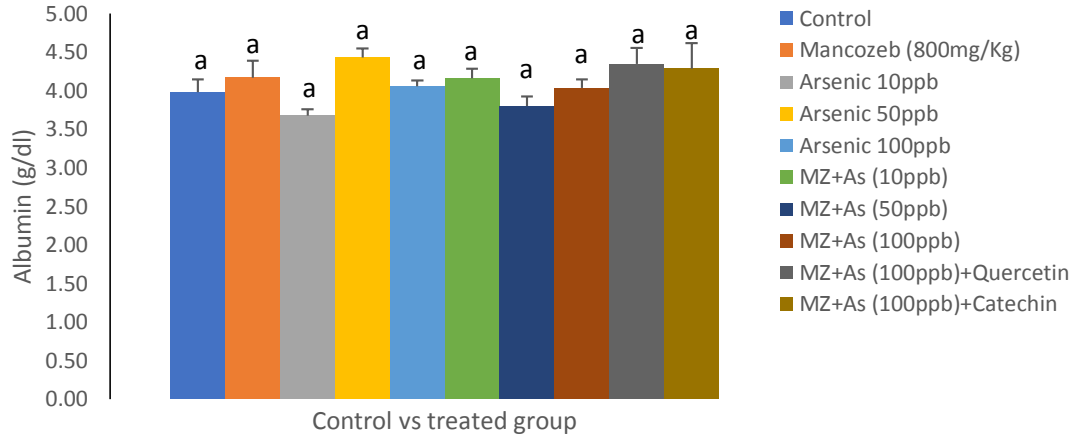


(b)

Figure 4.11: **Graphical representation of plasma biomarkers *viz.*LDH (a) and ALP (b) in hepatic tissue of rats following subacute exposure of mancozeb alone or in-combination with arsenic and its amelioration with quercetin and catechin.**



(a)



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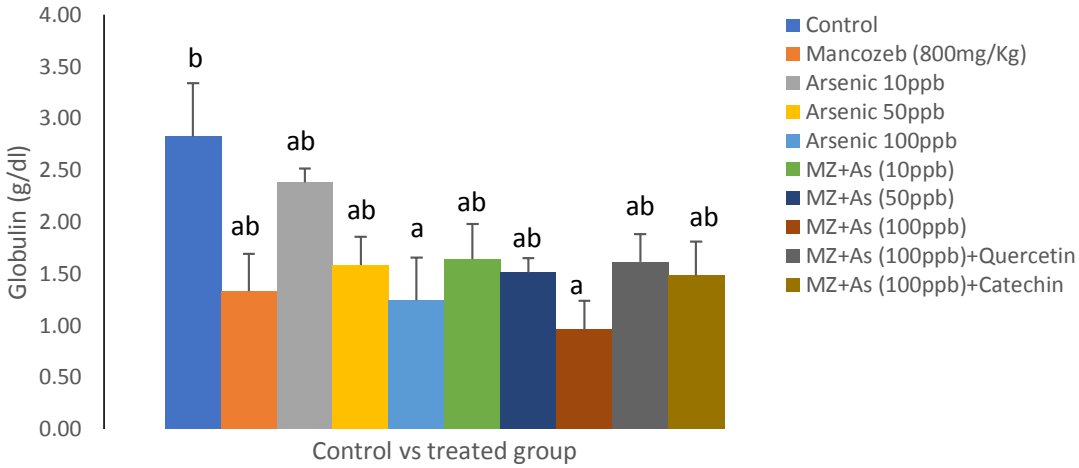
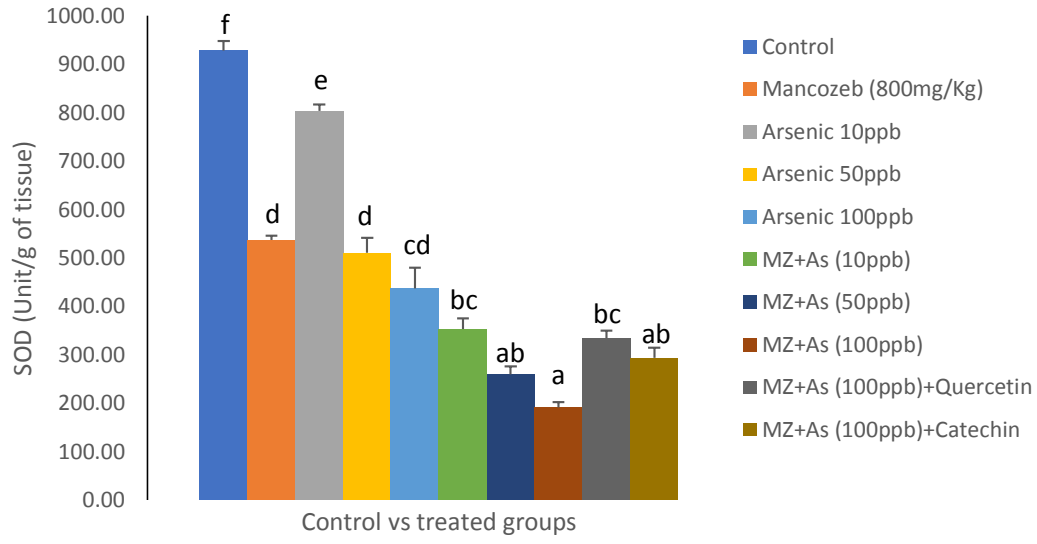
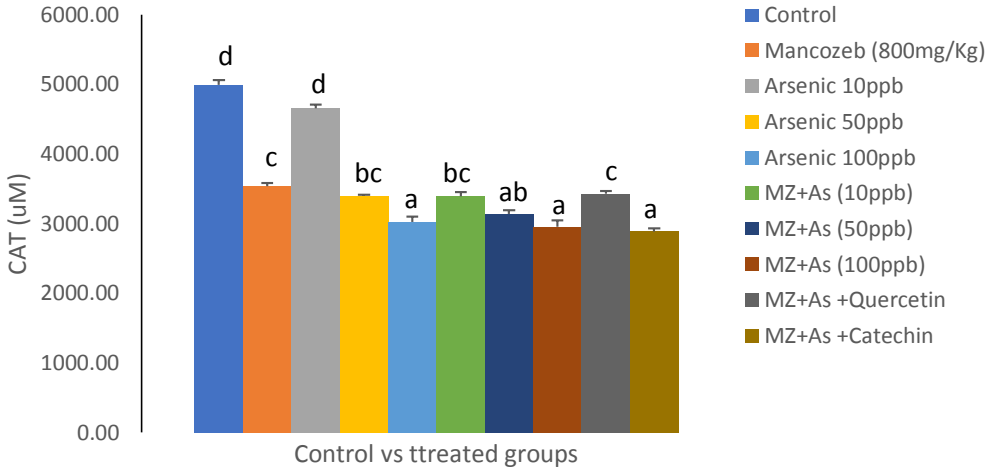


(c)

Figure 4.12: Graphical representation of plasma biomarkers *viz.* Total protein (a), Albumin (b) and Globulin (b) in hepatic tissue following subacute exposure of mancozeb alone or in-combination with arsenic and its attenuation with quercetin and catechin.



(a)



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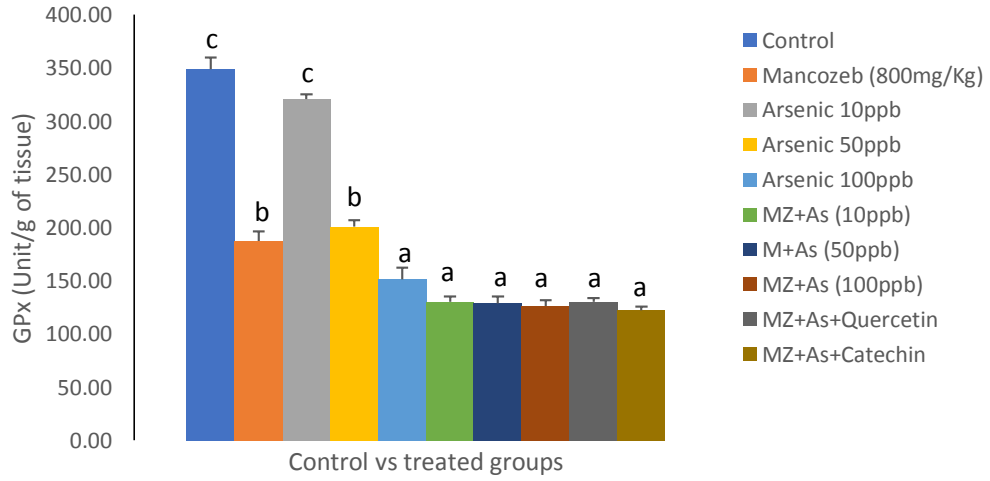
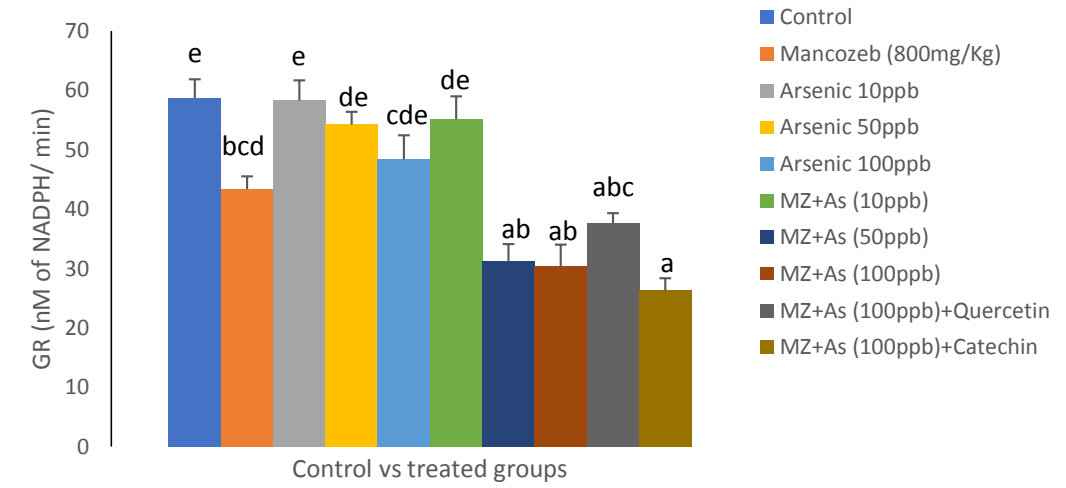


Figure 4.13: **Graphical representation of antioxidant biomarkers *viz.* SOD (a), CAT (b) and GPx (c) in hepatic tissue following subacute exposure of mancozeb alone or in-combination with arsenic and its amelioration with quercetin and catechin.**



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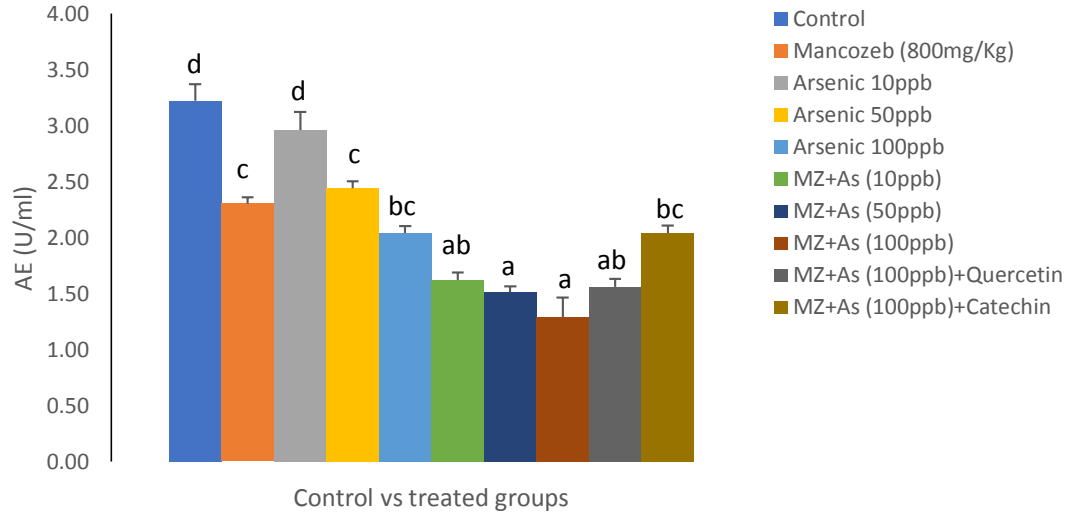

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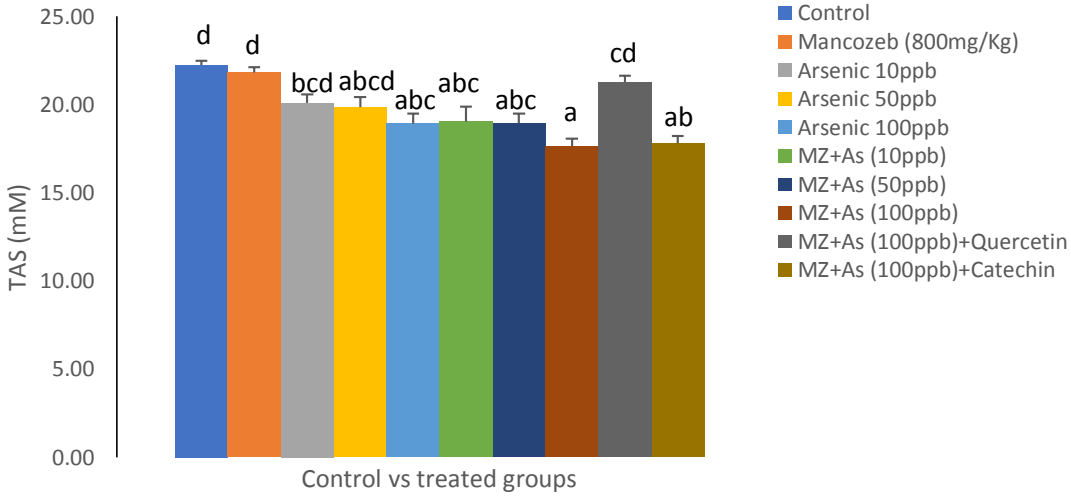
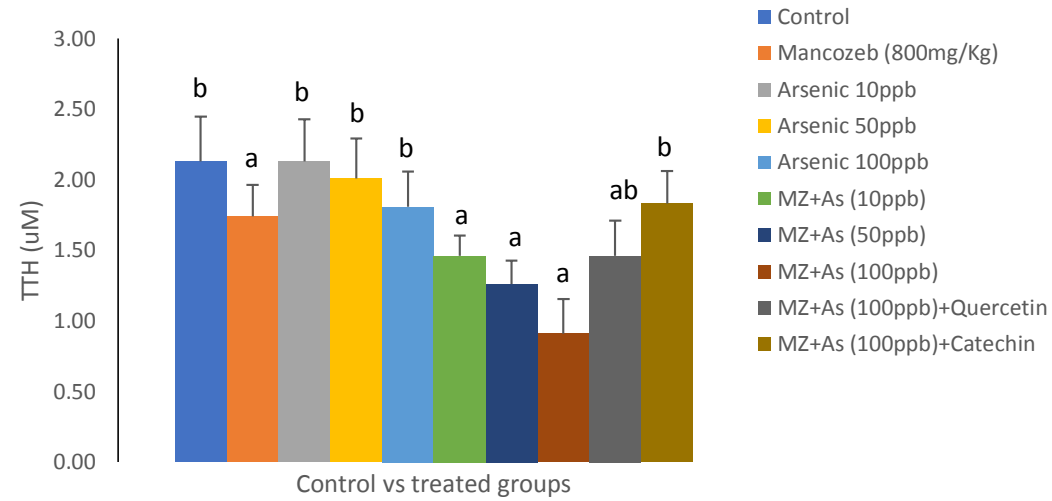
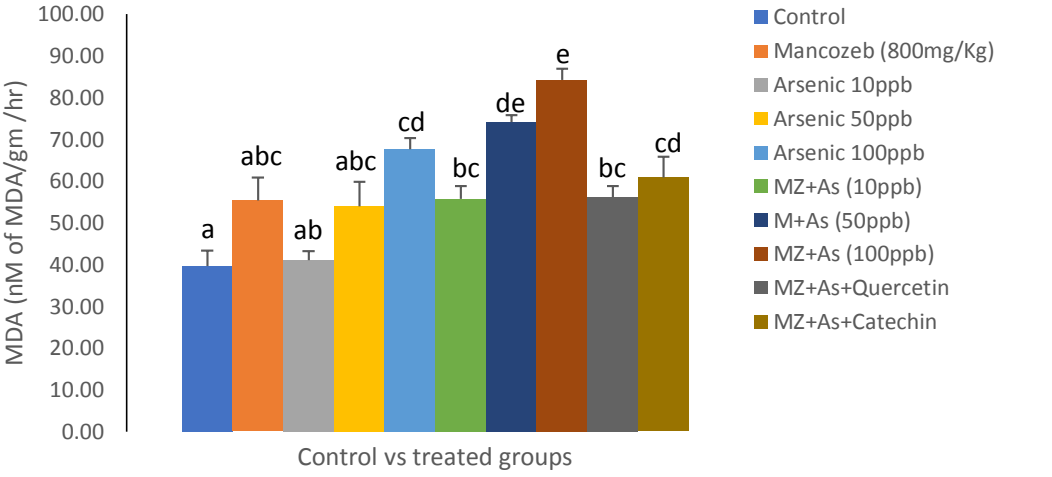


(c)

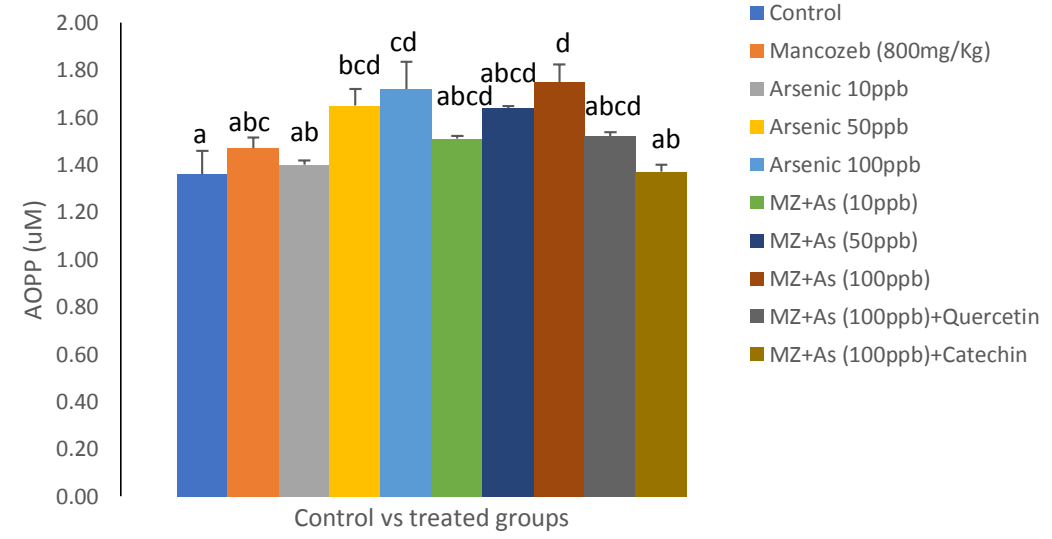
Figure 4.14: **Graphical representation of antioxidant biomarkers *viz.* GR (a), AE (b) and TAS (c) in hepatic tissue following subacute exposure of mancozeb alone or in-combination with arsenic and its attenuation with quercetin and catechin.**



(a)



(b)



(c)

Figure 4.15: Graphical representation of antioxidant biomarkers *viz.* TTH (a), MDA (b) and AOPP (c) in hepatic tissue of rats following subacute exposure of mancozeb alone or in-combination with arsenic and its amelioration with quercetin and catechin.

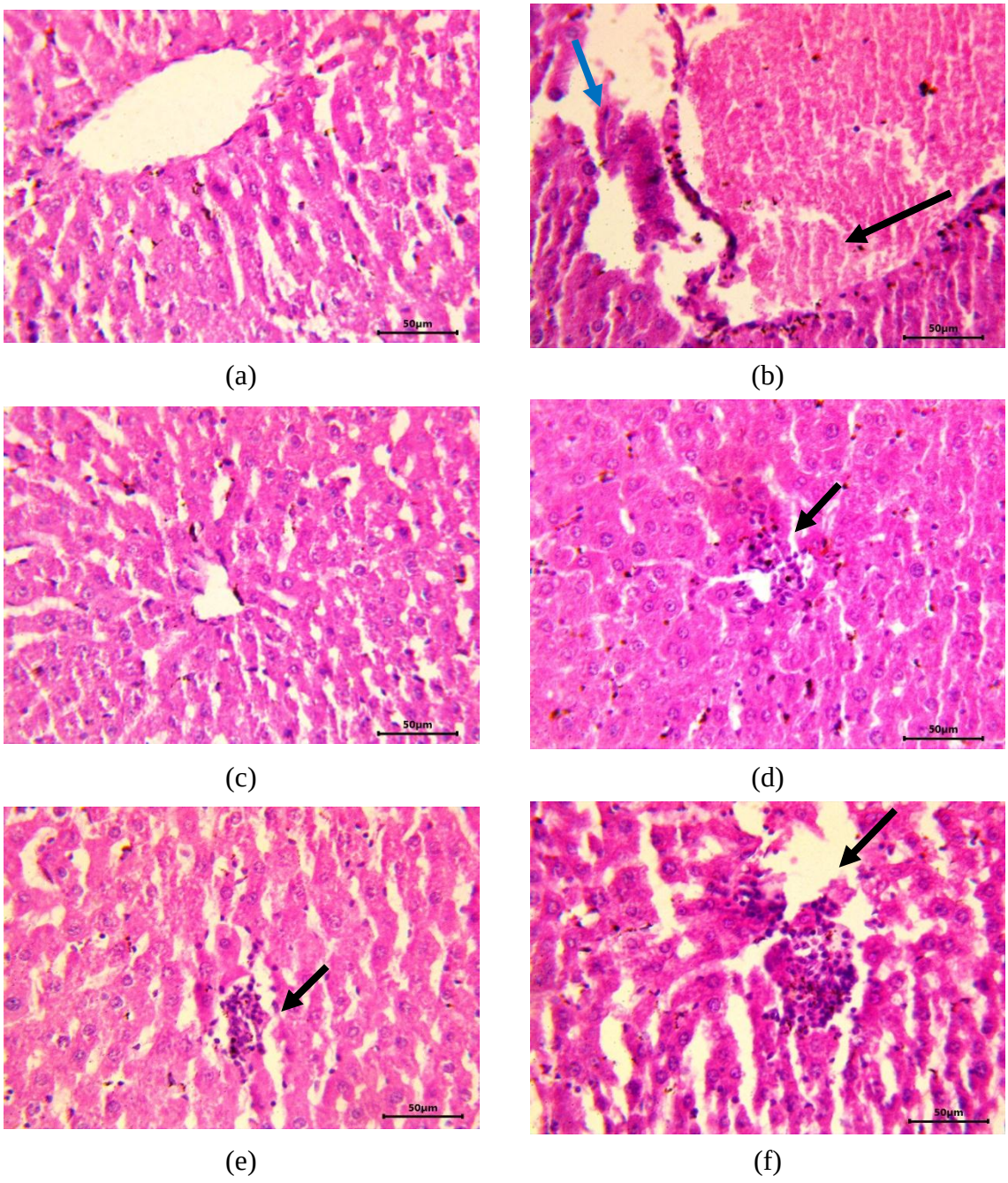
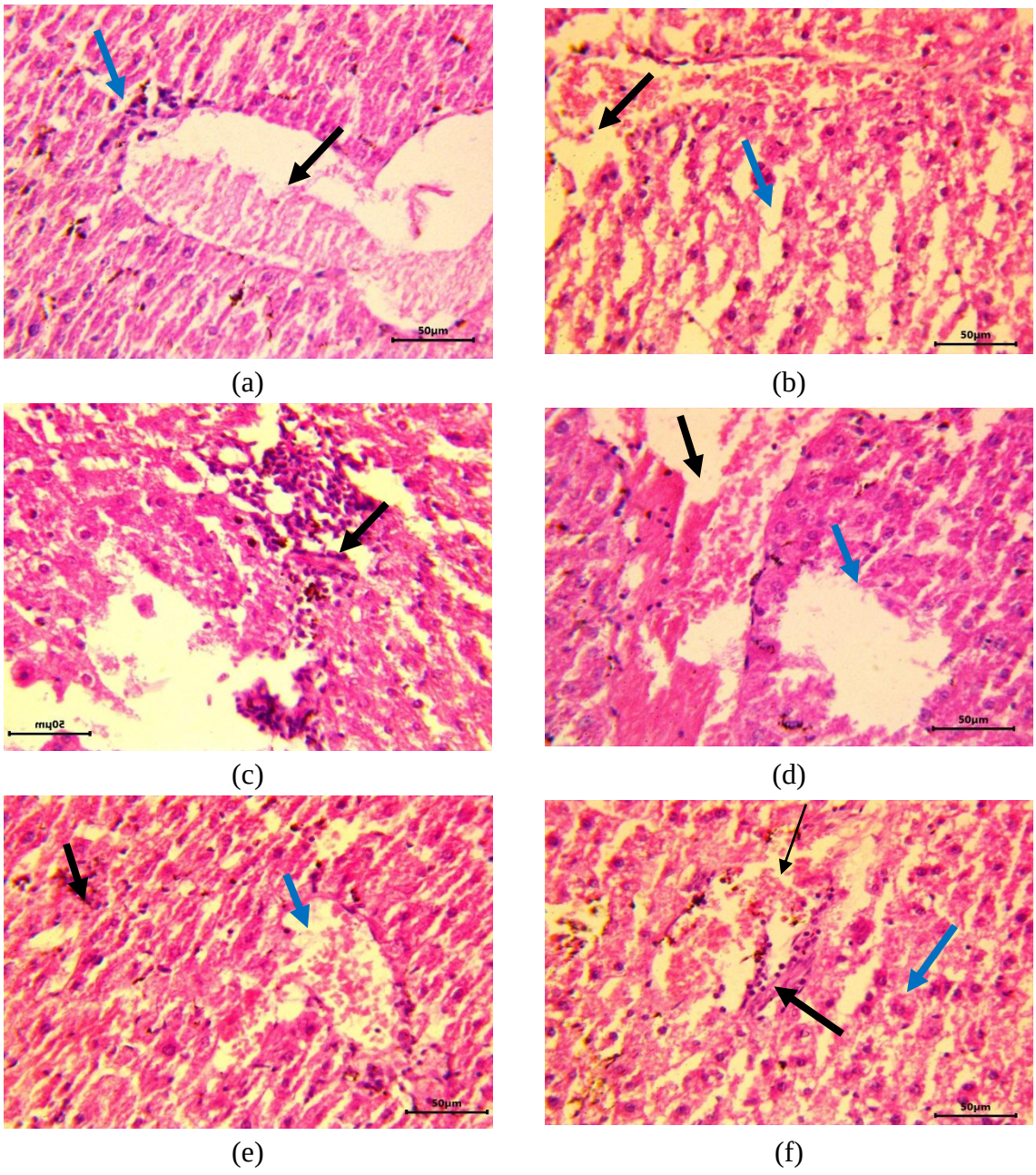


Plate 5: a. Liver sections of control rats showing healthy cords of hepatocytes radiating from central vein; b. G II rats had congested central veins (black arrow) which broke down frequently causing necrosis of centrilobular hepatocyte (blue arrow); c. Normal liver section of Group III rats; d. small focal area of hepatocyte necrosis (arrow) infiltrated by lymphocytes in Group IV rats; e. Mild hepatocellular degeneration and a focal area of hepatocellular necrosis secondarily infiltrated by lymphoid cells in Group V rats; f. Hepatocyte necrosis and lymphocytic infiltration (arrow) in G VI rat.



(a) (b) (c) (d) (e) (f)

Plate 6: a. Congestion (black arrow), degeneration of hepatocytes and infiltration of lymphocytes in centrilobular area (blue arrow) in Group VII rats; b. Congestion (black arrow) along with degeneration and atrophy of hepatocytes and dilatation of sinusoids (blue arrow) in G VIII; c. A large area of severe hepatocellular degeneration and necrosis being infiltrated by large aggregate of lymphocytes (black arrow) in G VIII rats; d. Congested central vein (black arrow) with necrosis of surrounding hepatocytes causing cystic cavitation in centrilobular areas (blue arrow) in G VIII; e. Congestion (blue arrow) along with hepatocyte degeneration and necrosis with infiltration of inflammatory cells (black arrow) in G IX rats; f. G X rats showing haemorrhage (thin arrow), degeneration (blue arrow) and necrosis of hepatocytes and lymphocyte infiltrates.

4.4. Effect on Plasma renal markers: In plasma urea, creatinine (CR) and uric acid (UA) levels were determine to assess the effect of toxicant on renal functions. Mean values of urea, CR and UA are presented in table 4.8 and their corresponding graphical representations are shown in figure 4.16. A significant ($p<0.05$) rise was observed in urea, CR and UA levels in group II as compared to control. Group III, IV and V witnessed an increase in urea, CR and UA levels in a dose dependent manner. In the interactive groups VI, VII and VIII, a significant increment was observed in urea levels in a dose dependent manner as compared to control. Group VIII showed a 323.4% increment in urea activity as compared to control. While as group IX and X increased urea levels to 109.9% and 232.9% respectively as compared to control. Meanwhile, both the groups (IX and X) decreased urea activity significantly compared to group VIII but group IX improved urea activity more efficiently. Similarly, group VIII showed 221.4% and 472.2% increment in CR and UA levels respectively as compared to control. While as group IX increased CR and UA values to 83.9% and 311.1% and X increased the levels to 17.9% and 400.9% as compared to control.

4.4.1. Effect on Kidney Antioxidant System:

4.4.1.1. Total antioxidant status (TAS) and total thiols (TTH): Mean values of TAS and TTH are presented in table 4.10 and their graphical representation is depicted in figure 4.18 and 4.19 respectively. A significant ($p<0.05$) fall in activity of TAS and TTH was noticed in group II as comparison to control. Group III, IV and V depicted a decrease in TAS and TTH levels in a dose dependent pattern as compared to control. The groups VII, VIII treated with the combinations of toxins revealed a significant decrease in TAS and a significant decline only in TTH levels of group VIII as compared to control. Group VIII showed a 42.80% and 75.87% fall in TAS and TTH activity respectively as compared to control. While as group IX decreased TAS and TTH levels to 40.25% &76.93% and X declined the levels to 15.49% and 62.25% as compared to control. This indicates group X restored TAS as well as TTH levels.

4.4.1.2. Levels of proteins (AOPP) and lipids oxidation (MDA): Mean values of MDA and AOPP are presented in table 4.10 and their graphical representation is portrayed in figure 4.19. The activity of MDA represented a significant ($p<0.05$) increase in group II. Group IV revealed a significant increase in MDA levels and group V witnessed a significant rise in MDA and AOPP levels as compared to

control. The toxicant interactive groups including VI showed significant increase in MDA activity and VII and VIII showed a significant increment in MDA as well as in AOPP levels when compared to control. Group VIII showed 113.6% and 38.5% increment in MDA and AOPP levels respectively as compared to control. While as group IX increased MDA and AOPP levels to 49.8% and 15.6% and X increased the levels to 103.9% and 8.9% as compared to control. Group IX decreased MDA levels and X reduced MDA as well as AOPP levels significantly compared to group VIII. The AOPP levels of group IX and X are non-significantly higher than control indicating improvement in AOPP levels.

4.4.1.3. Superoxide dismutase (SOD) and Catalase (CAT): The activities of SOD and CAT are presented in table 4.9 and their graphical representation is depicted in figure 4.17. A significant ($p<0.05$) reduction was observed in SOD and CAT activity of group II as compared to control. Group III revealed a significant decrease in CAT levels and a non-significant decrease in SOD activity in contrast to control. Group IV and V treated with sodium arsenite alone represented a significant fall in SOD and CAT activity in a dose dependent manner as compared to control. In the interactive groups VI, VII and VII, a significant curtailment in SOD and CAT levels was seen in a dose dependent manner as compared to control. Group VIII showed a 77.5% and 35.6% fall in SOD and CAT activity respectively as compared to control. While as group IX lowered the SOD and CAT values to 67.9% and 24.8% and X declined the levels to 73.2% and 32.7% as compared to control. This indicates group IX improved the SOD as well as CAT activity.

4.4.1.4. Activities of GPx GR and AE: Mean values of GPx, GR and AE are presented in table 4.9 and their graphical representation is revealed in figure 4.17 and 4.18 respectively. In Group `II, a significant ($p<0.05$) reduction was observed in GPx activity as compared to control. Group III, IV and V represented a significant decline in GPx values in a dose dependent manner as compared to control. The group VI witnessed a significant decrease in GPx levels and a significant decline in GPx as well as in GR activity was seen in group VII and VIII in a dose dependent manner in contrast to control. Group VIII showed a 69.1% and 43.2% decrease in GPx and GR activity respectively as compared to control. While as group IX decreased GPx and GR levels to 67.3% &18.8% and X decreased the levels to 50.2% and 19.7% as compared to control. Similarly, activity of AE witnessed a significant ($p<0.05$)

decrease in group II and V. The toxicant interactive groups including VI, VII and VIII showed a significant curtailment in the AE activity compared to control as well as alone treated toxicants. Group VIII showed a 55.5% decline in AE activity as compared to control. Meanwhile group IX and X decreased the enzyme activity to 41.9% and 27.4% respectively as compared to control.

4.4.2. Histopathological alterations in kidney

The control group kidneys contained healthy glomeruli with capillary tufts within Bowman's capsule besides normal renal tubules lined by cuboidal epithelium (Plate 7a). In group II, there was tubular necrosis, haemorrhages and oedema in the interstitium (Plate 7b). In group III only mild congestion was seen alongside hyalinised tubular casts (Plate 7c). Kidney of group IV revealed changes like congestion, haemorrhage and necrosis of tubules, presence of oedematous fluid in the interstitium (Plate 7d). Vacuolar degeneration of tubular epithelium and epithelial desquamation in lumen was observed in Group 5 (Plate 7e). In group VI, tubular degeneration, necrosis with haemorrhage was noted in the renal interstitium along with degeneration in glomeruli tuft and atrophy (Plate 7f). Similarly, in Group VII tubular degeneration and necrosis alongside calcification was seen (Plate 8a). Group VIII had the most severe pathological alterations which consisted of glomerular atrophy and synechia wherein glomerular tuft adhered to Bowman's capsule (Plate 8b). At the same time periglomerular lymphoid aggregates with oedema and tubular necrosis were appreciated (Plate 8b). Multifocally, the tubular interstitium showed degeneration in blood vessel walls with concomitant oedema and perivascular infiltration of lymphocytes (Plate 8c). Tubular epithelial lining showed a range of alterations from vacuolar degeneration to necrosis and flattening. Areas of severe necrosis which appeared as hyalinised masses, oedema and infiltration of inflammatory cells mainly lymphocytes were also seen (Plate 8d). Group IX showed less severe changes when compared to those seen in Group VIII as well as Group X which included congestion, haemorrhages and tubular degenerative changes (Plate 8e). In Group X rats the changes in kidneys included glomerular as well as tubular degeneration, perivascular oedema and haemorrhage (Plate 8f).

Table 4.8: Alterations in plasma renal biomarkers of rats following repeated administration of mancozeb (MZ) alone or in-combination with arsenic (As) and its amelioration with quercetin and catechin.

Treatment group	Plasma renal biomarkers		
	CR (mg/dl)	UA (mg/dl)	Urea (mg/dl)
Control	$0.56^{a}\pm0.115$	$1.08^{a}\pm0.055$	$37.55^{a}\pm0.616$
Mancozeb (800mg/kg)	$1.06^{cd}\pm0.060$	$3.70^{ef}\pm0.080$	$130.65^{c}\pm4.93$
Arsenic (10 ppb)	$0.60^{a}\pm0.036$	$1.26^{ab}\pm0.103$	$48.34^{a}\pm2.23$
Arsenic (50 ppb)	$1.41^{de}\pm0.108$	$1.98^{bc}\pm0.105$	$73.39^{b}\pm3.58$
Arsenic (100ppb)	$1.49^{ef}\pm0.137$	$3.02^{de}\pm0.307$	$88.41^{b}\pm2.02$
MZ + As (10ppb)	$1.40^{cde}\pm0.108$	$2.36^{cd}\pm0.079$	$117.37^{c}\pm2.75$
MZ + As (50ppb)	$1.56^{ef}\pm0.083$	$4.41^{f}\pm0.206$	$126.58^{c}\pm9.95$
MZ +As (100 ppb)	$1.80^{f}\pm0.087$	$6.18^{g}\pm0.193$	$159.00^{d}\pm3.86$
MZ+ As (100ppb) + Quercetin (50 mg/kg)	$1.03^{bc}\pm0.046$	$4.44^{f}\pm0.176$	$78.84^{b}\pm5.05$
MZ+ As (100ppb) + Catechin (50mg/kg)	$0.66^{ab}\pm0.013$	$5.41^{g}\pm0.320$	$125.00^{c}\pm8.91$

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

Values having different superscripts (a,b,c,d,e,f,g) in a column are statistically different from one another at 5% level of significance

CR-creatinine; UA-uric acid

Table 4.9: **Alterations in antioxidant biomarkers in renal tissue of rats following repeated administration of mancozeb (MZ) alone or in-combination with arsenic (As) and its amelioration with quercetin and catechin.**

Treatment group	Kidney antioxidant parameters				
	SOD	CAT	GPx	GR	AE
Control	$911.86^{f}\pm36.42$	$4748.89^{f}\pm46.93$	$337.04^{e}\pm7.19$	$54.89^{c}\pm3.13$	$3.03^{f}\pm0.192$
MZ (800mg/kg)	$454.84^{bc}\pm49.19$	$3608.96^{bc}\pm68.26$	$165.33^{c}\pm7.14$	$45.45^{bc}\pm3.64$	$2.55^{de}\pm0.037$
Arsenic (10 ppb)	$841.00^{ef}\pm15.12$	$4405.23^{e}\pm52.21$	$303.02^{d}\pm2.23$	$51.53^{c}\pm3.83$	$2.98^{f}\pm0.051$
Arsenic (50 ppb)	$695.31^{de}\pm35.24$	$3975.75^{d}\pm55.73$	$203.47^{c}\pm5.36$	$49.61^{c}\pm3.09$	$2.67^{ef}\pm0.062$
Arsenic (100ppb)	$557.07^{cd}\pm37.23$	$3636.20^{bc}\pm66.42$	$129.19^{ab}\pm6.80$	$48.86^{bc}\pm3.55$	$2.20^{cd}\pm0.044$
MZ + As (10ppb)	$338.13^{ab}\pm27.48$	$3881.73^{cd}\pm86.96$	$140.70^{bc}\pm5.15$	$42.82^{abc}\pm2.00$	$2.14^{bc}\pm0.086$
MZ + As (50ppb)	$263.28^{a}\pm40.08$	$3503.74^{b}\pm70.76$	$132.06^{ab}\pm6.81$	$36.05^{ab}\pm1.46$	$1.90^{bc}\pm0.058$
MZ +As (100 ppb)	$205.27^{a}\pm35.06$	$3057.09^{a}\pm39.67$	$104.31^{a}\pm5.46$	$31.20^{a}\pm2.08$	$1.35^{a}\pm0.032$
MZ+ As (100ppb) + Quercetin (50 mg/kg)	$291.89^{a}\pm31.02$	$3571.65^{b}\pm27.96$	$110.31^{a}\pm4.64$	$44.59^{bc}\pm1.63$	$1.76^{b}\pm0.105$
MZ+ As (100ppb) + Catechin (50mg/kg)	$244.31^{a}\pm26.19$	$3201.87^{a}\pm50.76$	$168.06^{c}\pm8.92$	$43.99^{abc}\pm2.17$	$2.20^{cd}\pm0.0707$

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

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

Values of SOD (Superoxide dismutase) are expressed in Unit/ g of tissue

Values of CAT (Catalase) are expressed in µmol H_2O_2 decomposed/ min/ g of tissue

GPx (glutathione peroxidase) are expressed in Unit/ g of tissue

Values of GR (glutathione reductase) are expressed nmol of NADPH/min

Activities of arylesterase (AE) expressed in U/ml

Table 4.10: Alterations in antioxidant biomarkers in renal tissue of rats following repeated administration of mancozeb (MZ) alone or in-combination with arsenic (As) and its amelioration with quercetin and catechin.

Treatment group	Kidney antioxidant parameters			
	TAS	TTH	MDA	AOPP
Control	$18.46^{d}\pm0.823$	$2.18^{c}\pm0.203$	$47.57^{a}\pm2.80$	$1.35^{a}\pm0.080$
MZ (800mg/kg)	$13.95^{b}\pm0.604$	$1.87^{b}\pm0.212$	$78.10^{cde}\pm1.50$	$1.44^{abc}\pm0.014$
Arsenic (10 ppb)	$17.20^{cd}\pm0.395$	$2.07^{bc}\pm0.263$	$58.02^{ab}\pm2.62$	$1.42^{ab}\pm0.018$
Arsenic (50 ppb)	$17.18^{cd}\pm0.617$	$1.64^{abc}\pm0.206$	$68.16^{bc}\pm3.07$	$1.61^{abcd}\pm0.018$
Arsenic (100ppb)	$16.70^{cd}\pm0.387$	$1.14^{abc}\pm0.173$	$86.60^{defg}\pm4.59$	$1.76^{cd}\pm0.180$
MZ + As (10ppb)	$16.34^{bcd}\pm0.127$	$1.66^{abc}\pm0.279$	$82.29^{cdef}\pm5.44$	$1.65^{abcd}\pm0.055$
MZ + As (50ppb)	$15.54^{bc}\pm0.094$	$1.41^{abc}\pm0.211$	$93.27^{efg}\pm2.30$	$1.74^{bcd}\pm0.067$
MZ +As (100 ppb)	$10.56^{a}\pm0.724$	$0.526^{a}\pm0.167$	$101.60^{g}\pm6.49$	$1.87^{d}\pm0.033$
MZ+ As (100ppb) + Quercetin (50 mg/kg)	$11.03^{a}\pm0.520$	$0.503^{a}\pm0.109$	$71.27^{bcd}\pm1.85$	$1.56^{abcd}\pm0.013$
MZ+ As (100ppb) + Catechin (50mg/kg)	$15.60^{bc}\pm0.339$	$0.823^{ab}\pm0.224$	$97.04^{fg}\pm2.40$	$1.47^{abc}\pm0.065$

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

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

Values of TAS (Total antioxidant status), expressed in mM

Values of TTH (Total thiols) expressed in µM

Values of advance oxidation protein product (AOPP) are expressed in µM of Chloramine-T

Values of malondialdehyde (MDA) are expressed in nmole of MDA formed/gm/hr

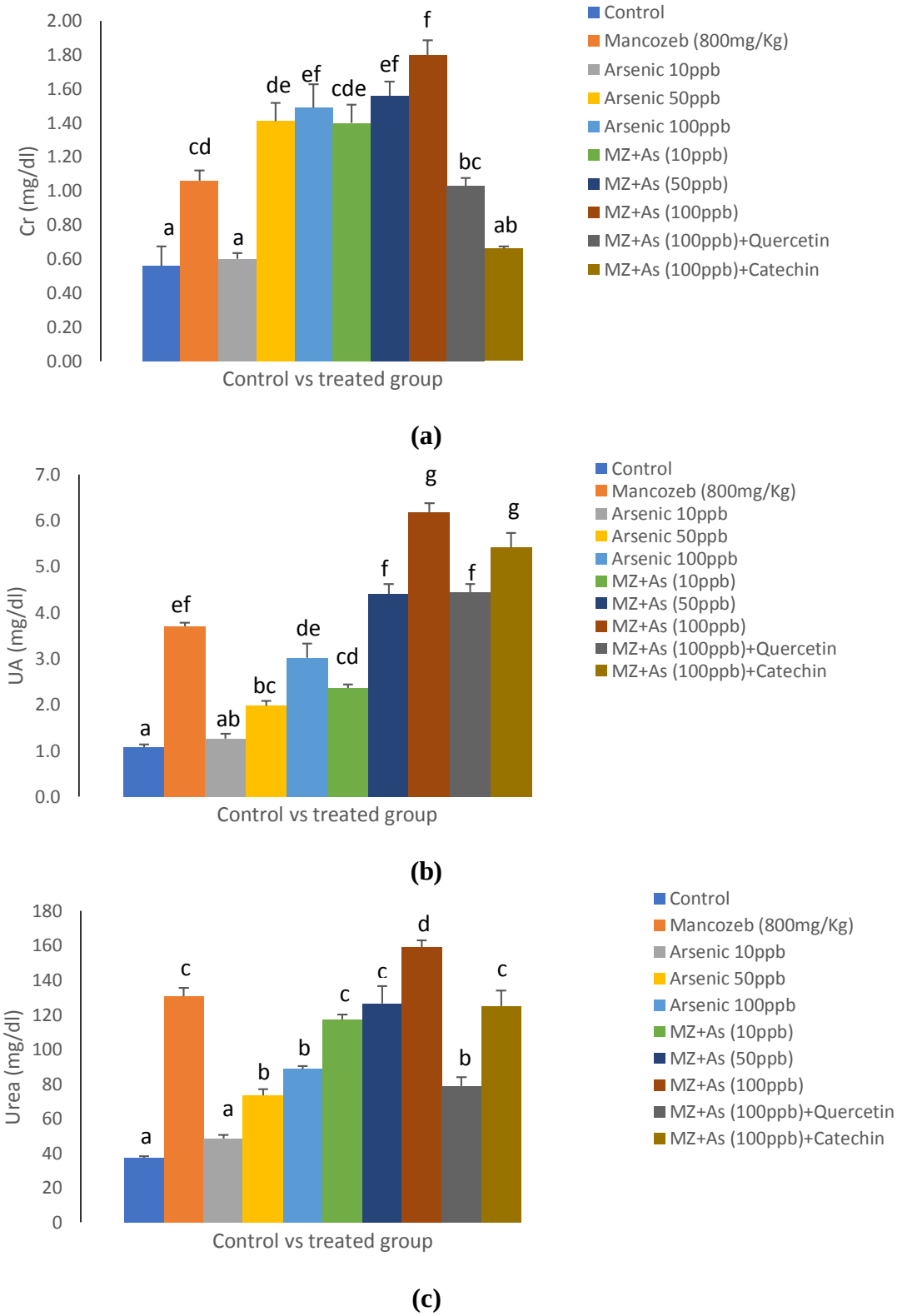
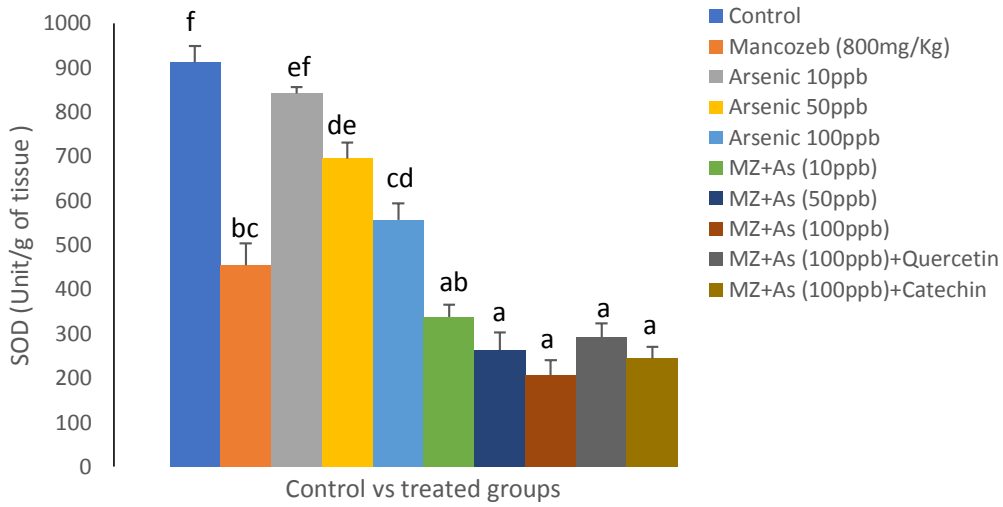
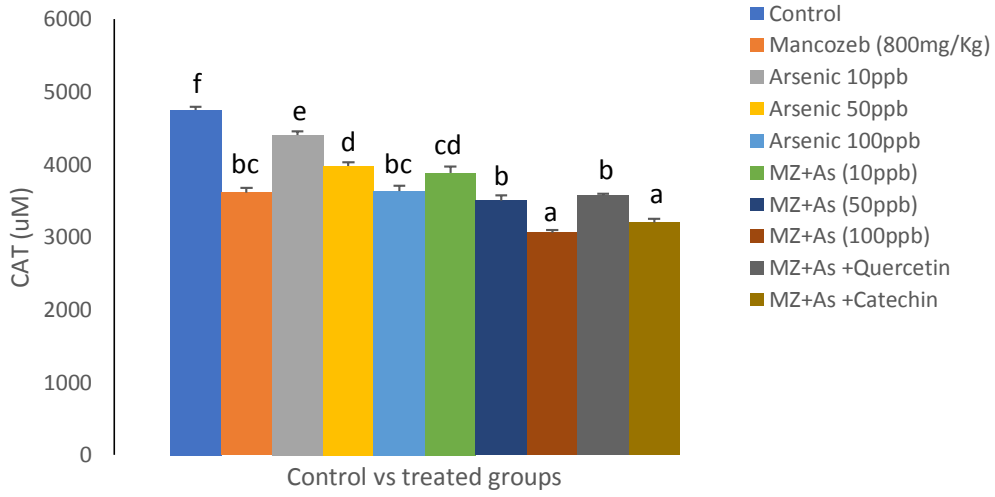


Figure 4.16: **Graphical representation of plasma renal biomarkers *viz.* Creatinine (a), Uric acid (b) and Urea (c) following subacute exposure of mancozeb alone or in-combination with arsenic and its amelioration with quercetin and catechin in rats.**



(a)



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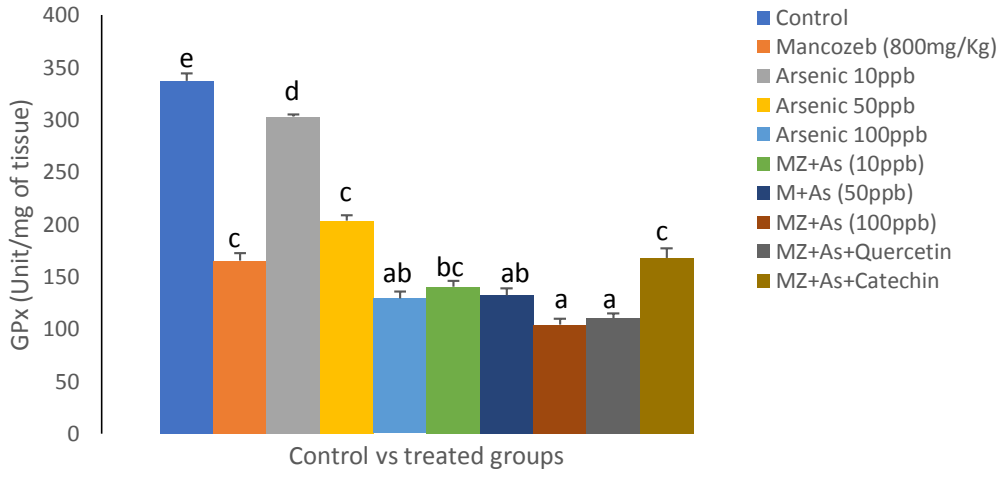
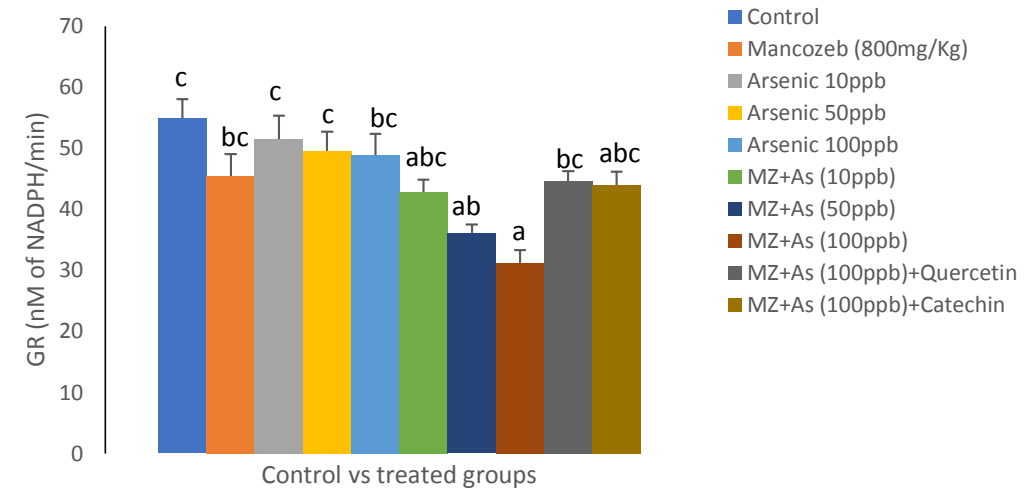


(c)

Figure 4.17: Graphical representation of antioxidant biomarkers *viz.* SOD (a), CAT (b) and GPx (c) in renal tissue of rats following subacute exposure of mancozeb alone or in-combination with arsenic and its attenuation with quercetin and catechin.



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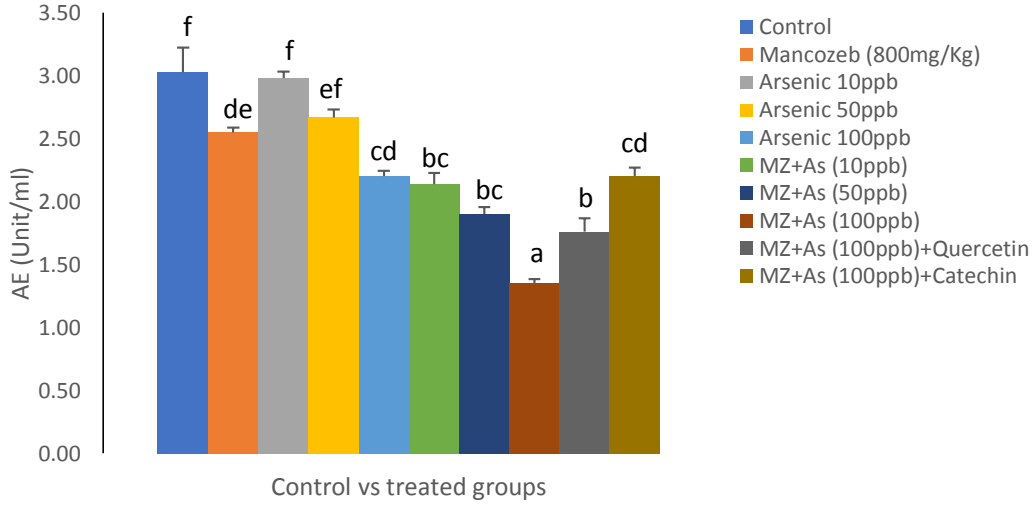

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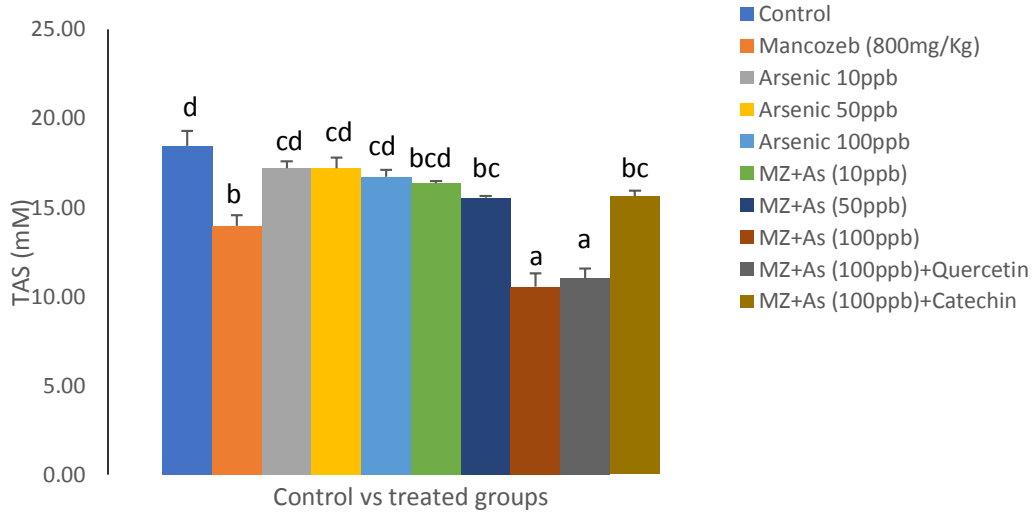
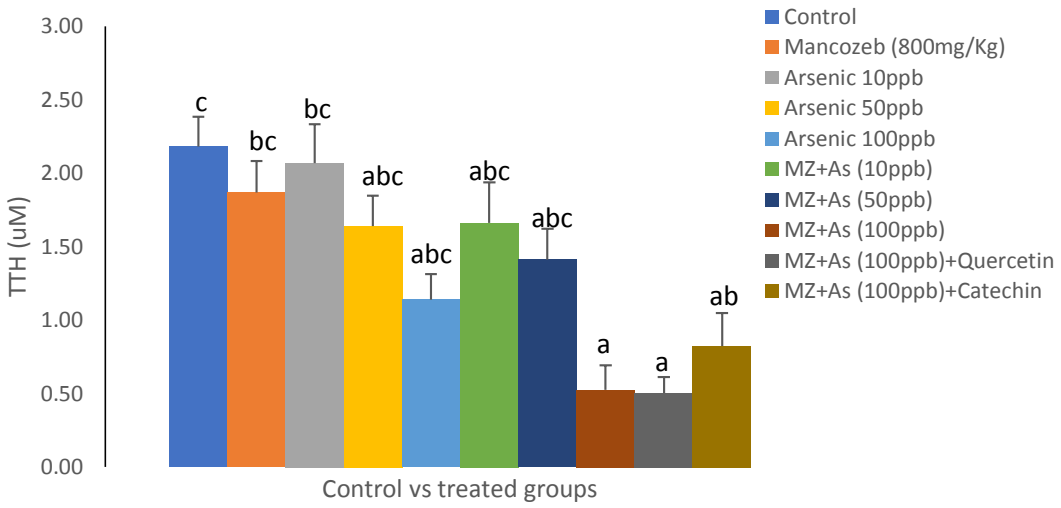
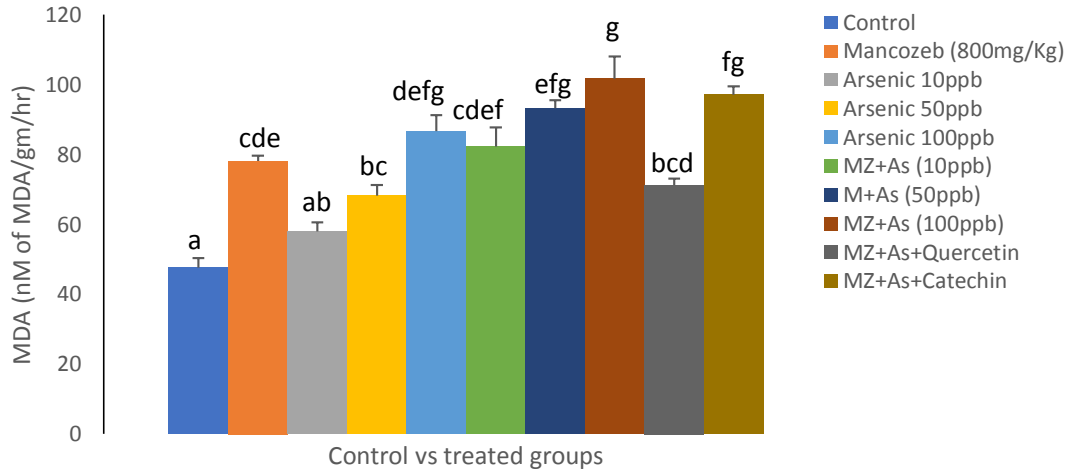


(c)

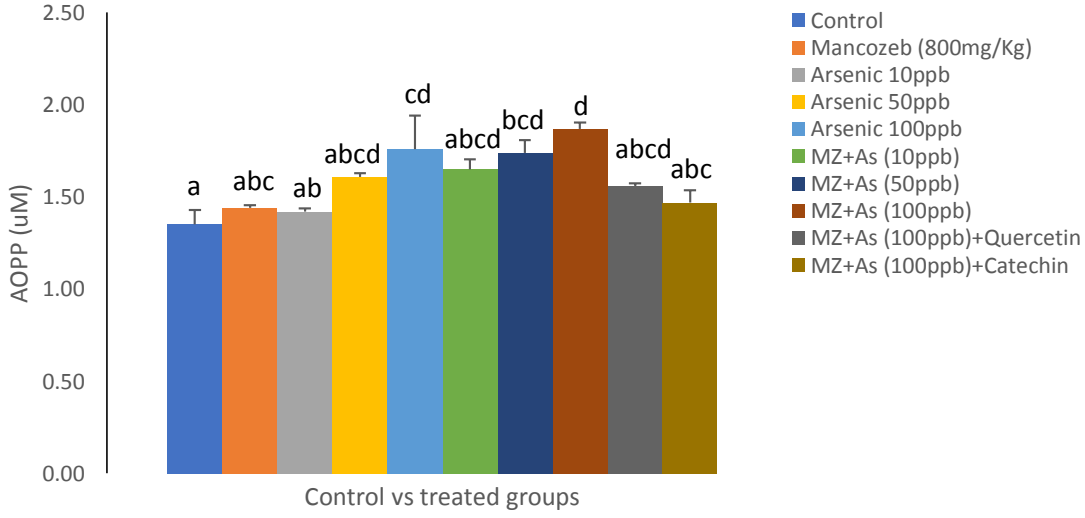
Figure 4.18: **Antioxidant biomarkers *viz.* GR (a), AE (b) and TAS (c) in renal tissue of rats following subacute exposure of mancozeb alone or in-combination with arsenic and its attenuation with quercetin and catechin.**



(a)

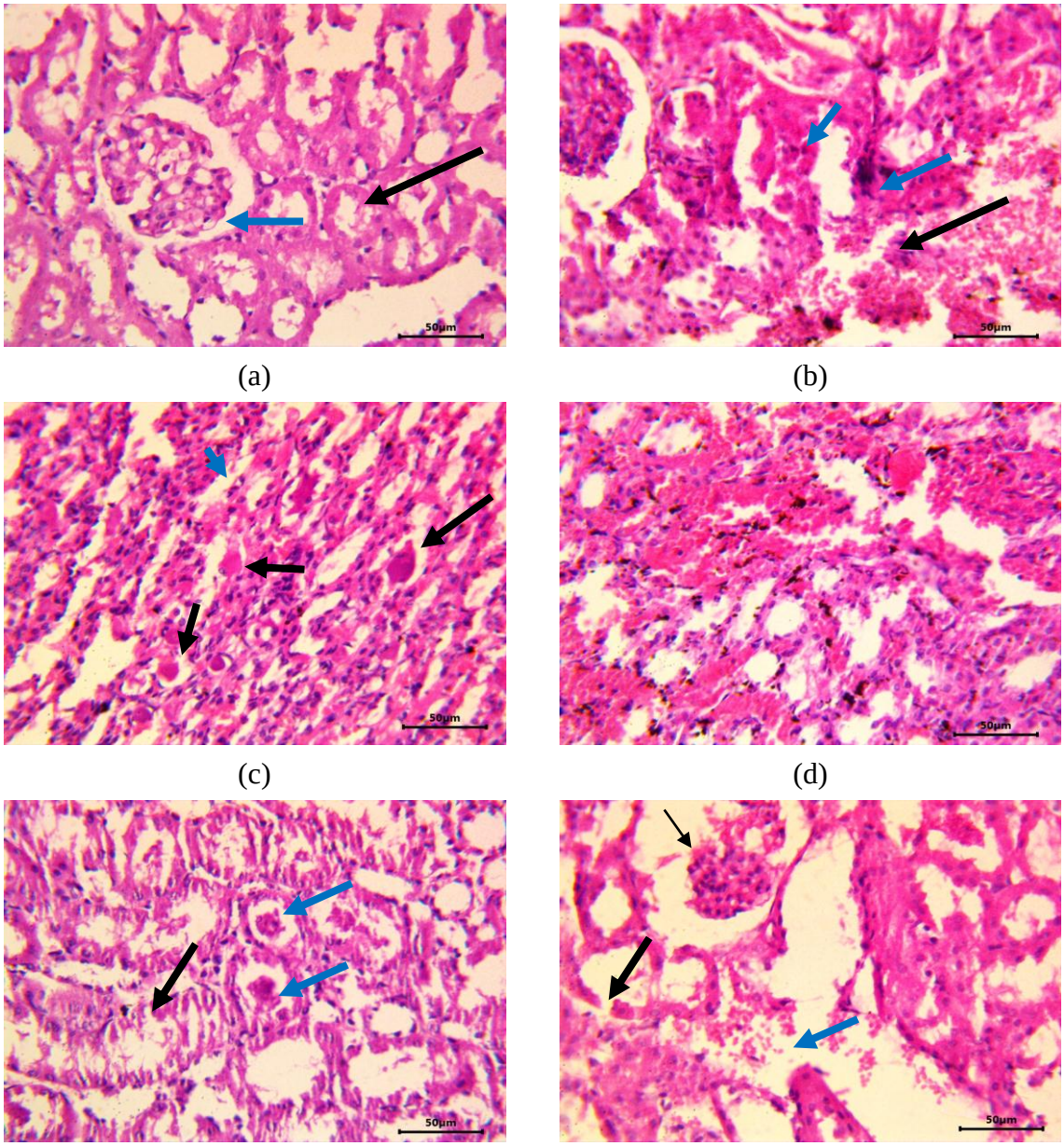


(b)



(c)

Figure 4.19: **Graphical representation of antioxidant biomarkers *viz.*TTH (a), MDA (b) and AOPP (c) in renal tissue of rats following subacute exposure of mancozeb alone or in-combination with arsenic and its attenuation with quercetin and catechin.**



(a) (b)

(c) (d)

(e) (f)

Plate 7: a. Normal architecture of Kidney in Group 1 rats showing glomerular capillaries inside bowman's capsule (blue arrow) and renal tubules lined by cuboidal epithelium (black arrow); b. G II rats showing haemorrhage (black arrow) in renal interstitium and necrosed renal tubules (blue arrow); c. Degenerative changes in renal tubular epithelium and renal casts (black arrow) in group III rats; d. Focal area of interstitial congestion and haemorrhage (blue arrow) along with degeneration in tubular epithelium (black arrow) in G IV rats; e. Degeneration and necrosis of epithelium (black arrow) with tubular casts (blue arrow) in G V rats; f. Glomerular atrophy (thin arrow), rhexis (black arrow) with interstitial haemorrhage (blue arrow) in group VI rats.

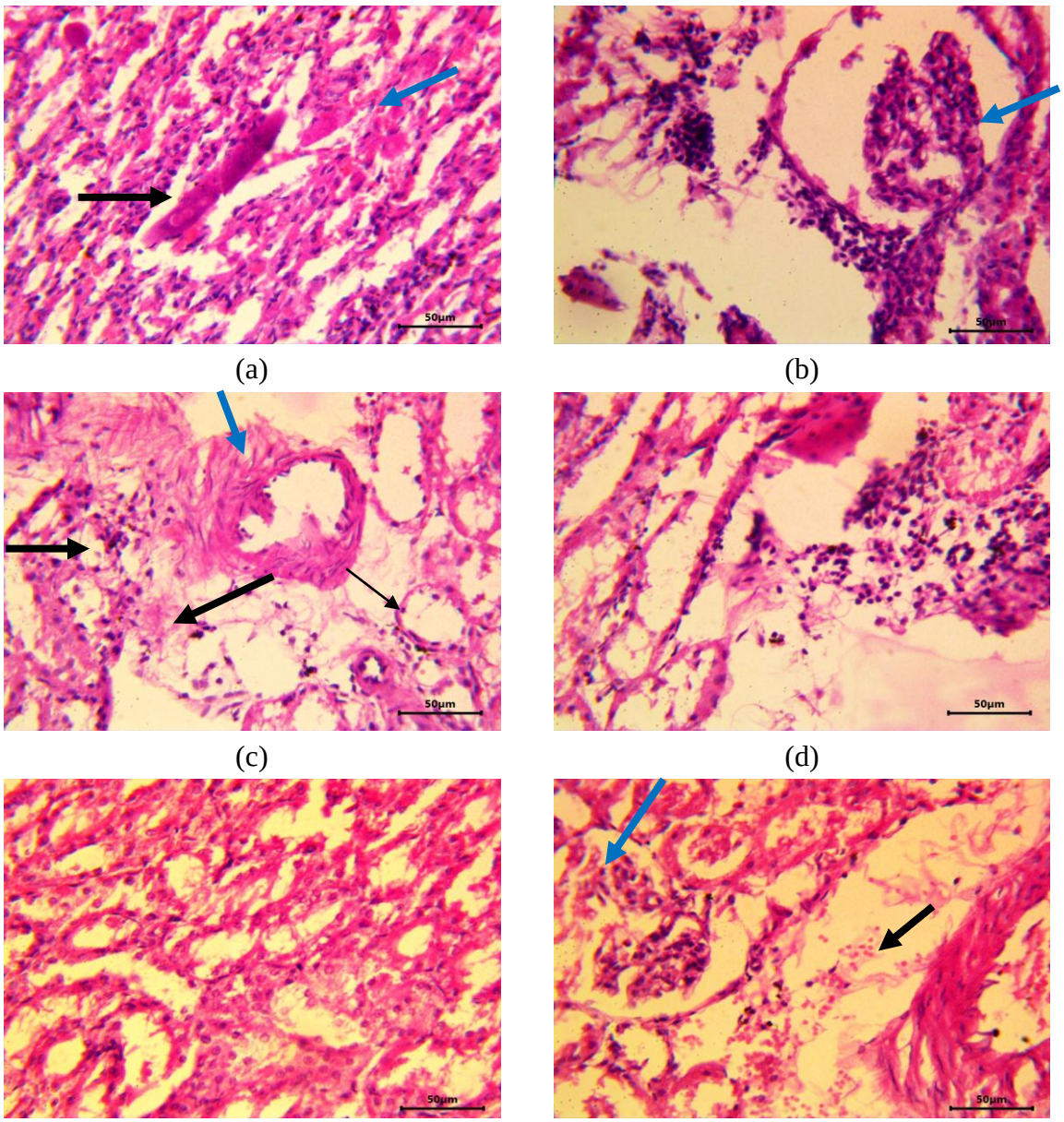


Plate 8: a. Degeneration and necrosis of renal tubules (blue arrow) with presence of renal casts and calcification (black arrow) in Group VII rats; b. Renal tubular necrosis, oedema, Periglomerular lymphoid aggregate (black arrow) and glomerular atrophy (blue arrow) in G VIII rats; c. Degenerative changes in renal arteriolar wall (blue arrow), oedema and infiltration of lymphocytes (black arrow) along with necrosis of tubular epithelium (thin arrow) in G VIII rats; d. Severe interstitial oedema (thin arrow) and infiltration of lymphocytes (black arrow) with tubular necrosis and hyalinisation of glomerular tuft (blue arrow) in G VIII rats; e. Degeneration and necrosis of tubular epithelium (arrow) in G IX rats; f. G X rats showing degeneration of renal tubules & glomerulus (blue arrow) with haemorrhage & oedema (black arrow) in the renal interstitial tissue.

4.5. Effect on antioxidant status of Testes:

4.5.1. Total antioxidant status (TAS) and total thiols (TTH): Mean values of TAS and TTH are given in table 4.12 and their graphical representation is illustrated in figure 4.21 and 4.22 respectively. A significant ($p<0.05$) decline in TAS and TTH activity was noticed in group II in comparison to control. Groups III, IV and V showed a fall in TAS and TTH levels in a dose dependent manner as compared to control. The groups VI, VII, VIII treated with the combinations of toxins depicted a significant decrease in TAS as well as TTH levels as compared to control. Group VIII showed a 45.9% and 54.1% fall in TAS and TTH activity, respectively as compared to control. While as group IX decreased the TAS and TTH values to 43.9% and 41.1% and X declined the levels to 31.9% and 39.6% as compared to control. This indicates group X alleviates the toxicity as far as TAS and TTH levels are concerned.

4.5.2. Levels of MDA and AOPP: Mean values of MDA and AOPP are presented in table 4.12 and their graphical representation is depicted in figure 4.22. The values of MDA and AOPP increased non-significantly in group II. In group III, IV and V, the MDA and AOPP activity increased in a dose dependent manner when compared to control. The MDA values of group V increased significantly compared to control. The toxicant interactive groups including VI, VII and VIII showed a significant increment in MDA as well in AOPP values compared to control. Group VIII represented a 137.7% and 41.5% rise in MDA and AOPP levels respectively as compared to control. While as group IX elevated the of MDA and AOPP values to 158.6% and 15.5% and X increased the levels to 169.1% and 5.7% as compared to control. This indicates only AOPP levels are restored and that too are prominently improved by group X.

4.5.3. Superoxide dismutase (SOD) and Catalase (CAT): Mean values of SOD and CAT are depicted in table 4.11 and their graphical representation is manifested in figure 4.20. A significant ($p<0.05$) reduction was observed in CAT activity and non-significant reduction was observed in SOD activity of group II as compared to control. Group III, IV and V represented a decreasing trend in SOD as well as CAT activity in a dose dependent manner as compared to control. In the interactive groups VI, VII and VII, a significant curtailment in SOD and CAT levels was seen in a dose dependent manner as compared to control. Group VIII showed a 69.4% and 66.5% fall in SOD and CAT activity respectively as compared to control. While as group IX

decreased the SOD and CAT levels to 46.4% and 49.9% and X declined the levels to 40.8% and 65.3% as compared to control. This indicates group IX improves the CAT activity more effectively and group X restored the SOD activity successfully.

4.5.4. Activities of GPx, GR and AE: Mean values of GPx, GR and AE are manifested in table 4.11 and their graphical representation is portrayed in figure 4.20 and 4.21 respectively. In Group II, a significant ($p<0.05$) reduction was observed in GPx and GR activity as compared to control. Group III, IV and V represented a decreasing trend in GPx and GR values when compared to control. The group VI, VII, VIII treated with the combinations of toxicants witnessed a significant fall in GPx as well as in GR levels in a dose dependent manner in contrast to control. Group VIII witnessed a 64.3% and 61.3% decline in GPx and GR activity, respectively, as compared to control. While as group IX decreased the GPx and GR levels to 21.9% and 36.7% and X declined the levels to 40.4% and 15.2% as compared to control. Similarly, the activity of AE witnessed a significant ($p<0.05$) decline in group II as compared to control. Group III, IV and V showed a decrease in AE activity in a dose dependent manner, compared to control. The toxicant interactive groups including VI, VII and VIII showed a significant fall in AE activity compared to control as well as alone treated toxicants (group III, IV and V). Group VIII witnessed a 63.6% decrease in AE activity as compared to control. While as group IX dropped the AE levels to 36.2% and X declined the levels to 34.2% as compared to control.

4.5.5. Histopathological alterations in testes: Testes of control rats were lined by thick capsule layer and contained healthy seminiferous tubules separated by thin fibrous connective tissue (Plate 9a). Interstituim also had Leydig cells which were cells with round nuclei and moderate amounts of cytoplasm. Seminiferous tubules were lined by intact basement membrane and housed normal populations of cells in various stages of spermatogenesis. Round spermatogonia and primary spermatocytes were recognized. Spermatids and mature spermatozoa were also present towards the lumen of the seminiferous tubules. Group III presented similar picture and apart from only mild interstitial congestion, nothing abnormal could be detected (Plate 9c). Testicals from Group II revealed moderately severe pathological changes which included congestion, interstitial oedema, and degeneration of seminiferous tubules along with reduction in their number (Plate 9b). The number of sperm cells was significantly reduced due to degeneration and necrosis of seminiferous tubules. Group

IV, showed congestion and oedema particularly in areas adjoining capsule (Plate 9d). Degeneration and necrosis in seminiferous tubules were also noted. Similarly, Group V revealed sub-capsular oedema, testicular haemorrhage and degeneration in semniferous tubules when compared with the control animals (Plate 9e). Lesions in combination group, Group VI, interstitial congestion and degeneration, necrosis and loss of seminiferous tubules (Plate 9f). Sub-capsular congestion, severe degeneration and necrosis of seminiferous tubules was seen in Group VII rats (Plate 10a). Whereas, coagulation necrosis was seen to affect a significant number of seminiferous tubules in almost all rats of Group VIII (Plate 10b). Also, necrotic fibrillar debris replaced stages of developing spermatozoa inside a number of seminiferous tubules while the majority of the tubules appeared atrophied hyalinised and devoid of any viable sperms in majority of the animals in G VIII (Plate 10c). In addition, necrotic tubules were frequently calcified in some rats (Plate 10d). Degree of pathological changes were partially ameliorated in Group IX and X when compared to that seen in group VIII. Group IX however still showed necrosis of many seminiferous tubules (Plate 10e). Changes in testicals of Group X rats were mainly mild degeneration in seminiferous tubules (Plate 10f).

Table 4.11: Alterations in antioxidant biomarkers in testes of rats following repeated administration of mancozeb (MZ) alone or in-combination with arsenic (As) and its amelioration with quercetin and catechin.

Treatment group	Antioxidant parameters in testes				
	SOD	CAT	GPx	GR	AE
Control	$433.77^{e}\pm15.58$	$1550.75^{e}\pm15.39$	$160.79^{e}\pm3.61$	$33.72^{e}\pm0.55$	$5.55^{e}\pm0.035$
MZ (800mg/kg)	$309.73^{de}\pm12.83$	$647.39^{ab}\pm37.79$	$126.87^{d}\pm5.94$	$26.76^{cd}\pm1.20$	$3.85^{c}\pm0.051$
Arsenic (10 ppb)	$423.82^{e}\pm11.64$	$1519.78^{e}\pm37.53$	$153.93^{de}\pm6.72$	$32.66^{de}\pm0.93$	$5.34^{e}\pm0.062$
Arsenic (50 ppb)	$359.39^{de}\pm20.07$	$1166.42^{d}\pm45.36$	$135.97^{de}\pm3.26$	$29.07^{de}\pm1.43$	$4.40^{d}\pm0.165$
Arsenic (100ppb)	$295.29^{cde}\pm27.12$	$1014.55^{c}\pm24.98$	$86.82^{bc}\pm5.52$	$21.31^{bc}\pm1.54$	$3.91^{c}\pm0.082$
MZ + As (10ppb)	$208.81^{abc}\pm26.37$	$626.19^{a}\pm22.93$	$82.53^{abc}\pm8.81$	$20.79^{bc}\pm0.38$	$3.16^{b}\pm0.10$
MZ + As (50ppb)	$173.81^{ab}\pm24.33$	$586.19^{a}\pm38.10$	$66.16^{ab}\pm5.89$	$19.13^{b}\pm1.80$	$2.85^{b}\pm0.058$
MZ +As (100 ppb)	$132.75^{a}\pm20.70$	$520.15^{a}\pm30.43$	$57.37^{a}\pm6.53$	$13.05^{a}\pm0.82$	$2.02^{a}\pm0.069$
MZ+ As (100ppb) + Quercetin (50 mg/kg)	$232.50^{bcd}\pm15.49$	$777.24^{b}\pm33.33$	$125.63^{d}\pm8.08$	$21.34^{bc}\pm2.04$	$3.54^{c}\pm0.062$
MZ+ As (100ppb) + Catechin (50mg/kg)	$256.87^{bcd}\pm9.60$	$537.69^{a}\pm11.82$	$95.79^{c}\pm5.73$	$28.61^{de}\pm1.06$	$3.65^{c}\pm0.057$

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

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

Values of SOD (Superoxide dismutase) are expressed in Unit/ g of tissue

Values of CAT (Catalase) are expressed in µmol H_2O_2 decomposed/ min/ g of tissue

GPx (glutathione peroxidase) are expressed in Unit/ g of tissue

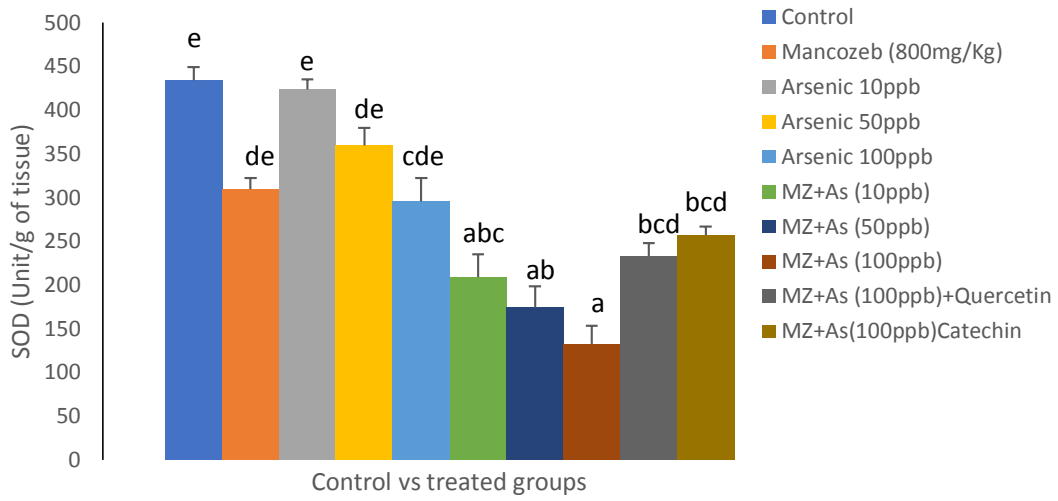
Values of GR (glutathione reductase) are expressed nmol of NADPH/min

Activities of arylesterase (AE) expressed in U/ml

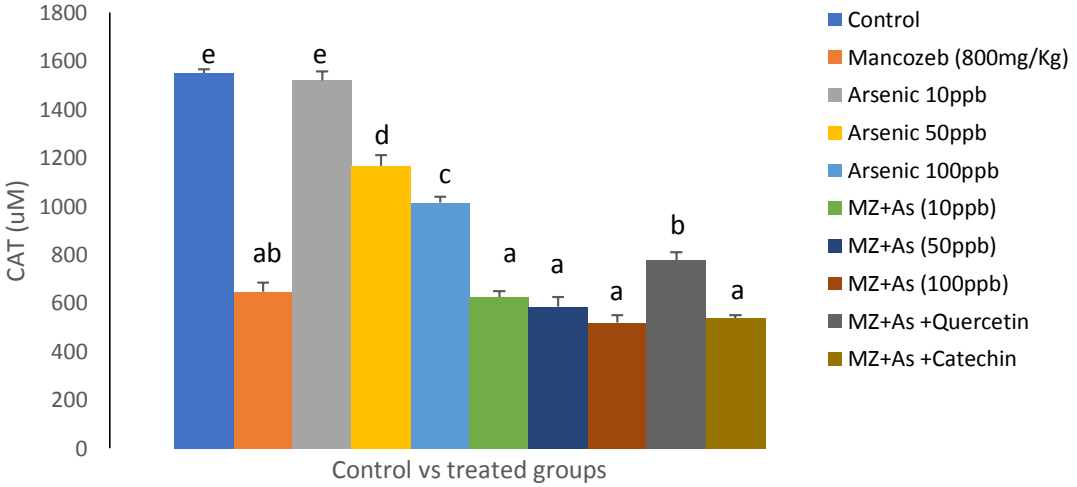
Table 4.12: Alterations in antioxidant biomarkers in testes of rats following repeated administration of mancozeb (MZ) alone or in-combination with arsenic (As) and its amelioration with quercetin and catechin.

Treatment group	Antioxidant parameters in testes			
	TAS	TTH	MDA	AOPP
Control	$21.61^{f}\pm0.160$	$3.33^{c}\pm0.046$	$26.04^{a}\pm1.28$	$1.23^{a}\pm0.066$
MZ (800mg/kg)	$18.09^{de}\pm0.280$	$1.84^{ab}\pm0.209$	$39.11^{abc}\pm2.99$	$1.34^{abc}\pm0.077$
Arsenic (10 ppb)	$19.72^{e}\pm0.112$	$3.22^{c}\pm0.218$	$27.42^{ab}\pm1.52$	$1.25^{a}\pm0.020$
Arsenic (50 ppb)	$17.93^{de}\pm0.511$	$2.65^{b}\pm0.280$	$38.64^{abc}\pm0.72$	$1.48^{abcd}\pm0.037$
Arsenic (100ppb)	$16.91^{d}\pm0.097$	$2.46^{b}\pm0.292$	$48.74^{cd}\pm2.81$	$1.52^{abcd}\pm0.066$
MZ + As (10ppb)	$14.00^{c}\pm0.588$	$2.01^{ab}\pm0.296$	$40.57^{bc}\pm3.74$	$1.55^{bcd}\pm0.027$
MZ + As (50ppb)	$13.76^{bc}\pm0.307$	$1.78^{ab}\pm0.244$	$49.75^{cd}\pm4.01$	$1.60^{cd}\pm0.017$
MZ +As (100 ppb)	$11.66^{a}\pm0.512$	$1.53^{a}\pm0.243$	$61.90^{de}\pm4.29$	$1.74^{d}\pm0.130$
MZ+ As (100ppb) + Quercetin (50 mg/kg)	$12.09^{ab}\pm0.508$	$1.96^{ab}\pm0.332$	$67.33^{e}\pm1.63$	$1.42^{abc}\pm0.042$
MZ+ As (100ppb) + Catechin (50mg/kg)	$14.70^{c}\pm0.535$	$2.01^{ab}\pm0.295$	$70.08^{e}\pm2.71$	$1.30^{ab}\pm0.032$

Values are given as mean ± SE of 6 animals unless otherwise stated
Values having different superscripts (a,b,c,d, e,f) in a column are statistically different from one another at 5% level of significance
Values of TAS (Total antioxidant status), expressed in mM
Values of TTH (Total thiols)expressed in µM
Values of advance oxidation protein product (AOPP) are expressed in µM of Chloramine-T
Values of malondialdehyde (MDA) are expressed in nmole of MDA formed/gm/hr



(a)



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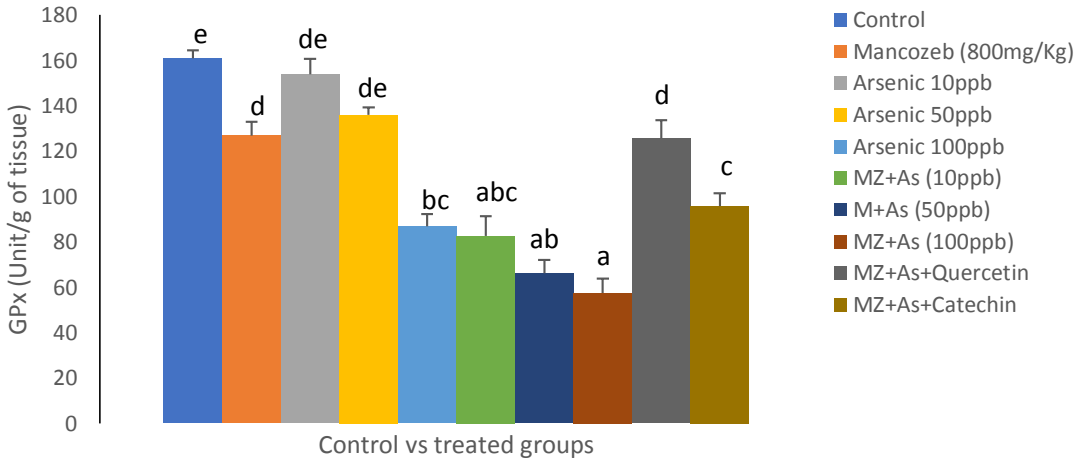
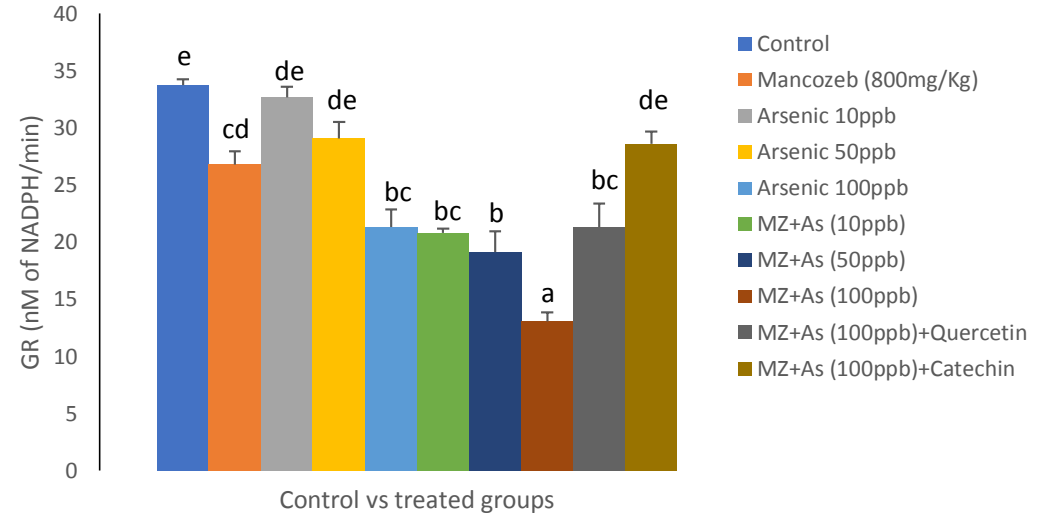


(c)

Figure 4.20: **Graphical representation of antioxidant biomarkers *viz.* SOD (a), CAT (b) and GPx (c) in testes of rats following subacute exposure of mancozeb alone or in-combination with arsenic and its amelioration with quercetin and catechin.**



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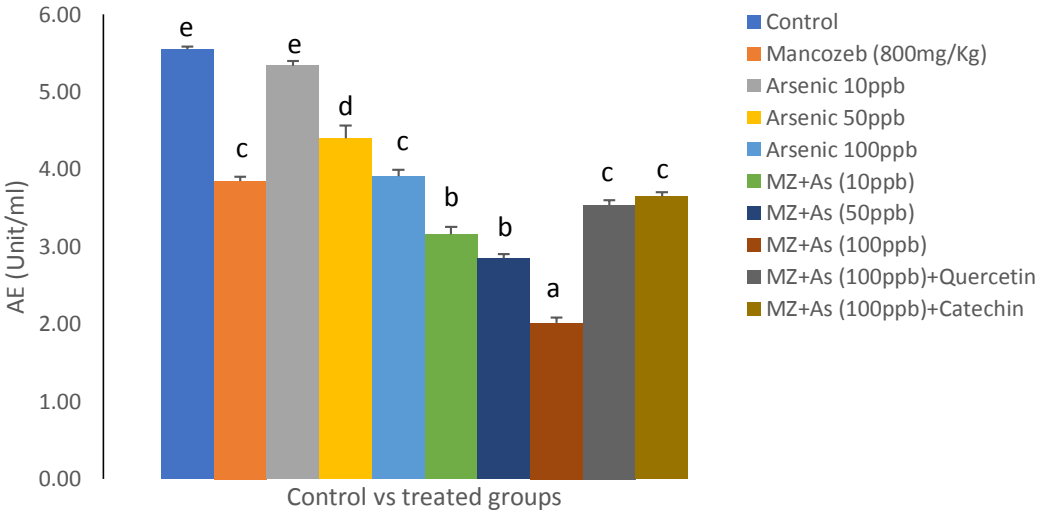

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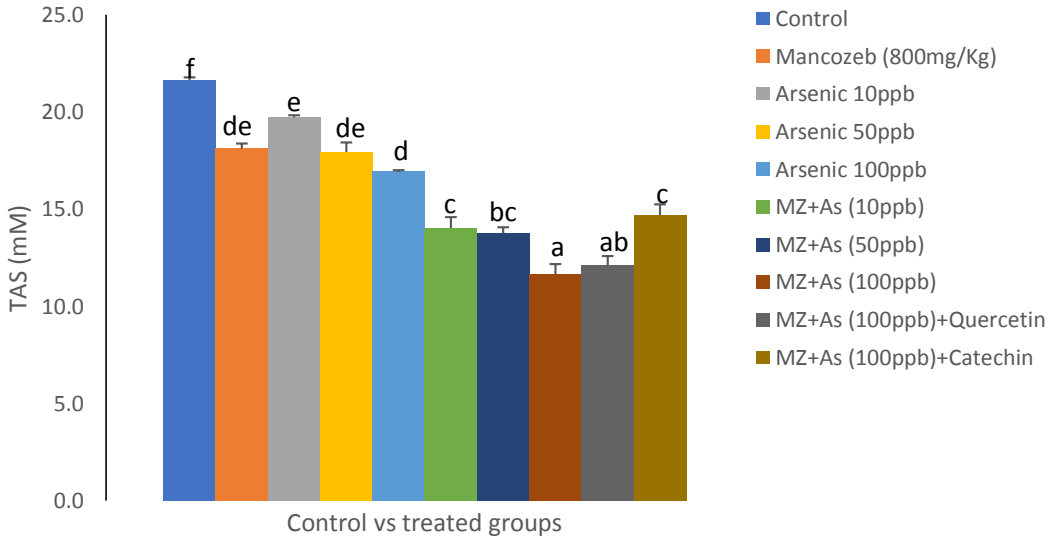
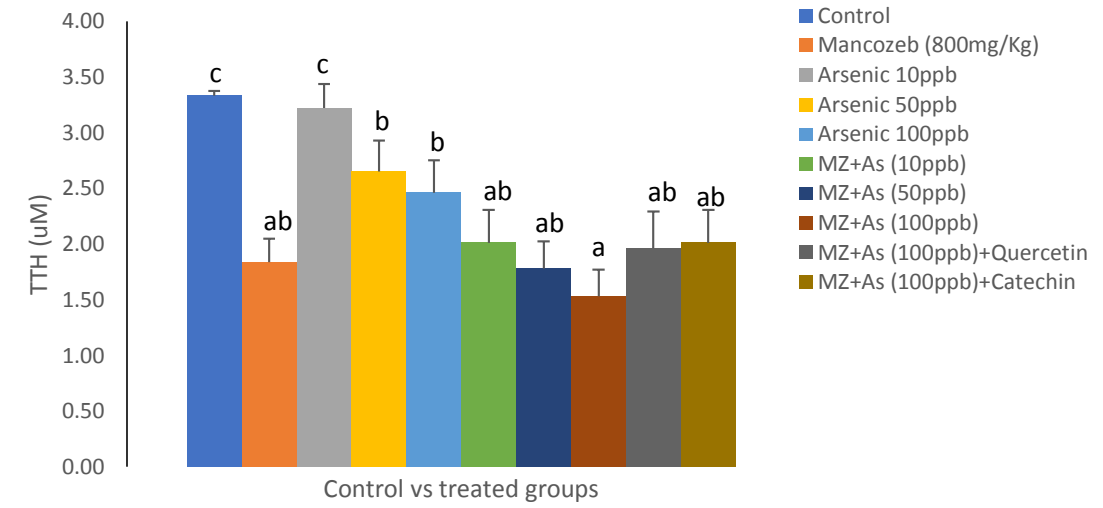
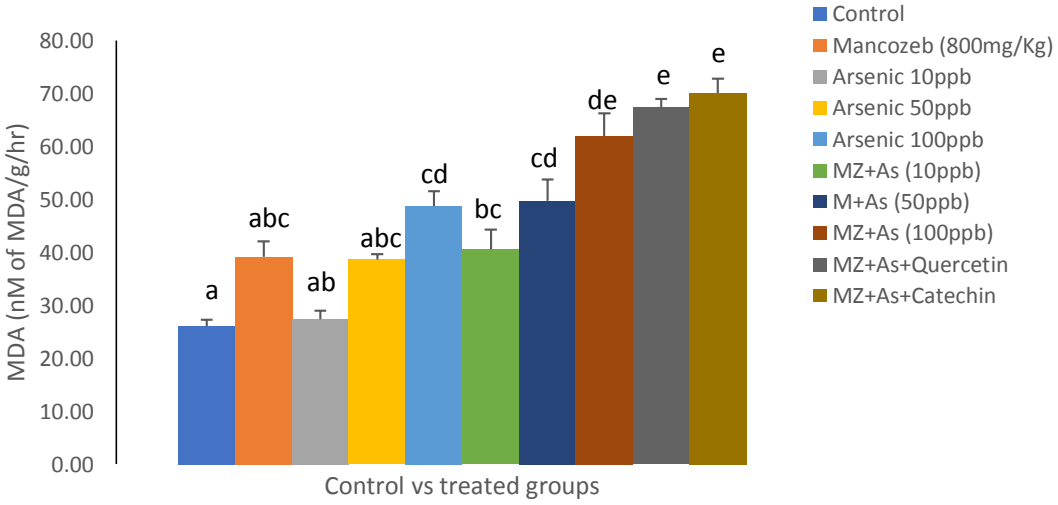


(c)

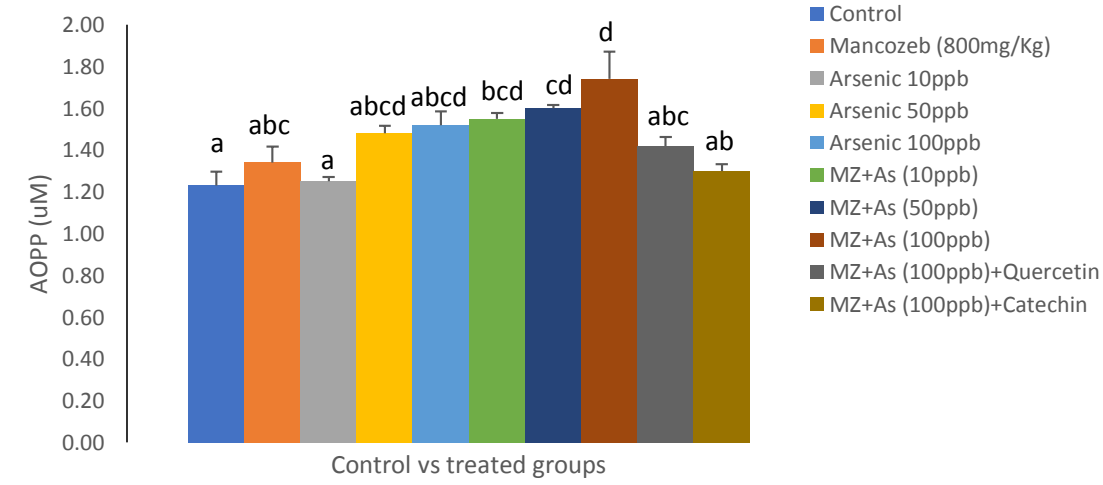
Figure 4.21: **Graphical representation of antioxidant biomarkers *viz.*GR (a), AE (b) and TAS (c) in testes of rats followingsubacute exposure of mancozeb alone or in-combination with arsenic and its amelioration with quercetin and catechin.**



(a)



(b)



(c)

Figure 4.22: **Graphical representation of antioxidant biomarkers *viz.*TTH (a), MDA (b) and AOPP (c) in testes of rats following subacute exposure of mancozeb alone or in-combination with arsenic and its amelioration with quercetin and catechin.**

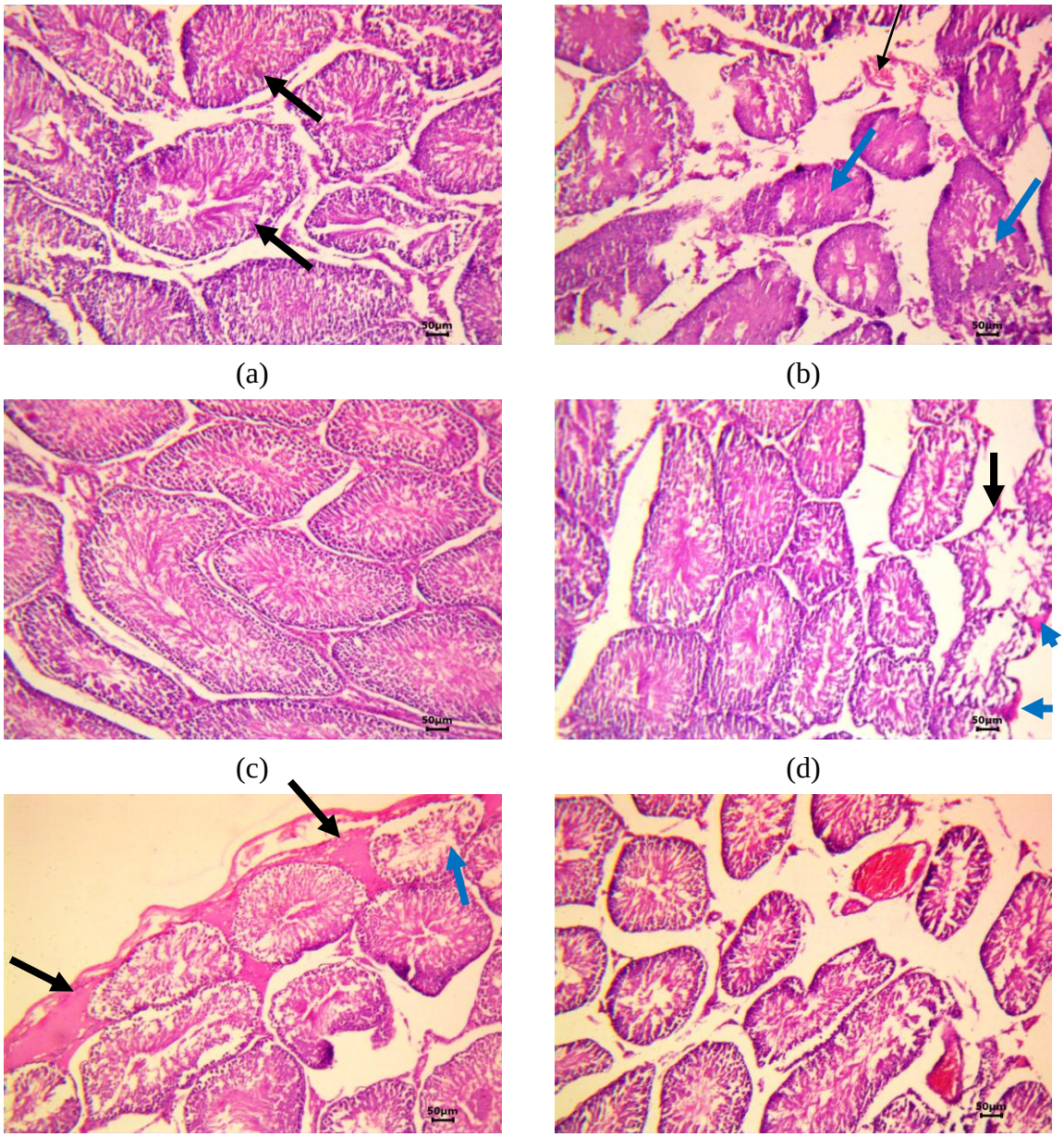
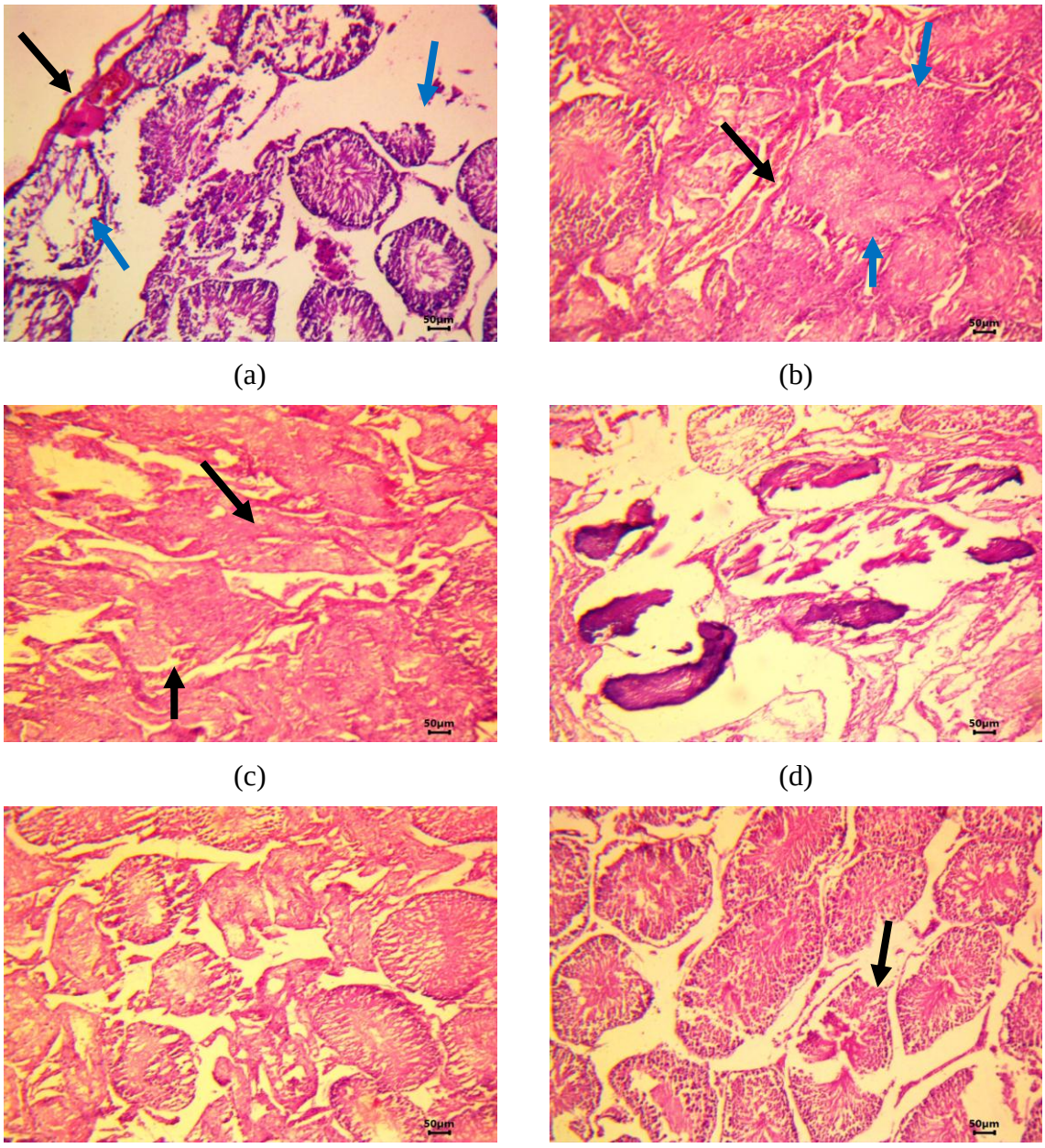


Plate 9: a. Seminiferous tubules (black arrows) in testes of G1 rats showing normal stages of spermatogenesis and scanty interstitium; b. Testes in G II rats with haemorrhage (thin arrow) reduced number of seminiferous tubules containing degenerating or necrotic contents (blue arrows) and increased interstitial space; c. Normal seminiferous tubules in testes of Group III rats; d. Mild subcapsular oedema (blue arrows) and degenerative changes (black arrow) in adjoining seminiferous tubules in Group IV rats; e. Accumulation of oedema beneath lining capsule (black arrow) along with degeneration in seminiferous tubules (blue arrow) in Group V rats; f. Testes from G VI rats showing interstitial congestion.



(a) (b)

(c) (d)

(e) (f)

Plate 10: a. Subcapsular oedema (black arrow), degeneration and necrosis of seminiferous tubules (blue arrows) in testes of Group VII rats; b. Congestion (black arrow) along with degeneration and necrosis of contents of seminiferous tubules (blue arrow) in G VIII; c. Severe necrosis and hyalinisation of seminiferous tubules (arrows) in G VIII rats; d. Destruction of seminiferous tubules in G VIII and replacement with eosinophilic fibrillar mass (blue arrow) and calcification (black arrows); e. Degeneration and necrosis of seminiferous tubules (black arrows) in G IX rats; f. G X rats showing mild degeneration (arrow) of seminiferous tubules.

Chapter-V

Discussion

Chapter 5

Discussion

The environment is complex fabric of biotic and abiotic components which vary with time and space. The abiotic components, including chemicals and metals which persist in environment cause serious health hazards. Humans and animals are exposed to a wide range of environmental contaminants due to exponential increment in the production, use and disposition of chemicals. Mancozeb, a fungicide belonging to EBDC group family has a broad-spectrum activity and is widely utilized for the control and management of variety of diseases of agricultural, horticultural, and ornamental importance (Sardoo *et al*., 2018). Non-judicious applications and environmental persistency of agrochemicals especially fungicides play a significant role in subtle effects on non-target species.

With regard to arsenic, it is widely distributed throughout the earth's crust and as such the natural exposure through contaminated groundwater to this metalloid has become unavoidable now-a-days. The health effects of arsenic toxicity are multidimensional in both human and animal population (Mahajan *et al.,* 2020). Arsenic is one of the most hazardous metalloids derived from natural environment and a potential human carcinogen (Coryell *et al*., 2018). The principal source of exposure being the food and contaminated water. Globally, at least 140 million people in 50 countries have drinking water containing arsenic above WHO recommendations.

According to recent reports, As is naturally present at high levels in the groundwater of several countries, including Bangladesh, Argentina, Chile, India, Vietnam, China, Taiwan, Argentina, Canada, Mexico, and the United States of America. As is a toxicant that affects virtually every organ or tissue in the body, its deleterious effects include, skin lesions, various types of cancer, as well as gastrointestinal, cardiovascular, and respiratory effects. To better understand the toxicity of this widely used fungicide individually and/or along with arsenic, present study was designed to evaluate whether consecutive sub-acute exposure can cause oxidative stress to erythrocytes, brain, liver, kidney and testes. Recently, much attention is being paid to the role of naturally occurring dietary substances viz. quercetin and catechin for the control and management of toxicant induced cellular oxidative damage due to their

potent scavenging potential as well as improving mammalian antioxidant defense system. Therefore, present study was an attempt to elucidate the individual toxicity of MZ or arsenic and their combination on different visceral organs and amelioration of combined toxicity with quercetin and catechin.

***5.1. Alterations in erythrocytes parameters following subacute exposure of mancozeb (MZ) and arsenic (As) alone and in-combination and its amelioration with quercetin and catechin*:** The fungicide, MZ has been an invaluable tool to growers worldwide for management of fungal pests in the crops for more than five decades (Gullino *et al*., 2010). With ever increasing use of MZ globally and presence of As in groundwater, it is now becoming clear that environmental exposures to humans are not just limited to single chemical rather they are simultaneously exposed to variety of chemicals. Oxidative stress and associated damage to cellular components occur when there is an imbalance between antioxidant defense system and the production of ROS. The term "ROS" includes both free radicals [molecules having an odd electron, *viz*. superoxide radical anion (O_2^-) and hydroxyl radical (OH)] and species that are not free radicals, such as singlet oxygen (O_2), hydrogen peroxide (H_2O_2) and ozone (O_3). Reactive nitrogen species (RNS) includes nitric oxide (NO) and peroxynitrite ($ONOO^-$) formed in a reaction of NO with O_2^-. The imbalance between the production of ROS or RNS, and the antioxidant defense system, in favour of pro-oxidants, is called oxidative and nitrative stress, respectively. Although their physiological concentrations are important as signaling molecules for cell growth, proliferation, differentiation and apoptosis, but their excess production may react with and damage all types of endogenous macromolecules including those of lipids, proteins, carbohydrates and nucleic acids. Antioxidant defense system consisting of enzymes like GSH, CAT, SOD, GPx and GR which offer protection to cells against pro-oxidant free radicals (Pavlovic *et al*., 2015; Grzesik *et al*., 2018; Hu *et al*., 2020; Maji *et al*., 2020; Khatun *et al*., 2020; Li *et al*., 2021b). In present study, a significant deduction was found in the antioxidant defense system alongside a decline in AE and AChE. GSH, is considered as the most abundant endogenous antioxidant, forming glutathione disulfide (GSSG) in its oxidized form. GSSG is recycled back by GR with an expenditure of one molecule of NADH (Ahmed *et al*., 2013). GSH has a strong nucleophilic character due the presence of a -SH group and thus it conjugates with electrophilic substrates like ROS/RNS, which are potentially

toxic to tissues and precipitate toxicity. Thus, decrease in GSH and GR activity in MZ or As treated animals indicated oxidative mediated damage. Co-exposure of MZ and As at different doses revealed a significant curtailment in the levels of GSH and GR. The significant fall in GSH and GR levels, has also been reported after treatment with As or imidacloprid and their combination (Mahajan *et al*., 2020). Also, the study by Salam *et al*., 2020 reported that the GSH content of human erythrocytes decreased on exposure to fungicide thiram. Similar studies were also reported by Lorio *et al*., 2015 on MZ exposure on mouse granulosa cells (GCs) which revealed ROS involvement in causing decreased p53 content, GSH levels, ATP and a depolarized membrane potential in GCs, suggesting low doses of MZ induces a mild oxidative stress. SOD and CAT are the two most important superoxide radical converting and defense enzymes during xenobiotics exposure. While, SOD convert superoxide radicals into hydrogen peroxide and molecular oxygen, CAT and GPx convert hydrogen peroxide to water. The difference between three enzymes lies in the fact that SOD and CAT do not need co-factors to function, while GPx requires several co-factors to function. Also, GPx scavenges free radicals at the cost of reduced GSH to form GSSG and hence along with GR help in maintaining the cellular ratio of reduced and oxidized GSH as 300:1 (Ahmed *et al*., 2013; Mahajan *et al*., 2020). A reduction in SOD, CAT, GPx and GR in MZ or As alone treated animals observed in present study clarifies the free radical mediated injury to erythrocytes and the decline was further intensified in the combination groups. The study by Tandon *et al*., 2012 in sodium arsenite induced toxicity in rat erythrocytes, liver, kidney and brain also revealed a decline in SOD and CAT activity alongside a fall in GSH content. Similar reports were provided by Balaji *et al*., 2014 and Sadowska-Woda *et al.*, 2010 on human erythrocytes on exposure to MZ and bifenthrin respectively. The study of Messarah *et al*., 2013 also manifested a decline in CAT and GPx activity on exposure to diazinon on rat erythrocytes and liver. Calviello *et al*., 2006 reported exposure of MZ on the fibroblasts and peripheral blood mono-nucleated cells (PBMC) isolated from Wistar rats. Both the cells produced dose-dependent induction of DNA single strand break (SSB) formation, measured via single cell gel electrophoresis (SCGE). There were elevated levels of oxidative markers of DNA oxidation, 8-hydroxy-2'-deoxyguanasine (8-OHdG) and of ROS, indicating pro-oxidant action of MZ. Thiols are sulfhydryl (-SH) group containing endogenous antioxidant molecules which protect against the free radical

mediated oxidative damage and maintain the homeostasis inside the cell. Total thiols occur in free form as oxidized or GSH or as bound to proteins as intracellular and extracellular form. A significant decline in TTH level was observed in animals administered MZ alone or in combination. Similarly, sub-acute exposure of As caused a fall in TTH levels in a dose dependent manner. Present study was in accordance with Mahajan *et al*., 2020, Salam *et al*., 2020 and Sharma *et al*., 2020b. The values of TAS that indicates the total antioxidant status in erythrocytes showed a significant fall in MZ treated animals, while as a dose dependent decrease was seen in As treated animals. The TAS levels were more prominently reduced in the combination groups. Meanwhile, plasma TAS which is accurate index measurement of total plasma defenses against ROS, declined significantly in combination groups. The results were consistent with reports of Selvi *et al*., 2013; Ogut *et al*., 2015 and Sharma *et al*., 2021b. In our study, we noticed a significant depression in AE activity alongside a curtailment in AChE activity in MZ or As administered rats. AE, an important free radical scavenger shows decline in its levels under oxidative stress conditions as per the studies by Sharma *et al*., 2021b. Present results were supported by Vieira *et al*., 2020 which reported a decline in AChE on exposure to MZ on Zebra fish. Similar studies were reported by Singh *et al*., 2020 in which there was a fall in AChE on exposure to As and quinalphos in renal tissue. The rise in MDA levels in erythrocytes occurs as they are more prone to oxidative mediated damage not only due to presence of high contents of polyunsaturated fatty acids in their membranes, but also due to the high cellular oxygen particularly in the presence of harmful xenobiotics (Balaji *et al*., 2014; Mahajan *et al.,* 2020). In present study, it was revealed by increment in MDA values, which is an endogenous product of enzymatic and oxygen radical-induced lipid peroxidation. The increment was more severe in combination groups. Similar findings were provided by Mansour *et al*., 2009 in rat erythrocytes on exposure to methomyl. The findings of Sutcu *et al*., 2007 also suggested the rise in MDA levels along with decline in SOD and GPx activity on exposure to diazinon on rat erythrocytes. AOPP is a dityrosine consisting of cross-linked protein product and a validated marker for protein oxidation. It has been found that AOPP produced by different oxidation patterns lead to the formation of either hydrogen peroxide or nitric oxide. In present study, significant hike in AOPP levels was seen in MZ treated animals and dose dependent increment was found in As treated rats. Combined

exposure of both the toxicants significantly elevated protein oxidation which leads to conclusion that the stress levels in erythrocytes were indeed exacerbated on co-administration of toxic agents. The findings of present study were consistent with reports of Singh *et al*., 2020; Mahajan *et al*., 2020 and Sharma *et al*., 2021b. Nitric oxide (NO), generated from L-arginine by NO synthase, is well known physiological messenger molecule and reactive free radical. Excessive generation of NO, however, is potentially toxic and has been involved in variety of pathological situations and chronic inflammation. Additionally, NO is cytotoxic agent and a mediator in inflammatory disorders and it is due to its cytotoxicity, that overproduction is deleterious to cell. In present study, administration of MZ or As caused a significant increase in NO production and might be responsible for erythrocytic damage. Co-exposure of both the chemicals significantly inflated NO levels recorded after individual administration of toxicants. Similar findings were reported by Rana *et al*., 2010 and Dkhil *et al*., 2020. In the ameliorative groups, treatment with quercetin in combined toxicant group restored plasma TAS, GSH, CAT, GR, AE, AChE and AOPP and NO as compared to group VIII. The reports were as per Balaji *et al*., 2014 in which quercetin reversed the elevated levels of MDA and decreased levels of SOD, CAT, GSH and GPx in human erythrocytes induced by MZ. In addition, during combined exposure along with quercetin and catechin individually, quercetin scavenged NO and peroxynitrite and also down regulates excessive production of NO by inducible form of NO synthase (iNOS). The results of present study were consistent with those of Rana *et al*., 2010 in which the administration of ascorbic acid inhibited the excess NO production in chronic arsenicosis. The study by Pavlovic *et al*., 2015 suggested role of ascorbic acid in alleviating ROS generation on MZ treated rat thymocytes. Tandon *et al*., 2012 suggested that aqueous extract of *Psidium guajava* leaves has protective potential against sodium arsenite induced toxicity in rat erythrocytes, liver, kidney, brain and restored GSH, SOD, CAT and MDA levels. Similarly various studies showed vitamin C, E and curcumin mitigated diazinon induced toxicity on rat erythrocytes by improving the antioxidant defense system (Sutcu, *et al*., 2007 and Messarah *et al*., 2013). In the present study catechin restored the GSH, AE, AChE activity along with MDA and AOPP in erythrocytes. Number of studies proved catechin to be a protective agent against oxidative insult caused by

either pesticides or heavy metals or their combination (Afolabi *et al*., 2016; Cheruku *et al*., 2018; Kayode *et al*., 2019; Mosolin *et al*., 2022).

Findings of study conducted suggested that repeated combined administration of MZ and As caused more profound oxidative damage in RBCs of rats when compared to individual application of any single toxicant as was depicted by a significantly larger deduction in activities of different antioxidants along with significantly higher NO, lipid and protein oxidation in the former case. Administration of quercetin along with combined toxicants upgraded plasma TAS, GSH, CAT, GR, AE, AChE and AOPP and NO. Meanwhile GSH, AE, AChE along with MDA and AOPP were improved by catechin in the toxicant interactive group, indicating quercetin to be effective as ameliorative agent in erythrocytes.

5.2. Alterations in antioxidant and histology of brain following subacute exposure of mancozeb (MZ) and arsenic (As) alone and in-combination and its amelioration with quercetin and catechin: Mancozeb, as per the available literature produces neurotoxic effects and Parkinson's like symptoms (Zhou *et al*., 2004), fetal brain development dysregulation (Nordby *et al*., 2005), and thyroid hormone disruption are highlighted (Goldner *et al*., 2010). Mancozeb has also been reported to have neurotoxic effects on both GABAergic neuronal cell populations and mesencephalic dopaminergic neurons (Domico, *et al*., 2007; Brody *et al*., 2013). On the other hand, arsenic exposure to unacceptable amounts is associated with increased risk of different types of ailments (Coryell *et al*., 2018; Sodhi *et al*., 2019). Moreover, data from previous experimental and clinical investigations suggest that arsenic intoxication injures neuronal architecture and significantly impairs neurophysiological processes which can predispose to cognitive dysfunctioning and dementia (Rahaman *et al*., 2021). The underlying mechanism by which MZ and As causes neurotoxicity is not clear. However, individual studies on both the chemicals have suggested oxidative insult due to excessive reactive oxygen and nitrogen species (ROS/RNS) generation and/or reduced radical scavenging capacity as a powerful contributor in development of MZ (Calviello *et al*., 2006; Costa-Silva *et al*., 2018; Saraiva *et al*., 2018) and As (Mishra *et al*., 2008; Li *et al*., 2021a; Thakur *et al*., 2021) induced neurological deficits. The brain tissue being highly susceptible to oxidative insult because it requires proportionately higher oxygen levels for its normal functioning as neurons derive energy only via oxidative phosphorylation. This along with presence of a

robust redox signaling, and a relative deficiency in antioxidant defenses make the brain more susceptible to oxidative stress (Prakesh *et al*., 2016; Cobley *et al*., 2018; Nurchi *et al*., 2020). Additionally, its richness in lipid content and lack of capacity for cellular regeneration or repair increase its vulnerability to oxidative damage induced by exposure to toxicants (Nirankari *et al*., 2016; Costa-Silva *et al*., 2018; Saraiva *et al*., 2018; Garza-Lombo *et al*., 2019). The generation of free radicals and subsequent oxidative stress are responsible for various toxic effects including neurodegenerative disorders through oxidizing mitochondrial DNA, lipids and proteins, increasing the permeability transitional pores of mitochondria; resulting in degeneration of neurons and hence death (Prakesh *et al*., 2016; Cobley *et al*., 2018; Nurchi *et al*., 2020). In CNS, a primary defense against ROS is offered by SOD, CAT and GPx. The enzymes convert superoxide radical into hydrogen peroxide and molecular oxygen and water. GPx also uses thiols-reducing power of GSH, so as to cause reduction of oxidized lipids and protein targets of ROS. Also, reduced TAS levels following administration of MZ or As in the present study indicate the suppression of antioxidant defenses of the brain tissue. The reduction in TTH content in the brain tissue caused by MZ (Calviello, *et al*., 2006) or As (Garza-Lombó *et al*., 2019; Thakur *et al*., 2021) treatment or their combination can be illustrated by the fact that thiol groups have affinity towards metal ions and are easily oxidizable by free radicals. AE also called as paraoxonase1 has a pivotal role in the catalysis of free radicals and its activity is impeded in response to oxidative stress such as those caused by either of the toxicants (Zargari, 2019). So as a matter of fact, the levels of TTH, SOD, CAT, GPx, GR and AE decreased in MZ induced neurotoxicity (Tsang and Trombetta, 2007; Saber *et al*., 2016; Costa-Silva *et al*., 2018; Saraiva *et al*., 2018; Leandro *et al*., 2021) or As induced neurotoxicity (Mishra *et al*., 2008; Zhao *et al*., 2017; Nageshwar *et al*., 2019; Hu *et al.*, 2020; Maji *et al*., 2020 and Khatun *et al*., 2020; Metwally *et al*., 2020; Peruru *et al*., 2020; He *et al*., 2021; Sharma *et al*., 2021b). Co-administration of MZ and As in rats triggered significant decrease in activities of CAT, SOD, GPx, and GR alongside a decline in TAS, TTH and AE values, compared to individual exposure of either MZ or As. Previous studies involving co-exposure exposure of pesticides, drugs, metals or their combination include those of Peng *et al*., 2007; Feng *et al*., 2010; Khan *et al*., 2015; Sing *et al*., 2017; Farkhondeh *et al*., 2020; Zhao *et al*., 2021. Meanwhile, the levels of AChE decreased in individual toxicant groups which were

further suppressed in combined toxicity groups. This enzyme is indispensable for the regulation of transmission of acetylcholine which coordinates the neuronal networks of the cholinergic modulation area of the brain (Leandro *et al*., 2021). Cholinergic dysfunction is reported to be associated with the impairment in motor coordination and learning, which may be the major etiological factor of Alzheimer's disease. Reduced AChE along with the degeneration of cholinergic neurons have been reported in MZ or arsenic-exposed rats (Chandravanchi *et al*., 2018; Zizza *et al*., 2018; Leandro *et al*., 2021). Oxidative mediated damage and subsequent neurodegeneration was also revealed by increment in the levels of NO. A significant increase was seen in NO levels in MZ treated rats and As treated rats in a dose dependent manner. The role of NO in the neurotoxicity due to environmental toxicants has been reported from various studies (Flora *et al*., 2008; Goudarzi *et al*., 2018). NO is a high reactive molecule that is derived from four distinct isoforms of NO synthase neuronal NOS-1 (nNOS), inducible NOS-2 (iNOS), endothelial NOS-3 (eNOS) and mitochondrial (mtNOS). NOS-1 and NOS-3 are constitutively expressed and controlled by intracellular calcium calmodulin whereas NOS-2 is inducible at gene transcription level, and is expressed by macrophages and other cells in response of pro-inflammatory mediators. NO in the brain tissue not only regulates various physiological processes like cognitive function and synaptic plasticity (nNOS), but also controls brain blood flow, hence, promotes angiogenesis and maintains cellular redox potential, immunity and neuronal survival (eNOS) (Luiking *et al*., 2010; Levine *et al*., 2012). However excessive production of NO (iNOS) alone or in combination with other superoxide radicals leads to neurotoxicity and consequent neurodegeneration (Saber *et al*., 2016). Role of iNOS and consequent elevated levels of NO in pathological conditions has been reported in studies by Levine *et al*., 2012; Sharma *et al*., 2013; Khazan *et al*., 2014; Saber *et al*., 2016. Very high levels of NO in the combination group in our study may be generated upon inflammatory iNOS induction that often competes with O_2 causing NO-dependent hypoxia/nitroxia. Nitroxia leads to the generation of excess levels of ROS/RNS which in turn can shut down of mitochondrial respiration by irreversible inhibition of ETC complexes resulting in depleted levels of ATP and consequent cytotoxic effects. Irreversible inhibition of ETC results in apoptosis and necrosis of brain tissue via energy depletion which may end up in multiple organ failure associated with accelerated and refractory

anaerobic metabolism (Levine *et al*., 2012). Excess NO causes post-translational modifications in proteins by S-nitrosylation of the thiols amino acids, which regulate protein function physiologically. The process of protein nitrotyrosination is irreversible that ultimately results in accumulation of the modified proteins which contribute to the progression of neurodegeneration like the case of Alzheimer's disease or Parkinson's disease (Khazan *et al*., 2014; Dawson *et al*., 2018). This gaseous molecule can pass neuronal membranes easily leading damage to lipids, proteins and nucleic acids. This damage to lipids and proteins was elicited by significant increment in MDA and AOPP levels in brain tissue following exposure to MZ or As. AOPP levels represent ROS induced protein oxidation and it is reported that AOPP generated by different oxidation patterns cause production of either hydrogen peroxide or nitric oxide (Mahajan *et al*., 2020). So severe increment in levels of AOPP, NO and MDA in combination groups inferred that brain tissue was put under heightened oxidative and carbonyl stress after concomitant treatment with MZ and As. Present study was in accordance with Souza *et al* 2018; Metwally *et al*., 2020; Peruru *et al*., 2020. The overwhelmingly high lipid peroxidation may be the cause of severe pathological lesions in tissue sections of brain as revealed from histopathology. Both cerebrum and cerebellum revealed changes viz. congestion, hemorrhages, degeneration, necrosis, spongiosis, gliosis, and perivascular oedema. Additionally, cerebellum revealed changes like neuronophagia, focal areas of encephalomalacia and liquefaction. These changes were moderate in MZ treated animals and were mild-moderate in arsenic treated animals and were severe in combination groups (Saber *et al*., 2016; Prakesh *et al*., 2016; Goudarzi *et al*., 2018; Nageshwar *et al*., 2019; Metwally *et al*., 2020).

The role of chelating and complexing agents and plant-based interventions on individual toxicity of MZ and/or As is well reported, but there is no report of their action on multiple exposure of MZ and As. Therefore, present study was designed to use quercetin and catechin against multiple exposure of MZ and As. Administration of quercetin to the combined toxicity group alleviated the declined activity of CAT, AChE and suppressed the elevated levels of MDA, AOPP and NO as compared to toxicant interactive group. On the other hand, catechin improved the SOD, GR, AE, AChE, TAS, TTH contents along with AOPP and NO levels. Present study was supported by Nageshwar *et al*., 2019 which showed that quercetin reversed the

sodium arsenite induced oxidative damage, behavioral and histological alterations in brain tissue of rat. As per Cheruku *et al*., 2018, catechin caused a significant restoration of time-induced memory deficit induced by doxorubicin in Wistar rat model (*in vivo* and *in vitro*). Study of Josiah *et al.,* 2022 depicted catechin and quercetin possess potential relevance in treatment of Parkinson's disease through optimization of dopamine metabolism, alleviation of redox stress, modulation of anti-inflammatory and anti-apoptotic pathways in rotenone-induced Parkinsonism in male Wistar rats. Clinical investigations which used other antioxidants and chelating agents to attenuate MZ (Domico *et al*., 2007; Tsang and Trombetta, 2007; Saber *et al*., 2016) or As (Yadav *et al*., 2010; Goudarzi *et al*., 2018; Metwally *et al*., 2020; Peruru *et al*., 2020; He *et al*., 2021) induced neurotoxicity have been reported. A study by Domico *et al*., 2007 also suggested that MZ associated free radical damage to mesencephalic dopamine and GABA cell populations was markedly attenuated when carried out in presence of ascorbate. Tsang and Trombetta, 2007 evaluated the neurotoxic potential of MZ in rat hippocampal astrocytes exposed for 1 hour and protective role of various antioxidants and butylated hydroxytoluene (BHT) was reported to be most protective against MZ insult. Another study by Metwally *et al*., 2020 proposed neuroprotective potential of chlorogenic acid against sodium arsenite induced toxicity and showed the substance has protective efficacy by altering antioxidant parameters like SOD, CAT, GPx, GR and MDA and NO. Peruru *et al*., 2020 reported neuroprotective efficacy of devil's claw (*Harpagophytum procumbens*) against arsenic induced neurotoxicity in female rats and revealed motor in-coordination related behavioral changes and significant change in antioxidant biomarkers like SOD, CAT, MDA and NO, which were improved by treatment with devil's claw. The present study indicates that combined exposure to MZ and As resulted in marked imbalance in oxidative status and aggravated histopathological response in the brain tissue of rats as compared to administration of either of toxicants. These alterations were, however, mitigated by treatment with quercetin and catechin. Meanwhile, catechin proved to have better protective activity to resist against oxidative insult and nervous tissue damage. The study, therefore, showed that supplementation with catechin has beneficial effects on toxicity evoked by co-exposure to MZ and As on nervous tissue.

5.3. Alterations in antioxidant and histology of liver following subacute exposure of mancozeb (MZ) and arsenic (As) alone and in-combination and its amelioration

***with quercetin and catechin*:** The liver is a vital and primary organ responsible for the homeostasis and removal of toxins and poisons, and it is greatly affected by many harmful xenobiotics that lead to an increase in the liver enzymes (Hashem *et al*., 2018). In the present study, MZ and As were administered to Wistar rats alone and in combination to induce hepatotoxicity and quercetin and catechin were used as ameliorative agents. The rats individually or co-exposed to MZ and As showed increased activity of liver enzymes which may be attributed to hepatocellular damage caused by ROS generation resulting from exposure of either or both of the toxicants. The extent of damage was evaluated by the levels of hepatic enzymes viz. ALT, AST, ALP, LDH and GGT. These enzymes manifest the functional status of hepatic tissue. The magnitude of elevated ALT activity is proportional to the number of affected hepatocytes, which leak the enzyme into the extra-cellular space and finally into circulation. ALT is predominantly found in the peripheral zone of the liver and has primary role in gluconeogenesis and amino acid metabolism. The marked increase in the ALT enzyme activity reflects irreversible cell damage and necrosis, while as mild rise may indicate mostly membrane blebbing and reversible cell damage. The increment in the levels of AST and LDH in our study clearly indicates the extent of damage to liver tissue. The results are consistent with the study of Rana *et al*., 2010; Yahia *et al*., 2019 and Dallognol *et al*., 2021 which demonstrated that there was increase in indicator enzymes like AST, ALT in As or MZ induced hepatotoxicity. GGT as such is elevated in all forms of liver damage but it primarily appears from the hepato-biliary damage and in present study significant hike was seen in animals treated with MZ alone or in combination with As. The decreased levels of total protein, albumin and globulin reflected the severity of damage to liver caused by these toxins. The increment in the activity of ALT, AST, LDH and reduced albumin and globulin levels after the concurrent exposure of MZ and As at higher doses was clear evidence of severe hepatocellular damage and defeat of structural and functional integrity of the hepatic membrane and hence plasma membrane damage (Sakr, 2007; Saber *et al*., 2019; Dkhil *et al*., 2020; Renu *et al*., 2020). Similar findings were proposed by Santra *et al*., 2000 in hepatic tissue caused by chronic As toxicity (3.2 mg/l) in mice model administered *via* drinking water. Changes like fatty liver, increased liver weight with elevated levels of serum ALT and AST were found. Another study by Simakani *et al*., 2018 suggested MZ exposure caused biochemical

effects in *Cyprinus carpio* (common carp) that was estimated by decrease in plasma proteins like albumin and globulin along with increased ALT and AST levels. The increase in ALP activity in arsenic alone treated animals may be due to the increased hepatic functions and present results are consistent with the studies of Dkhil *et al*., 2020; Renu *et al*., 2020; Ishaq *et al*., 2021 in which sodium arsenite/arsenate induced hepatotoxicity caused elevated levels of ALP along with AST and ALT activity. Meanwhile, decrease in ALP activity in MZ alone treated animals and in combination may be attributed to the reduced osteoblastic activity of bone by MZ, since it is present and synthesized in the osteoblasts. Similar findings were observed by Joshi *et al*., 2005.

Oxidative imbalance induced by MZ and As has been well documented, consequently due to increase in free radical generation and depletion of antioxidant defense system that is caused by both of these toxicants. Even though liver plays a definite role in detoxification, but prolonged exposure to xenobiotics and their derivatives makes it susceptible to oxidative damage (Domico *et al*., 2007; Lorio *et al*., 2015; Khatun *et al*., 2020; Li *et al*., 2021b). The oxidative stress induced by MZ may be due to its chemical structure since it contains coordinated transitional metals which catalyses the formation of free radicals through the Fenton reaction (Calviello *et al*., 2006). Additionally, EBDCs are lipophilic and hence can penetrate the cell membrane easily and induce oxidative damage (Saber *et al*., 2019). Furthermore, findings by Dkhil *et al*., 2020 and Renu *et al*., 2020 demonstrated that sodium arsenite induced hepatotoxicity caused oxidative stress due to the excessive production of free radicals, particularly ROS, resulting in hepatic damage. In current study, treatment with MZ and As alone or their co-exposure resulted in the inhibition of all the antioxidant components measured in the liver tissue. Inhibition of antioxidant enzyme activity has been found following exposure of animals to either MZ or As or their combination. Studies have revealed that several factors may help to preserve and defend the body cells by removing free radicle species to decrease oxidative stress (Lorio *et al*., 2015; Khatun *et al*., 2020; Hu *et al*., 2020; Maji *et al*., 2020). Among such factors, antioxidant markers including CAT, SOD, GPx and GR are thought to play a main role in alleviating ROS-induced oxidative stress. In the current study the levels of the various antioxidant biomarkers viz. TAS, TTH, SOD, CAT, GPx, GR and AE were decreased along with rise in MDA and AOPP levels after exposure to MZ or As alone

and in-combination in a dose dependent manner. The reduction in TAS by MZ and As co-administration is an indication of the depletion of the antioxidant system capacity in the liver tissue. The decline in TTH content in the liver tissue in the present study can be illustrated by the fact that thiols groups have affinity towards metal ions and are easily oxidizable by free radicals. Interestingly, both MZ and As have affinity towards the thiols group and our results are consistent with the studies of Kayode *et al*., 2019 and Bahrami *et al*., 2020. Aryl esterase belongs to the family of hydrolases that specifically act on carboxy esters and the most important feature being they are antioxidants and are involved in the metabolism of many xenobiotics. Epidemiological and biochemical studies suggest their involvement in the removal of the biologically active products of lipid peroxidation (Cebeci *et al*., 2012; Eren *et al*., 2014). Thus, in current study similar reason may be responsible for fall in AE levels. The activity of SOD, CAT and GPx decreased as compared to control because these are the major and primary enzymes which convert superoxide radicals such as H_2O_2 and lipid peroxides to nontoxic products like water. The depleted GPx and GR concentration caused by increased oxidative stress burden could induce lipid and protein oxidation. In present study, this is reflected by the marked increase in MDA and AOPP contents in the co-exposed rats. The increased ROS production may be the reason for the generation of oxidized proteins observed in hepatic tissue after exposure of MZ or As or their combination. The results are in accordance with the finding of Kayode *et al*., 2019 in which the multiple exposure of deltamethrin and arsenic to Wistar rats caused increased levels of MDA and AOPP and marked decline was seen in the contents of TAS, SOD, CAT and GPx. The rise in level of MDA produced after the peroxidation of lipids and polyunsaturated fatty acids is the most important indicator of oxidative damage in response to individual or combined MZ or As induced stress to cells that indirectly measures the degree of tissue and cell injury. Previously, it was reported that ROS including H_2O_2 and MDA contents were significantly elevated in the hepatic tissue homogenates of As-intoxicated rats (Renu *et al*., 2020; Ishaq *et al*., 2021). Souza *et al*., 2018 reported effects of As in diabetic rats and results revealed that arsenate exposure declined the antioxidant activity and increased the MDA and carbonyl protein levels. Similarly, elevated levels of MDA were reported for MZ alongside a decline in the antioxidant enzymes (SOD and CAT) activities (Calviello *et al*., 2006; Srivastava *et al*., 2012 and Hashem *et al*., 2018).

Dkhil *et al*., 2020 also observed a marked increment in MDA and NO and a significant decrease in the levels of GSH, SOD, CAT, GPx and GR in the liver of rats exposed to sodium arsenite. Histopathological alterations in liver induced by MZ, As or their combination include congestion, hepatocellular degeneration, hemorrhages, centrilobular hepatic necrosis, dilation of sinusoids and infiltration of inflammatory cells. These results further validated present findings of oxidative injury to liver tissue and are in accordance with previous studies (Yahia *et al*., 2019; Dkhil *et al*., 2020 and Renu *et al*., 2020). The toxic effects elicited by co-exposure to MZ and As were all significantly reduced by quercetin and catechin as compared to group VIII. Although reports are not available about the protective effect of quercetin and catechin against simultaneous exposure of MZ and As induced toxicity, but there are reports of their ability to protect against individual chemical induced oxidative stress and inflammation (Uzun and Kalender, 2013; Afolabi *et al*., 2016; Cheruku *et al*., 2018; Akinmoladun *et al*., 2020; Owumi *et al*., 2019; Mosolin *et al*., 2022; Arslan *et al*., 2022). In the present study, quercetin restored the plasma hepatic biomarkers viz. AST, LDH, GGT, TP, GLO and activities of the antioxidant enzymes SOD, CAT, TAS, while suppressing the generation of MDA and AOPP in the liver tissue. Meanwhile, catechin improved the enzyme activity including AST, GGT, LDH, ALP, TP, GLO and antioxidant biomarkers such as AE, TTH, MDA and AOPP. The improvement of the hepatic biomarkers in MZ and As co-exposed rats by quercetin and catechin indicated that the polyphenols effectively scavenged free radicals and suppressed the oxidative damage. Histopathological changes were milder in quercetin treated animals compared to moderate changes in catechin treated rats which further validated the protective role of both the agents. Previous studies have shown quercetin to be an effective agent able to scavenge and remove deleterious toxic free radicals that could disrupt antioxidant enzyme defense system and cause tissue injury (Akinmoladun *et al*., 2020; Owumi *et al*., 2019; Mosolin *et al*., 2022; Arslan *et al*., 2022). Studies of Afolabi *et al*., 2016; Cheruku *et al*., 2018; Kayode *et al*., 2019; Mosolin *et al*., 2022 proved catechin to be a potential agent against oxidative insult caused by either pesticides or heavy metals or their combination. The study by Mosolin *et al*., 2022 revealed that concurrent treatment with green tea and quercetin attenuated Cd induced oxidative damage, serum biochemical changes and histopathological alterations related to hepatic damage in rats. As per study by Arslan

et al., 2022 quercetin possess ameliorative effects against Pb induced oxidative stress, biochemical changes and apoptosis in laying Japanese quails. Blood biochemical markers like ALT, AST, ALP, total protein, albumin and globulin were improved and oxidative markers like CAT, GPx, GSH and MDA in liver were also corrected towards control. Afolabi *et al*., 2016 reported that catechin protected against arsenic induced dyslipidaemia and hepatic damage in male Wistar rats when administered concurrently with arsenic and improved AST, ALT and ALP levels. In the study by Ishaq *et al*., 2021, turmeric-extracted curcumin effectively restored liver functions as well as attenuated the oxidative injury that occurred after the intoxication of sodium arsenate in male rats. Another study by Sakr, 2007 suggested that ginger improved the biochemical and histopathological changes induced in liver by MZ. Furthermore, Maji *et al*., 2020 found turmeric and Chinese tea vine (*Paederia foetida*) powder had ameliorative potential on experimentally induced arsenic toxicity in sheep and found that administration of both the test drugs not only increased elimination of arsenic from feces, urine and wool but also improved hematological, biochemical (AST, ALT, blood urea nitrogen and serum creatinine) and antioxidant (SOD and CAT) parameters, compared to group treated with arsenic only. The ameliorative role of quercetin and catechin was further substantiated from the histopathological assessment as only mild to moderate changes were manifested in these animals. Previous study also revealed that administration of both quercetin and catechin restored the hepatic damage caused by the exposure of chlorpyripos and cadmium respectively (Uzun and Kalender, 2013; Mosolin *et al*., 2022). Findings of present study concluded that repeated multiple exposure of MZ and As caused more pronounced oxidative damage to liver of Wistar rats when compared to individual application of any single toxicant as was depicted by a significantly larger reduction in activities of different antioxidants along with significantly higher MDA and AOPP in the former case. Among the ameliorative agents, quercetin proved to be more effective as far as the restoration of activity of antioxidant biomarkers and histopathology is concerned.

***5.4. Alterations in antioxidant and histology of kidney following subacute exposure of mancozeb (MZ) and arsenic (As) alone and in-combination and its amelioration with quercetin and catechin*:** Kidney plays a pivotal role in the maintenance of total fluid volume, composition, acid-base balance by process of selective re-absorption of

various ions and solutes. However, a variety of environmental pollutants including pesticides, heavy metals, anticancer drugs and other chemicals have been found to alter the structure and functions of the kidney drastically. Arsenic compounds have been shown to cause serious toxic effects in almost all the target organs including kidney (Rizwan *et al*., 2014; Shahid *et al*., 2014; Mahajan *et al*., 2018b). Experimental evidences and epidemiological investigations have demonstrated that both acute and chronic exposure to arsenic compounds can cause injury to the kidney and pose risk of renal cancer (Shahid *et al*., 2014; Prakash *et al*., 2018; Thangapandiyan *et al*., 2019; Jin *et al*., 2020; Ramadan *et al*., 2022). However, the first comprehensive and molecular analysis of MZ induced nephrotoxicity was provided by Zhang *et al*., 2022 in mice using transcriptomics and metabolomics. Many different substances like chelating agents have been explored for their protective potential in various toxicities. However, majority of them have not yet proved safe for clinical application. Nowadays, the use of naturally occurring dietary substances for prevention of toxic effects of environmental toxicants and chemicals is gaining interest. Quercetin is a natural polyphenolic compound that is widely distributed in kingdom plantae and abundantly found in fruits and vegetables (Uzun *et al*., 2010: Baltaci *et al*., 2016). It has found application in treatment of many pathological conditions including cancer, obesity, chronic inflammation, cardiovascular and nervous disorders (Zhang *et al*., 2011; Satyendra *et al*., 2012). It has a strong antioxidant property due to its chelating property and free radical scavenging ability. Quercetin can prevent oxidative damage to DNA and also to cell membranes thus stabilizing lipid membranes by preventing lipid peroxidation by free radicals (Nirankari *et al*., 2016). Similarly, catechin is also a phenolic compound that is widely distributed in tea and beverages. It has been used since ancient times for promoting human health. Both clinical and epidemiological studies have found catechin to be effective as anti-oxidant, anti-inflammatory, anti-bacterial, anti-viral, immuno-stimulatory, cardio-protective, diuretic, anti-carcinogenic, anti-mutagenic, anti-tumorigenic, anti-allergic, anti-diabetic and anti-hypertensive (Uzun *et al*., 2010; Bharrhan *et al*., 2011; Chen *et al*., 2016; Wongmekiat *et al*., 2018; Cosarca *et al*., 2019). The versatile structures of catechins endow them with strong antioxidant activity. Because catechins have ortho dihydroxy or ortho trihydroxy in their structure, they could form half quinone free radicals which upon acting on free

radicals lead to their inactivation (Grzesik *et al*., 2018). So, in view of wide spread use of MZ globally and occurrence of arsenic toxicity in many parts of world, the present investigation was undertaken to evaluate the multifaceted adverse effects of MZ or As and their combination and attenuation of combined toxicity by quercetin and catechin by analyzing certain parameters, antioxidant enzymes of kidney along with histopathology.

MZ administration significantly increased plasma CR, UA and urea as compared to control, indicating MZ-induced nephrotoxicity as reported earlier (Sakr *et al.*, 2013; Zhang *et al*., 2022). These biomarkers are diagnostic indicators of kidney damage as their elevated levels indicate either failure of renal tissue to excrete nitrogenous waste or increased breakdown of dietary protein or tissue (Rizwan *et al*., 2014; Mahajan *et al*., 2018b; Zhang *et al*., 2022). A dose dependent increase in CR, UA and urea was seen in As administered rats, when compared to control. Similar reports were evidenced from previous studies (Naqshbandi *et al*., 2013; Rizwan *et al*., 2014; Shahid *et al*., 2014; Dutta *et al*.,2018; Turk *et al*., 2019). These studies validated elevated serum CR and urea levels in arsenic induced toxicity in experimental animals including Wistar rat and mice. A study by Prakash *et al*., 2018 revealed rise in serum CR, urea, UA and GGT levels after oral administration of arsenic in rats. Increased CR levels are mainly associated with cardiac and skeletal muscle degeneration. Impaired renal function in present study may be due to oxidative damage induced by either MZ or arsenic in mammals (Rizwan *et al*., 2014; Shahid *et al*., 2014; Mahajan *et al*., 2018b; Turk *et al*., 2019; Zhang *et al*., 2022). Co-exposure of the toxicants further intensified the CR, UA, and urea levels than alone treated toxicants which undoubtedly indicate more severe renal dysfunctions which may be due to oxidative insult. The findings were supported by reports of Baba *et al*., 2016; Mahajan *et al*., 2018b; Singh *et al*., 2020 and Wan *et al*., 2021 which exposed the Wistar rats to combination of metals or pesticides or their combination. The underlying mechanism by which MZ or As causes nephrotoxicity is not well understood. However, there are individual reports of both of them suggesting that ROS are important mediators of MZ or As nephropathy (Rizwan *et al*., 2014; Dutta *et al*., 2018; Prakash *et al*., 2018; Turk *et al*., 2019; Zhang *et al*., 2022). MZ has been found to produce toxicological manifestations in different cells by formation of ROS which is due to its chemical structure, since it contains transitional metals known to induce ROS production

through Fenton's reaction (Calviello *et al*., 2006; Runkle *et al*., 2013). In the same manner metabolism of As in the cells leads to the generation of ROS like superoxide anion (O_2), hydrogen peroxide (H_2O_2), singlet oxygen (O) hydroxyl radical (OH), and peroxyl radicals, which are responsible for numerous toxic effects (Rao and Balachandran, 2002; Ratnaike, 2003; Hu *et al*., 2020) including oxidative insult by disturbance of antioxidant response and damage to major macromolecules including DNA, proteins and lipids (Rizwan *et al*., 2014). The antioxidant defense system is the main cellular defense mechanism that the cell has against any toxicant. Previous studies have found that both enzymatic and non-enzymatic defense response help to protect the cells by removing and clearing ROS. Among enzymatic components, a major cellular defense against ROS is delivered by SOD and CAT, which work together in converting superoxide radicals first to H_2O_2 and finally to molecular oxygen and water. Another enzyme viz. GPx employs thiol reducing power of GSH to reduce oxidized proteins and lipid targets of ROS. GSH, an abundant non-protein thiols found in cells, plays a crucial role against oxidative stress by minimizing the cellular ROS levels (Dutta *et al*., 2018; Turk *et al*., 2019). In present study, the reduction in antioxidant components like GSH, SOD, CAT, GPx and GR after individual treatment with MZ or As might be either due to increased concentration of free radicals or decreased concentration of reduced GSH, a substrate for different antioxidant enzymes like GPx, and GR. Similar reports were provided by Naqshbandi *et al*., 2013; Sakr *et al*., 2013; Rizwan *et al*., 2014; Dutta *et al*., 2018; Prakash *et al*., 2018; Turk *et al*., 2019; Jin *et al*., 2020; Liu *et al*., 2020; Wan *et al*., 2021; Zhang *et al*., 2022. Both MZ and As has been shown to enhance MDA and AOPP, both being indicators of tissue injury and to deplete TTH, TAS and AE. The decline in TTH content of the renal tissue caused by treatment with MZ or As can be explained by the fact that MZ has affinity for thiols and also thiols are quite reactive towards metal ions and are easily oxidizable by ROS. AE is an important enzyme that is involved in catalysis of free radicals and its activity gets impeded in presence of any toxicant (Sharma *et al*., 2021b) and present study also revealed the decline in AE levels in MZ or As treated rats, which was more pronounced in combination groups. Diminished AE, TTH and TAS content has been manifested upon exposure of tissues to pesticides, heavy metals or drugs (Flora *et al*., 2008; Sharma *et al*., 2021b). The increased free radicals generated during metabolism of MZ and As suppressed

cellular free radicals scavenging capacity as evident from reduced TTH and TAS which might be responsible for free radicals induced lipid and protein oxidation in renal tissue. The present results revealed that co-administration of MZ and As caused damage to renal tissue mediated by ROS which was apparent by further reduction in the TAS, TTH, GSH, SOD, CAT, GPx, GR and AE contents accompanied with increased MDA and AOPP. Present results were further validated by concurrent exposure of As or other elements with pesticides (Baba *et al*., 2016; Mahajan *et al*., 2018b; Kayode *et al*., 2019; Singh *et al*., 2020). The membrane polyunsaturated fatty acids (PUFA) are extremely susceptible to free radical mediated oxidative damage. Interaction of ROS with PUFA initiates the self-propagating lipid peroxidation cascade responsible for impaired membrane function and integrity (Rizwan *et al*., 2014; Dutta *et al*., 2018; Turk *et al*., 2019; Wan *et al*., 2021). Observations revealed that co-treatment with MZ and As led to surfeit lipid peroxidation resulting in overwhelmingly high levels of MDA in the renal tissues (Mahajan *et al*., 2018b; Thangapandiyan *et al*., 2019; Turk *et al*., 2019). Thus, exposure of MZ or As or their combination induced lipid peroxidation and hence lead to the degradation of phospholipids and ultimately results in the renal cellular deterioration. Present study was validated by histopathological assessment and it was found administration of either toxicant caused histopathological changes like hemorrhages in renal interstitium, congestion, necrosis, degeneration in renal tubular epithelium with presence of renal casts. Similar results were found by Prakash *et al*., 2018; Dutta *et al*., 2018; Thangapandiyan *et al*., 2019; Turk *et al*., 2019. Co-exposure to MZ and As produced more pronounced changes in renal tissue as compared to individual toxicants and additionally oedema, glomerular atrophy, calcification, hyalinization of glomerular tuft and focal infiltration of lymphocytes was observed. Current results were in accordance to nephrotoxicity produced by multiple exposure of pesticides with metals (Baba *et al*., 2016; Mahajan *et al*., 2018b; Singh *et al*., 2020). When quercetin was administrated to combined toxicant treated rats renal biomarkers like UA, urea was restored along with CAT, GR, AE and MDA as compared to co-exposed group. While as, catechin administered in the multiple toxicant exposed groups improved CR, urea and further alteration in levels of TAS, GPx, GR, AE and AOPP was prevented. The protection by quercetin and catechin against combined toxicity can be attributed to their intrinsic biochemical and natural antioxidant

properties, possibility by chelating and free radical scavenging property of quercetin and catechin (Nirankari *et al*., 2016; Grzesik *et al*., 2018). The protective property of quercetin and catechin was also revealed by histopathology as only mild and moderate changes were seen in catechin and quercetin treated animals, respectively. In a study by Wongmekiat *et al*., 2018, it was clearly demonstrated that catechin proved effective against mitochondrial dysfunction with subsequent oxidative stress and inflammation in cadmium induced toxicity in male Wistar rat. Similar studies which used different antioxidants and dietary substances to mitigate metal, pesticide or drug induced toxicity include Naqshbandi *et al*., 2013; Sakr., *et al* 2013; Dutta *et al*., 2018; Thangapandiyan *et al*., 2019; Turk *et al*., 2019; Jin *et al*., 2020; Liu *et al*., 2020 and Ramadan *et al*., 2022. To conclude, the present investigation clearly demonstrated that MZ and As produces nephrotoxicity and caused profound damage to renal proximal tubules and glomerulus. However, damage was severe in toxicant co-administered groups. In contrast, quercetin and catechin appear to improve membrane integrity and functions of renal tissue. Catechin has accelerated the repair or regeneration of injured renal tissue more efficiently as evident by plasma renal, antioxidant biomarkers and histopathological studies.

***5.5. Alterations in antioxidant and histology of testes following subacute exposure of mancozeb (MZ) and arsenic (As) alone and in-combination and its amelioration with quercetin and catechin*:** Exponential increment in the production, use and disposition of chemicals have a profound environmental impact and creates unforeseen hazards to well being of man (Joshi *et al*., 2005). In particular, it is worth noting in induction of sterility, decreased sperm count and increased frequency of sperm with aberrant head morphological changes (Calviello *et al*., 2006). Similarly, exposure to arsenic has been reported to increase risk of various disorders including cardiovascular dysfunctions, diabetes mellitus, neurotoxicity, nephrotoxicity and hepatotoxicity (Singh *et al*., 2011). The potential of arsenic to act as a carcinogen is of serious concern as it increases risk of various cancers such as those of skin, kidney, urinary bladder, lungs and prostate along with type 2 diabetes and depression (Mazumder, 2008; Sodhi *et al*., 2019). With reference to its effect on male reproductive system, recent studies have clearly confirmed its deleterious effects on testicular tissue (Renu *et al*., 2018; Fouad *et al*., 2018; Mehrzadi *et al*., 2018; Zeng *et al*., 2019; Nava-Rivera *et al*., 2021). As per the recent data and scientific literature is

available both MZ (Girish *et al*., 2018; Sardoo *et al*., 2018) and As (Fouad *et al*., 2018; Mehrzadi *et al*., 2018; Zeng *et al*., 2019; Erkan *et al*., 2021) are thought to cause testicular toxicity by involvement of ROS and inactivation of sulfhydryl group. Oxidative stress, the imbalance between generation of free radicals and antioxidant capacity is considered as the potential underlying mechanism of toxicity of pesticides or metals in different diseases, including male reproductive toxicity and male infertility. Male reproductive system is highly susceptible to oxidative insult because the testicular tissue has high rate of cell division, high mitochondrial oxygen demand, high concentrations of polyunsaturated fatty acids with low level of oxygen partial pressure probably due to partially weakened vessels (Mehrzadi *et al*., 2018; Sardoo *et al*., 2018; Saddein *et al*., 2019). In the present study, treatment with either MZ or As decreased the activity of antioxidant enzymes viz. SOD, CAT, GPx, GR, AE alongside a decline in TTH and TAS with increased peroxidation of lipids and proteins, as compared to control animals. SOD, CAT and GPx are abundantly antioxidant enzymes which form the first line of defense against ROS. Therefore, curtailment in their activity indicates the failure of the primary antioxidant system to act against generation of free radicals. Present results were consistent with Girish *et al*., 2018 and it was revealed that MZ significantly decreased GSH, SOD, CAT and increased LPO in testes Wistar rat. Similar findings were reported by Kwon *et al*., 2018; Elsharkawy *et al*., 2019; Mahdi *et al*., 2019 and Saddein *et al*., 2019. As per the reports by Sardoo *et al*., 2018, MZ caused dose dependent decrease in sperm motility and count with increase in lipid peroxidation, reduced antioxidant enzymes activities like GSH content. Studies that confirmed arsenic as a suppressor of antioxidant defense system include those of Fouad *et al*., 2018; Mehrzadi *et al*., 2018; Zeng *et al*., 2019; Concessao *et al*., 2020; Erkan *et al*., 2021 and Sharma *et al*., 2021a. The decline in the levels of TTH may be due the disruption of the protein's thiol groups by both MZ (Calviello *et al*., 2006) and As (Sodhi *et al*., 2019). Co-administration of MZ and As further suppressed the antioxidant defense response which was depicted as more reduction in antioxidant biomarkers like SOD, CAT, GPx, GR, AE and marked elevation in MDA and AOPP levels than the individual toxicant group. Current results were in agreement with studies of concurrent exposure of pesticides or metals or their combination (Mahajan *et al*., 2018a; Ramos-Treviño *et al*., 2018; Kayode *et al*., 2019; Lovaković *et al*., 2020; Liu *et al*., 2021; Raeeszadeh *et al*., 2021; Wu *et al*., 2021)

which also represented a significant curtailment in TTH, SOD, CAT, GPx, GR, and GST activities along with increased AOPP and MDA levels. The marked increase in lipid peroxidation in the combined toxicant groups may result from the inactivation of antioxidant enzymes and reduction in the content of antioxidant GSH, which can be the causative factor for damage to cellular membrane and cytoplasmic membrane structures. The damage and induction of oxidative injury in testicular tissue was further confirmed by histopathological changes like congestion, hemorrhages, degeneration, necrosis, increased interstitial, hyalinization, reduced number of seminiferous tubules, and sub-capsular oedema, when compared to control rats. The results are consistent with reports of Kackar *et al*., 1997a; Girish *et al*., 2018; Sardoo *et al*., 2018; Elsharkawy *et al*., 2019; Mahdi *et al*., 2019; Saddein *et al*., 2019; Zeng *et al*., 2019; Concessao *et al*., 2020; Erkan *et al*., 2021; Sharma *et al*., 2021a. In a study by Kackar *et al*., 1997a, it was depicted that chronic oral administration of orally MZ on gonads of male rats caused noticeable increase in testes weight and decrease in epididymis weight. Changes like degeneration in seminiferous tubules and epididymal tubules were observed with loss of sperms, corroborated with decrease in gonadal acid phosphatase (ACP), succinic dehydrogenase (SDH) and increase in alkaline phosphatase (ALP), LDH and elevated serum cholesterol. In combination groups, histopathological alterations were more pronounced and are consistent as per investigations on combined toxicity effects by Mahajan *et al*., 2018a; Lovaković *et al*., 2020; Liu *et al*., 2021; Raeeszadeh *et al*., 2021; Ramos-Treviño *et al*.,2018; Wu *et al*., 2021.

Since oxidative stress and DNA damage are considered as major factors in male infertility and for achieving a healthy pregnancy, there is a definite rationale behind the use of antioxidants and development of antioxidant therapy for infertility (Mehrzadi *et al*., 2018; Sardoo *et al*., 2018). So, in present study quercetin and catechin were used to attenuate the combined toxicity effect of MZ and As. Administration of quercetin in the multiple exposure group refined SOD, CAT, GPx, AE and AOPP and catechin improved SOD, GPx, GR, AE, TAS and AOPP as compared to combined toxicity induced group. Additionally, histopathology observations supported catechin as more protective for testicular tissue than quercetin as only mild changes were manifested in catechin treated group. Studies in support of quercetin as protective agent for various toxicities include those of Uzun *et al*., 2010;

Bu *et al*., 2011; Zhang *et al*., 2011; Satyendra *et al*., 2012; Baltaci *et al*., 2016; Nirankari *et al*., 2016; Izak-Shirian *et al*., 2022. Meanwhile, for catechin, reports of Uzun *et al*., 2010; Bharrhan *et al*., 2011; Chen *et al*., 2016; Grzesik *et al*., 2018; Wongmekiat *et al*., 2018; Cosarca *et al*., 2019; Cheng *et al*., 2020; Aparna *et al*., 2021; Arslan *et al*., 2022; Josiah *et al*., 2022 supported its protective role for alleviating various toxic effects. Reports of Akinmoladun *et al*., 2020 found ameliorative effects of catechin, quercetin, and taxifolin against rotenone-induced toxicity and revealed improvement in testicular weight, splenic weight and oxidative damage in rats. As per Kayode *et al*., 2019 supplementation with catechin attenuated combined toxicity effects of As and deltamethrin and improved the parameters like GSH, TAS, MDA, AOPP, SOD, CAT and GPx in testis. Other clinical studies that investigated possible beneficial effects of other antioxidants in reproductive toxicity and male infertility cover those of Fouad *et al*., 2018; Girish *et al*., 2018; Hashem *et al*., 2018; Mehrzadi *et al*., 2018; Sardoo *et al*., 2018; Mahdi *et al*., 2019; Saddein *et al*., 2019; Concessao *et al*., 2020; Raeeszadeh *et al*., 2021 Sharma *et al*., 2021a. The study by Girish *et al*., 2018 depicted that forskolin showed protective effect against MZ-induced reproductive toxicity in adult male Wistar rats and improved the decreased GSH, SOD, CAT levels and increased LPO activity in testes and epididymis. Another study by Sardoo *et al*., 2018 reported protective role of N-acetylcysteine on sub-chronic effects of MZ on testis of male albino mice administered orally at different doses. N-acetylcysteine showed ameliorative role in reducing oxidative stress against lipid peroxidation, reduced antioxidant enzymes activities like GSH content. Additionally, Aziz *et al.,* 2018 found ameliorative potential of quercetin and L-carnitine against atrazine-induced reproductive toxicity in male Albino rats. Co-administration with low dose of quercetin and high doses of L-carnitine counteracted the negative effects of atrazine on serum oxidative stress indicators. Moreover, the reports of Sharma *et al*., 2021a suggested the effects of coenzyme Q_{10} and vitamin E on As induced sub-acute testicular toxicity and DNA damage in Swiss Albino mice and found that GSH, TTH, SOD and total protein levels were improved by coenzyme Q_{10} and vitamin E compared to arsenic treated group. In nutshell, the present investigation emphasis on the individual and combined toxicity effects of MZ and As on testicular tissue and antioxidant suppressing ability of toxins as a key mechanism for the toxic effects. However, the co-exposure of toxicants

increased ROS generation and subsequent testicular damage. So, the study mainly highlights that co-exposure of As and MZ pose an ecological risk and might have a synergistic effect on testicular toxicity. Meanwhile, treatment with quercetin and catechin mitigates the harmful combined effects of MZ and As on the testes by modifying oxidative stress and expression of ROS modulating genes. Although, both quercetin and catechin proved to be effective to alleviate testicular toxicity, but catechin was more effective in reducing the load of free radicals and associated damage as manifested from histopathological assessment.

Chapter-VI

Summary and Conclusions

Chapter 6

Summary & Conclusions

The widespread use of MZ globally and presence of other environmental contaminants has resulted in deleterious and multidimensional health effects both in human and animal population. Co-exposure to different environmental contaminants may cause synergistic or additive toxicity. In the present study MZ and arsenic (As) alone and in-combination were administered to Wistar rats to determine the type of toxic interaction after their concurrent exposure. Also, ameliorative potential of quercetin and catechin was evaluated against these toxicants when both the toxic agents were administered in combination. Most of exogenous contaminants produce deleterious effects either by compromising antioxidant mechanisms or increasing the production of reactive intermediate metabolites during the biotransformation process. MZ has been found to produce toxicological manifestations in different tissues by formation of ROS which is due to its chemical structure, since it contains transitional metals known to induce ROS production through Fenton reaction. Similarly, arsenic especially its trivalent form has higher affinity for thiol groups and inhibits enzymes like GPx, GR, enzymes, etc. And as per scientific data and literature, majority of disorders in mammalian tissue occurs due to imbalance in oxidant and antioxidant ratio. Excessive accumulation of these radicals in blood or tissues induces oxidative damage to erythrocytes and important visceral organs *viz.* liver, kidney, brain and testes. The salient findings of the study conducted are listed as under:

a. Oral administration of MZ or As (100ppb) alone and in-combination significantly ($P<0.05$) elevated the levels of ALT, AST, GGT, Urea, CR, UA in plasma but decreased the levels of TP, GLO, as compared to control animals, however, these changes were more severe in combination groups. In erythrocytes the levels of TAS, TTH, GSH, GR, AE and AChE were significantly decreased and NO level were significantly increased in MZ and As (100ppb) alone exposed groups. While as, in-combination groups, there was a significant decline in TAS, TTH, GSH, SOD, CAT, GPx [MZ + As (50ppb and 100ppb], GR, AE, AChE and significant increment in MDA, AOPP [MZ + As (50 ppb &100 ppb)] and NO levels as

compared to control. Repeated administration of quercetin (50 mg/kg, PO) along with combined toxicants restored plasma TAS, GSH, CAT, GR, AE, AChE and AOPP and NO as compared to co-exposed group. Whereas, restoration of GSH, AE, AChE along with MDA and AOPP were by repeated administration of catechin (50 mg/kg, PO) in the toxicant interactive group.

b. In brain tissue, a significant decline was seen in TAS, CAT, GPx, AChE and a significant elevation was seen in MDA and NO levels in MZ and As alone (50ppb &100ppb) treated rats. Co-administration of MZ and As caused a significant curtailment in TAS, TTH, SOD, CAT, GPx, GR [MZ + As (50ppb and 100ppb), AE, AChE and significant increment in MDA, AOPP (MZ + As (50ppb and 100ppb) and NO levels as compared to control. Histopathology of brain tissue (cerebrum and cerebellum) witnessed changes like necrosis, spongiosis, gliosis, perivascular edema, hemorrhage and neuronal degeneration. Combination groups showed the more severe changes, while as mild and moderate changes were seen in catechin and quercetin co-treated animals, respectively. Simultaneous administration of quercetin to the co-toxicant exposed group improved the declined activity of CAT, AChE and suppressed the elevated levels of MDA, AOPP and NO as compared to toxicant interactive group. On the other hand, catechin restored the SOD, GR, AE, AChE, TAS, TTH contents along with AOPP and NO levels.

c. In liver, the levels of CAT, SOD, GP_X, GR, AE, were significantly ($P<0.05$) decreased in MZ treated rats, as compared to control and in As (100ppb) treated animals, additionally, the levels of TAS were decreased and MDA and AOPP were significantly increased. On the other hand, co-exposure of MZ and As caused significant deduction in TAS, TTH, SOD, CAT, GPx, GR [MZ+As (50ppb and100ppb)], AE and a significant hike in MDA and AOPP [MZ+As (100ppb)] levels as compared to control. Repeated administration of quercetin restored the plasma hepatic biomarkers *viz.* AST, LDH, GGT, TP, GLO and activities of the antioxidant defense system *viz.* SOD, CAT, TAS, while suppressing the MDA and AOPP levels in the liver tissue as compared to toxicant combined group (group VIII). Meanwhile, catechin improved the enzyme activity including AST, GGT, LDH, ALP, TP, GLO and antioxidant biomarkers such as AE, TTH, MDA and AOPP. Histopathology of liver revealed changes like congestion, hepato-cellular

degeneration, hemorrhages, necrosis and dilation of sinusoids. More severe changes were seen in combination groups and mild-moderate in ameliorative groups.

d. In kidney, there was a significant fall in TAS, SOD, CAT, GPx, AE and a significant hike was seen in MDA levels in MZ treated rats. In As treated animals, a dose dependent alteration was noticed in antioxidant biomarker and additionally, we found significant rise in AOPP levels in As (50ppb and100ppb) treated animals. However, multiple exposure of MZ and As inferred a significant reduction in antioxidant biomarkers viz., TAS (MZ+As (50ppb and100ppb)], TTH (MZ +As (100ppb), SOD, CAT, GPx, GR [MZ+As (50ppband100ppb)], AE and a significant hike in MDA and AOPP levels. Histopathological assessment of renal tissue revealed changes like congestion, hemorrhage, edema, renal casts, atrophy, tubular degeneration, necrosis & focal infiltration of lymphocytes. Meanwhile catechin and quercetin alleviated the damage as compared to toxicant interactive group. In ameliorative group *viz.* quercetin administration to combined toxicant group, serum biomarkers like UA, urea was restored along with CAT, GR, AE and MDA as compared to group VIII. While as catechin improved CR, urea and TAS, GPx, GR, AE and AOPP levels as compared to group VIII.

e. In testes, the levels of TAS, CAT, GPx, GR, AE, were significantly ($P<0.05$) decreased in MZ and As alone treated animals as compared to control. Co-administration of MZ and As revealed a sharp decline in TAS, TTH [MZ+As (50 ppb & 100 ppb)], SOD, CAT, GPx, GR (MZ+As @ 50 ppb & 100 ppb), AE and a significant elevation was seen in MDA and AOPP levels as compared to control. Histopathology observations manifested alterations like congestion, degeneration, necrosis, increased intercellular spaces and hyalinization of seminiferous tubules. Also, the histopathological alterations were more severe in combination groups as compared to either toxicant exposed group and less severe in ameliorative groups. Administration of quercetin refined SOD, CAT, GPx, AE and AOPP, while as catechin restored levels of SOD, GPx, GR, AE, TAS and AOPP in testes as compared to combined toxicity induced group (MZ + As).

In the nut shell, observations of present study indicated that repeated co-exposure of MZ and As (100ppb) produced significant alterations in plasma biomarkers, oxidative parameters and histoarchitecture of brain, liver, kidney and testes in Wistar rats. These

changes were more severe after co-exposure as compared to those produced by exposure of either toxicant. Treatment with quercetin and catechin mitigated the harmful combined effects of MZ and As on different tissues probably by modifying the cellular oxidative insult. Quercetin proved to be effective to alleviate the toxicants induced toxicity in erythrocytes and liver, while as, catechin was more effective in reducing the toxicant associated damage in brain, kidney and testes as manifested from plasma and antioxidant biomarkers and histopathological assessment.

References

References

Adjrah, Y., Karou, S.D., Agbonon, A., Eklu-Gadegbeku, K., de Souza, C., and Gbeassor, M. 2013. Effect of mancozeb-treated lettuce (*Lactuca sativa*) on Wistar rat liver. *Ethiopian Journal of Environmental Studies and Management*, **6**(1):67-73.

Aebi, H.E. 1983. Catalase, In: Bergmeyer HU (EDS). *Methods of Enzymatic Analysis*, New York, Academic Press. pp 276-286.

Afolabi, O.K., Ugbaja, R.N., Arinola, A., Olorunnisola, O.S., and Adeleke, G.E. 2016. Catechin Protects Against Dyslipidemia and Nephro-Hepatototoxicity Induced in Rats Exposed to Arsenic. *Jordan Journal of Biological Sciences*, **9**(3).

Ahmed, M.E., Khan, M.M., Javed, H., Vaibhav, K., Khan, A., Tabassum, R., and Islam, F. 2013. Amelioration of cognitive impairment and neurodegeneration by catechin hydrate in rat model of streptozotocin-induced experimental dementia of Alzheimer's type. *Neurochemistry International*, **62**(4):492-501.

Akinmoladun, A.C., Olaniyan, O.O., Famusiwa, C. D., Josiah, S.S., and Olaleye, M.T. 2020. Ameliorative effect of quercetin, catechin, and taxifolin on rotenone-induced testicular and splenic weight gain and oxidative stress in rats. *Journal of Basic and Clinical Physiology and Pharmacology*, **31**(3) dio: 10.1515/jbcpp-2018-0230.

Aparna, K., Madhuri, D., Lakshman, M., Anilkumar, B., Swathi, B., and Ravikumar, Y. 2021. Study of ameliorative effect of quercetin on methotrexate induced toxicity on body weights and haematological parameters in albino Wistar rats. *The Pharma Innovation*, **10**(7):1434-1436.

Arslan, A.S., Seven, I., Mutlu, S.I., Arkali, G., Birben, N., and Seven, P.T. 2022. Potential ameliorative effect of dietary quercetin against lead-induced oxidative stress, biochemical changes, and apoptosis in laying Japanese quails. *Ecotoxicology and Environmental Safety*, **231**:113200.

Asparch, Y., Svartz, G., and Coll, C.P. 2020. Toxicity characterization and environmental risk assessment of mancozeb on the South American common toad *Rhinella arenarum*. *Environmental Science and Pollution Research*, **27**(3): 3034-3042.

Axelstad, M., Boberg, J., Nellemann, C., Kiersgaard, M., Jacobsen, P.R., Christiansen, S., Hougaard, H.S., and Hass, U. 2011. Exposure to the widely used fungicide mancozeb causes thyroid harmone disruption in rat dams but no behavioral effects in the offspring. *Toxicology Sciences,* **120**(2):439-446.

Aziz, R.L.A., Abdel-Wahab, A., El-Ela, F.I.A., Hassan, N.E.H.Y., El-Nahass, E.S., Ibrahim, M.A., and Khalil, A.T.A. 2018. Dose-dependent ameliorative effects of quercetin and l-Carnitine against atrazine-induced reproductive toxicity in adult male Albino rats. *Biomedicine & Pharmacotherapy*, **102**:855-864.

Baba, N., Raina, R., Verma, P., and Sultana, M. 2016. Free radical-induced nephrotoxicity following repeated oral exposure to chlorpyrifos alone and in conjunction with fluoride in rats. *Turkish Journal of Medical Sciences*, **46**(2):512-517.

Bahrami, A., Sathyapalan, T., Moallem, S.A., and Sahebkar, A. 2020. Counteracting arsenic toxicity: Curcumin to the rescue. *Journal of Hazardous Materials*, **400**:123160.

Bailey, D.C., Todt, C.E., Orfield, S.E., Denney, R.D., Snapp, I.B., Negga, R., and Fitsanakis, V.A. 2016. *Caenorhabditis elegans* chronically exposed to a Mn/Zn ethylene-bis-dithiocarbamate fungicide show mitochondrial Complex I inhibition and increased reactive oxygen species. *Neurotoxicology*, **56**:170-179.

Balaji, B., Rajendar, B., and Ramanathan, M. 2014. Quercetin protected isolated human erythrocytes against mancozeb-induced oxidative stress. *Toxicology and Industrial Health*, **30**(6):561-569.

Baligar, P.K and Kaliwal, B.B. 2001. Introduction of gonadal toxicity to female rats after chronic exposure to mancozeb. *Industrial Health*, **39**:235-243.

Baltaci, B.B., Uygur, R., Caglar, V., Aktas, C., Aydin, M., and Ozen, O.A. 2016. Protective effects of quercetin against arsenic induced testicular damage in rats. *Andrologia*, **48**(10):1202-1213.

Behari, J.R. and Prakash, R. 2006. Determination of total arsenic content in water by atomic absorption spectroscopy (AAS) using vapour generation assembly (VGA). *Chemosphere*, **63**(1):17-21.

Belpoggi, F., Soffritti, M., Guarino. M., Lambertini, L., Cevolani, D., and Maltoni, C. 2002. Results of long-term experimental studies on the carcinogenicity of

ethylene-bis-dithiocarbamate (Mancozeb) in rats. *Annals New York Academy of Sciences,* **982**:123-136.

Beutler, E. 1975. Red cell metabolism: a manual of biochemical methods 2nd Edn, pp. 67-69, Grune Stratton, New York.

Bharrhan, S., Koul, A., Chopra, K., and Rishi, P. 2011. Catechin suppresses an array of signalling molecules and modulates alcohol-induced endotoxin mediated liver injury in a rat model. *PLoS One*, **6**(6): e20635.

Bianchi, S., Nottola, S.A., Torge, D., Palmerini, M.G., Necozione, S., and Macchiarelli, G. 2020. Association between female reproductive health and mancozeb: systemic review of experimental models. *International Journal of Environmental Research and Public Health*, **17 (7)**:2580.

Bjorklund, G., Aaseth, J., Chirumbolo, S., Urbina, M.A., and Uddin, R. 2017. Effects of arsenic toxicity beyond epigenetic modifications. *Environmental Geochemistry and Health*, **40** (3):955-965.

Brinkel, J., Khan, M.H., and Kraemer. 2009. A systemic review of arsenic exposure and its social and mental health effects with special reference to Bangladesh. *International Journal of Environmental Research and Public Health*, **6**(5):1609-1619.

Brody, A.H., Chou, E., Gray, J.M., Pokyrwka, N.J., and Susman, K.M.R. 2013. Mancozeb-induced behavioural deficits precede structural neural degeneration. *Neuro Toxicology*, **34**:74-81.

Bu, T., Mi, Y., Zeng, W., and Zhang, C. 2011. Protective effect of quercetin on cadmium-induced oxidative toxicity on germ cells in the brain of mice exposed to arsenic at environmentally relevant levels. *Journal of Occupational Health*, **47**:445-449.

Burlina, A., Michielin, E., and Galzigna, L. 1977. Characteristics and behaviour of arylesterase in human serum and liver. *European Journal of Clinical Investigation*. **7**:17-20

Calviello, G., Piccioni, E., Boninsegna, A., Tedesco, B., Maggiano, N., Serini, S., Wolf, F.I., and Palozza, P. 2006. DNA damage and apoptosis induction by the pesticide mancozeb in rat cells: involvement of the oxidative mechanism. *Toxicology and Applied Pharmacology*, **211**:87-96.

Carlberg, I., and Mannervik B. 1975. Glutathione reductase. *Methods in Enzymology*, **113**:484–490.

Cebeci, E., Alibaz-Oner, F., Usta, M., Yurdakul, S., and Erguney, M. 2012. Evaluation of oxidative stress, the activities of paraoxonase and arylesterase in patients with subclinical hypothyroidism. *Journal of Investigative Medicine*, **60**(1):23-28.

Chandravanshi, L.P., Gupta, R. and Shukla, R.K. 2018. Arsenic-induced neurotoxicity by dysfunctioning cholinergic and dopaminergic system in brain of developing rats. *Biological Trace Element Research*, **189**(1):118-133.

Chen, S.K., Edwards, C.A., and Subler, S. 2001. Effects of the fungicides benomyl, captan and chlorothalonil on soil microbial activity and nitrogen dynamics in laboratory incubations. *Soil Biology and Biochemistry*, **33**(14):1971-1980.

Chen, X.Q., Hu, T., Han, Y., Huang, W., Yuan, H.B., Zhang, Y.T., and Jiang, Y.W. 2016. Preventive effects of catechins on cardiovascular disease. *Molecules*, **21**(12):1759.

Cheng, Z., Zhang, Z., Han, Y., Wang, J., Wang, Y., Chen, X., and Wu, Z. 2020. A review on anti-cancer effect of green tea catechins. *Journal of Functional Foods*, **74**:104172.

Cheruku, S.P., Ramalingayya, G.V., Chamallamudi, M.R., Biswas, S., Nandakumar, K., Nampoothiri, M., and Kumar, N. 2018. Catechin ameliorates doxorubicin-induced neuronal cytotoxicity in in vitro and episodic memory deficit in in vivo in Wistar rats. *Cytotechnology*, **70**(1):245-259.

Cobley, J.N., Fiorello, M.L., and Bailey, D.M. 2018. 13 reasons why the brain is susceptible to oxidative stress. *Redox Biology*, **15**:490-503.

Concessao, P., Bairy, L.K., and Raghavendra, A.P. 2020. Effect of aqueous seed extract of *Mucuna pruriens* on arsenic-induced testicular toxicity in mice. *Asian Pacific Journal of Reproduction*, **9**(2):77.

Corsini, E., Viviani, B., Birindelli, S., Gilardi, F., Torri, A., Codecà, I., and Colosio, C. 2006. Molecular mechanisms underlying mancozeb-induced inhibition of TNF-alpha production. *Toxicology and Applied Pharmacology*, **212**(2):89-98.

Coryell, M., McAlpine, M., Pinkham, N.V., McDermott, T.R., and Walk, S.T. 2018. The gut microbiome is required for full protection against arsenic toxicity in mouse models. *Nature Communications*, **9** (1):1-9.

Coșarcă, S., Tanase, C., and Muntean, D.L. 2019. Therapeutic aspects of catechin and its derivatives–an update. *Acta Biologica Marisiensis*, **2**(1):21-29.

Costa-Silva, D.G., Lopes, A.R., Martins, I.K., Leandro, L.P., Nunes, M.E.M., de Carvalho, N.R., and Franco, J.L. 2018. Mancozeb exposure results in manganese accumulation and Nrf2-related antioxidant responses in the brain of common carp *Cyprinus carpio*. *Environmental Science and Pollution Research*, **25**(16):15529-15540.

Cycoń, M., Piotrowska-Seget, Z., Kaczyńska, A., andKozdrój, J. 2006. Microbiological characteristics of a sandy loam soil exposed to tebuconazole and λ-cyhalothrin under laboratory conditions. *Ecotoxicology*, **15**(8):639-646.

da Silva Gündel, S., Dos Reis, T.R., Copetti, P.M., Favarin, F.R., Sagrillo, M.R., da Silva, A.S., and Ourique, A.F. 2019. Evaluation of cytotoxicity, genotoxicity and ecotoxicity of nanoemulsions containing Mancozeb and Eugenol. *Ecotoxicology and Environmental Safety*, **169**: 207-215.

Dacie, J.V., and Lewis, S.M. (Eds). 1968. *Practical Haematology*. J. & A, Churchill, U.K. p 37.

Dallagnol, J.C., Pezzini, M.F., Uribe, M.S., and Joveleviths, D. 2021. Systemic effects of the pesticide mancozeb- A literature review. *European Review for Medical and Pharmacological Sciences*, **25**:4113-4120.

Dawson, T.M., and Dawson, V.L. 2018. Nitric oxide signaling in neurodegeneration and cell death. *Advances in Pharmacology*, **82**:57-83.

Dkhil, M.A., Abdel Moneim, A.E., Bauomy, A.A., Khalil, M., Al-Shaebi, E.M., and Al-Quraishy, S. 2020. Chlorogenic acid prevents hepatotoxicity in arsenic-treated mice: role of oxidative stress and apoptosis. *Molecular Biology Reports*, **47**(2):1161-1171.

Domico, L.M., Cooper, K.R., Bernard, L.P., and Zeevalk, G.D. 2007. Reactive oxygen species generation by the ethylene-bis-dithiocarbamate (EBDC) fungicide mancozeb and its contribution to neuronal toxicity in mesencephalic cells. *Neuro Toxicology*, **28**:1079-1091.

Drury, R.A.B WEA. 1980. Carleton's Histological Technique. Open Library.

Dutta, S., Saha, S., Mahalanobish, S., Sadhukhan, P., and Sil, P.C. 2018. Melatonin attenuates arsenic induced nephropathy via the regulation of oxidative stress

and inflammatory signalling cascades in mice. *Food and Chemical Toxicology*, **118**:303-316.

Edward, I.R., Ferry, D.G., and Temple, W.A. and Kamrin, M.A. (Eds.). 1997. *Thiodithiocarbamates. In: Pesticide Profiles: Toxicity, Environmental Impact, and Fate.* CRC Lewis Publishers, New York. pp. 91-132.

Ehrenstein, O.S.V., Podder, S., Yuan, Y., Mazumder, D.G., and Smith, A.H. 2007. Children's intellectual function in relation to arsenic exposure. *Epidemiology*, **18**(1):44-51.

Elsharkawy, E.E., Abd El-Nasser, M., and Bakheet, A.A. 2019. Mancozeb impaired male fertility in rabbits with trials of glutathione detoxification. *Regulatory Toxicology and Pharmacology*, **105**:86-98.

Eren E, Abuhandan M, Solmaz A., and Taşkın A. 2014. Serum paraoxonase/ arylesterase activity and oxidative stress status in children with metabolic syndrome. *Journal of Clinical Research and Paediatric Endocrinology*, **6**(3):163-168.

Erkan, M., Aydin, Y., Yilmaz, B.O., and Yildizbayrak, N. 2021. Arsenic-induced oxidative stress in reproductive systems. *Toxicology*, 145-155.

Farkhondeh, T., Mehrpour, O., Forouzanfar, F., Roshanravan, B., and Samarghandian, S. 2020. Oxidative stress and mitochondrial dysfunction in organophosphate pesticide-induced neurotoxicity and its amelioration: a review. *Environmental Science and Pollution Research*, **27**(20):24799-24814.

Feng, X., Xia, Q., Yuan, L., Yang, X., and Wang, K. 2010. Impaired mitochondrial function and oxidative stress in rat cortical neurons: implications for gadolinium-induced neurotoxicity. *Neurotoxicology*, **31**(4):391-398.

Flora, S.J.S., Mittal, M., and Mehta, A. 2008. Heavy metal induced oxidative stress & its possible reversal by chelation therapy. *Indian Journal of Medical Research*, **128**(4):501.

Fouad, A.E.A., Fouad, A.A., and Al-Melhim, W.N. 2018. Protective effect of baicalin in rats exposed to arsenic-induced testicular toxicity. *Indian Journal of Forensic and Medical Toxicology*, **12**(2):256-61.

Furlong, C.E., Richter, R.J., Seidel, S.L., and Motulsky, A.G. 1988. Role of genetic polymorphism of human plasma paraoxonase/arylesterase in hydrolysis of the

insecticide metabolites chlorpyrifos oxon and paraoxon. *American Journal of Human Genetics*. **43**:230-238.

Garza-Lombó, C., Pappa, A., Panayiotidis, M.I., Gonsebatt, M.E., and Franco, R. 2019. Arsenic-induced neurotoxicity: a mechanistic appraisal. *JBIC Journal of Biological Inorganic Chemistry*, **24**(8):1305-1316.

Ghosh, A., Awal, M.A., Majumder, S., Sikder, M.H. and Rao, D.R. 2012. Arsenic residues in broiler meat and excreta at arsenic prone areas of Bangladesh. *Bangladesh Journal of Pharmacology*, **7**(3):178-185.

Girish, B.P., and Reddy, P.S. 2018. Forskolin ameliorates mancozeb induced testicular and epididymal toxicity in Wistar rats by reducing oxidative toxicity and by stimulating steroidogenesis. *Journal of Biochemical and Molecular Toxicology*, **32**(2):e22026.

Goldner, W.S., Sandler, D.P., Yu, F., Hoppin, J.A., Kamel, F., and LeVan, T.D. 2010. Pesticide use and thyroid disease among women in the Agricultural Health Study. *American Journal of Epidemiology*, **171**(4):455-464.

Goswami, S.K., Singh, V., Chakdar, H., and Choudhary, P. 2018. Harmful effects of fungicides-current status. *International Journal of Agriculture, Environment and Biotechnology*, **11**:1011-1019.

Goudarzi, M., Amiri, S., Nesari, A., Hosseinzadeh, A., Mansouri, E., and Mehrzadi, M. 2018. The possible neuroprotective effect of ellagic acid on sodium arsenate-induced neurotoxicity in rats. *Life Sciences*, **198**:38-45.

Grzesik, M., Naparło, K., Bartosz, G., and Sadowska-Bartosz, I. 2018. Antioxidant properties of catechins: Comparison with other antioxidants. *Food Chemistry*, **241**:480-492.

Gullino, M.L., Tinivella, F., Garibaldi, A., Kemmitt, G.M., Bacci L., and Sheppard B. 2010. Mancozeb past, present and future. *The American Phytopathological Society*, **94**(9):1077-1087.

Gurol, M.A., Arman, S., and Yon, N.D. 2020. Effects of mancozeb on the testicular histology of the zebrafish (*Danio rerio*). *Annales Limnology -International Journal of Limonology*, **56**(10):1-5.

Hafeman, D.G., Sunde, R.A. and Hoekstra, W.G. 1974. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. *Journal of Nutrition*, **104**(5):580-587.

Hashem, M.A., Mohamed, W.A., and Attia, E.S. 2018. Assessment of protective potential of Nigella sativa oil against carbendazim-and/or mancozeb-induced hematotoxicity, hepatotoxicity, and genotoxicity. *Environmental Science and Pollution Research*, **25**(2):1270-1282.

Hass, U., Boberg, J., Christiansen, S., Jacobsen, P.R., Vinggaard, A.M., Taxvig, C., and Axelstad, M. 2012. Adverse effects on sexual development in rat offspring after low dose exposure to a mixture of endocrine disrupting pesticides. *Reproductive Toxicology*, **34**(2): 261-274.

He, Z., Zhang, Y., Zhang, H., Zhou, C., Ma, Q., Deng, P., and Yu, Z. 2021. NAC antagonizes arsenic-induced neurotoxicity through TMEM179 by inhibiting oxidative stress in Oli-neu cells. *Ecotoxicology and Environmental Safety*, **223**:112554.

Hu, Y., Li, J., Lou, B., Wu, R., Wang, G., Lu, C., Wang, H., Pi, J., and Xu, Y. 2020. The role of reactive oxygen species in arsenic toxicity. *Biomolecules*. **10**:240.

Ishaq, A., Gulzar, H., Hassan, A., Kamran, M., Riaz, M., Parveen, A., and Fahad, S. 2021. Ameliorative mechanisms of turmeric-extracted curcumin on arsenic (As)-induced biochemical alterations, oxidative damage, and impaired organ functions in rats. *Environmental Science and Pollution Research*, **28**(46):66313-66326.

Islam, M.S., and Islam, F. 2010. Arsenic contamination in groundwater in Bangladesh: an environmental and social disaster. www.iwa-network.org.

Izak-Shirian, F., Najafi-Asl, M., Azami, B., Heidarian, E., Najafi, M., Khaledi, M., and Nouri, A. 2022. Quercetin exerts an ameliorative effect in the rat model of diclofenac-induced renal injury through mitigation of inflammatory response and modulation of oxidative stress. *European Journal of Inflammation*, **20**: 1721727X221086530.

Jacobsen, P.R., Axelstad, M., Boberg, J., Isling, L.K., Christiansen, S., Mandrup, K.R., and Hass, U. 2012. Persistent developmental toxicity in rat offspring after low dose exposure to a mixture of endocrine disrupting pesticides. *Reproductive Toxicology*, **34**(2):237-250.

Jamova, K., Jenisova, Z., Feszterova, M., Baros, S., Liska, J., Hudecova, D., Rhodes, C.J., and Valko, M. 2010. Arsenic: toxicity, oxidative stress and human disease. *Journal of Applied Toxicology*, **31**:95-107.

Jiang, Z., Zhang, J., Cai, Y., Huang, J., and You, L. 2017. Catechin attenuates traumatic brain injury induced blood–brain barrier damage and improves longer term neurological outcomes in rats. *Experimental Physiology*, **102**(10):1269-1277.

Jin, W., Xue, Y., Xue, Y., Han, X., Song, Q., Zhang, J., and Chu, L. 2020. Tannic acid ameliorates arsenic trioxide-induced nephrotoxicity, contribution of NF-κB and Nrf2 pathways. *Biomedicine & Pharmacotherapy*, **126**:110047.

Joshi, S.C., Gulati, N., and Gajraj, A. 2005. Evaluation of toxic impacts of mancozeb on testis in rats. *Asian Journal of Experimental Sc*iences, **19**(1):73-83.

Josiah, S.S., Famusiwa, C.D., Crown, O.O., Lawal, A.O., Olaleye, M.T., Akindahunsi, A.A., and Akinmoladun, A.C. 2022. Neuroprotective effects of catechin and quercetin in experimental Parkinsonism through modulation of dopamine metabolism and expression of IL-1β, TNF-α, NF-κB, IκKB, and p53 genes in male Wistar rats. *NeuroToxicology*, **90**:158-171.

Kackar, R., Srivastava, M.K., and Raizada, R.B. 1997a. Induction of gonadal toxicity to male rats after chronic exposure to mancozeb. *Industrial Health*, **35**:104-111.

Kackar, R., Srivastava, M.K., and Raizada, R.B. 1997b. Studies on rat thyroid after oral administration of mancozeb: morphological and biochemical evaluations. *Journal of Applied Toxicology*, **17**(6):369-375.

Kanchi, S., Singh, P., and Bisetty, K. 2013. Dithiocarbamates as hazardous remediation agent: A critical review on progress in environmental chemistry for inorganic species studies of 20th century. *Arabian Journal of Chemistry*, **7**:11-25.

Kayode, A.O., Abimbola, A., Adesola, A.F., Teslim, F.D., and Dorcas, W.A. 2019. Catechin Attenuates the Effect of Combined Arsenic and Deltamethrin Toxicity by Abrogation of Oxidative Stress and Inflammation in Wistar Rats. *Advances in Biochemistry*, **7**(2):51.

Khan, M.H.A., and Parvez, S. 2015. Hesperidin ameliorates heavy metal induced toxicity mediated by oxidative stress in brain of Wistar rats. *Journal of Trace Elements in Medicine and Biology*, **31**, 53-60.

Khan, M.M.H., Sakauchi, F., Sonoda, T., Wshio, M., and Mori, M. 2003. Magnitude of arsenic toxicity in tube-well drinking water in Bangladesh and its adverse

effects on human health including cancer: Evidence from review of the literature. *Asian Pacific Journal of Cancer Prevention*, **4**:7-14.

Khatun, M.K., Hasan. M.M., Islam, R., Sarkar, S., and Haque, M.A. 2020. Effect of spirulina (*Spirulina platensis*) and vitamin E on arsenic induced toxicity in Quail. *Asian Journal of Medical and Biological Research*, **6**(1):93-98.

Khazan, M., and Hdayati, M. 2014. The role of nitric oxide in health and diseases. *Scimetry*, **3**(1).

Kim, Y.J., and Kim, J.M. 2015. Arsenic toxicity in male reproduction and development. *Journal of the Korean Society of Developmental Biology*, **19**(4):167-180.

Ksheersagar, R.L., and Kaliwal, B.B. 2003. Temporal effects of mancozeb on testes, accessory reproductive organs and biochemical constituents in albino mice. *Environmental Toxicology and Pharmacology*, **15**:9-17.

Kumar, K., Sabarwal, A., and Singh, R.P. 2019. Mancozeb selectively induces mitochondrial-mediated apoptosis in human gastric carcinoma cells through ROS generation. *Mitochondrion*, **48**:1-10.

Kwon, D., Chung, H.K., Shin, W.S., Park, Y.S., Kwon, S.C., Song, J.S., and Park, B.G. 2018. Toxicological evaluation of dithiocarbamate fungicide mancozeb on the endocrine functions in male rats. *Molecular & Cellular Toxicology*, **14**(1):105-112.

Leandro, L.P., Mello, R.S.d., Costa-Silva, D.G.d., Nunes, M.E.M., Lopes, A.R., Martins, I.K., Posser, T., and Franco, J.L. 2021. Behavioural changes occur earlier than redox alterations in developing zebrafish exposed to mancozeb. *Environmental Pollution*, **268**:115783.

Levine, A.B., Punihaole, D., and Levine, T.B. 2012. Characterization of the role of nitric oxide and its clinical applications. *Cardiology*, **122**(1):55-68.

Li, Z., Liu, Y., Wang, F., Gao, Z., Elhefny, M.A., Habotta, O.A., Moneim, A.E.A. and Kassab, R.B. 2021a. Neuroprotective effects of protocatechuic acid on sodium arsenate induced toxicity in mice: role of oxidative stress, inflammation, and apoptosis. *Chemico-Biological Interactions*, **337**:109392.

Li, Z., Shah, S.W.A., Zhou, Q., Yin, X., and Teng, X. 2021b. The contributions of miR-25-3p, oxidative stress, and heat shock protein in a complex mechanism

of autophagy caused by pollutant cadmium in common carp (*Cyprinus carpio* L.) hepatopancreas. *Environmental Pollution*, **287**:117554.

Liu, P., Li, R., Tian, X., Zhao, Y., Li, M., Wang, M., and Yan, X. 2021. Co-exposure to fluoride and arsenic disrupts intestinal flora balance and induces testicular autophagy in offspring rats. *Ecotoxicology and Environmental Safety*, **222**:112506.

Liu, P., Xue, Y., Zheng, B., Liang, Y., Zhang, J., Shi, J., and Chu, L. 2020. Crocetin attenuates the oxidative stress, inflammation and apoptosis in arsenic trioxide-induced nephrotoxic rats: Implication of PI3K/AKT pathway. *International Immunopharmacology*, **88**:106959.

Lorio, R., Castellucci, A., Rossi, G., Cinque, B., Cifone, M.G., Macchiarelli, G., and Cecconi, S. 2015. Mancozeb affects mitochondrial activity, redox status and ATP production in mouse granulosa cells. *Toxicology in Vitro*, **30**:438-445.

Lovaković, B.T. 2020. Cadmium, arsenic, and lead: elements affecting male reproductive health. *Current Opinion in Toxicology*, **19**:7-14.

Luiking, Y.C., Engelen, M.P., and Deutz, N.E. 2010. Regulation of nitric oxide production in health and disease. *Current Opinion in Clinical Nutrition and Metabolic Care*, **13**(1):97.

Mahadevaswami, M.P., Jadaramkunti, U.C., Hiremath, M.B., and Kaliwal, B.B. 2000. Effect of mancozeb on ovarian compensatory hypertrophy and biochemical constituents in hemicastrated albino rat. *Reproductive Toxicology*, **14**:127-134.

Mahajan, L., Verma, P., Sharma, P., Raina, R., Sood, S., and Agarwal, S. 2020. Antioxidant biomarkers and oxidative damage in lipids and proteins of blood following exposure of arsenic and imidacloprid in Wistar rats. *Journal of Veterinary Pharmacology and Toxicology*, **19**(2/32):40.

Mahajan, L., Verma, P.K., Raina, R., and Sood, S. 2018a. Potentiating effect of imidacloprid on arsenic-induced testicular toxicity in Wistar rats. *BMC Pharmacology and Toxicology*, **19**(1):1-8.

Mahajan, L., Verma, P.K., Raina, R., Pankaj, N.K., Sood, S., and Singh, M. 2018b. Alteration in thiols homeostasis, protein and lipid peroxidation in renal tissue following subacute oral exposure of imidacloprid and arsenic in Wistar rats. *Toxicology Reports*, **5**:1114-1119.

Mahdi, H., Tahereh, H., Esmaiel, S., andMassood, E. 2019. Vitamins E and C prevent apoptosis of testicular and ovarian tissues following mancozeb exposure in the first-generation mouse pups. *Toxicology and Industrial Health*, **35**(2):136-144.

Maji, C., Biswas, S., Sarkar, P.K., Patra, P.H., Bandyopadhyay, S., Sarkar, S., Chattopadhyay., and Mandal, T.K. 2020. Evaluation of ameliorative effect of two selected plant drugs on experimentally induced arsenic toxicity in sheep. *Environmental Science and Pollution Research*, **27**(29):36744-36753.

Manach, C., Texier, O., Morand, C., Crespy, V., Régérat, F., Demigné, C., and Rémésy, C. 1999. Comparison of the bioavailability of quercetin and catechin in rats. *Free Radical Biology and Medicine*, **27**(11-12):1259-1266.

Mansour, S.A., Mossa, A.T.H., and Heikal, T.M. 2009. Effects of methomyl on lipid peroxidation and antioxidant enzymes in rat erythrocytes: in vitro studies. *Toxicology and Industrial Health*, **25**(8):557-563.

Marklund, S., and Marklund, G.1974. Involvement of superoxide anion radical in autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, **47**:469-474.

Mazumder, D.N.G, 2008. Chronic arsenic toxicity and human health. *Indian Journal of Medical Research*, **128**(4):436-447.

Mehrzadi, S., Bahrami, N., Mehrabani, M., Motevalian, M., Mansouri, E., and Goudarzi, M. 2018. Ellagic acid: A promising protective remedy against testicular toxicity induced by arsenic. *Biomedicine and Pharmacotherapy*, **103**:1464-1472.

Messarah, M., Amamra, W., Boumendjel, A., Barkat, L., Bouasla, I., Abdennour, C., and Feki, A.E. 2013. Ameliorating effects of curcumin and vitamin E on diazinon-induced oxidative damage in rat liver and erythrocytes. *Toxicology and Industrial Health*, **29**(1):77-88.

Metwally, D.M., Alajmi, R.A., El-Khadragy, M.F., Yehia, H.M., Al-Megrin, W.A., Akabawy, A.M.A., Amin, H.K., and Moneim. 2020. Chlorogenic acid confers robust neuroprotection against arsenic toxicity in mice by reversing oxidative stress, inflammation, and apoptosis. *Journal of Functional Foods*, **75**:1-9.

Mishra, D., and Flora, S.J.S. 2008. Differential oxidative stress and DNA damage in rat brain regions and blood following chronic exposure. *Toxicology and Industrial Health*, **24**:247-256.

Modica-Napolitano, J.S., and Singh, K. 2002. Mitochondria as targets for detection and treatment of cancer. *Expert Reviews in Molecular Medicine*, **4**(9):1-19.

Mosolin, H.O., Mehana, E.S.E., Oda, S.S., Elblehi, S.S., and Gad El-Karim, D.R. 2022. Ameliorative Effect of Green tea and Quercetin against Cadmium-Induced Hepato-Renal Toxicity in Rats. *Alexandria Journal for Veterinary Sciences*, **73**(1).

Motchnik, A.P., Frei, B., and Ames, N.B. 1994. Measurement of antioxidants in human blood plasma, Protein thiols. In: Packer, L. (Ed). *Oxygen radicals in biological systems*, *Methods in Enzymology*. California, Academic Press, **234**(D):273-274.

Mukherjee, S.C., Rahman, M.M., Chowdhary, U.K., Sengupta, M.K., Lodh, D., Chanda, C.R., Saha, K.C., and Chakraborti, D. 2003. Neuropathy in arsenic toxicity from groundwater arsenic contamination in West Bengal, India. *Journal of Environmental Science and Health*, **38**(1):165-183.

Muñoz-Leoz, B., Ruiz-Romera, E., Antigüedad, I., and Garbisu, C. 2011. Tebuconazole application decreases soil microbial biomass and activity. *Soil Biology and Biochemistry*, **43**(10):2176-2183.

Nageshwar, M., Umamaheshwari, P., and Reddy, K.P. 2019. Quercetin reverses sodium arsenate induced oxidative stress, behavioural and histological alterations in brain of rat. *Journal of Pharmaceutical Sciences and Research*, **11**(6):2267-2274.

Naqshbandi, A., Rizwan, S., and Khan, F. 2013. Dietary supplementation of flaxseed oil ameliorates the effect of cisplatin on rat kidney. *Journal of Functional Foods*, **5**(1):316-326.

Nava-Rivera, L.E., Betancourt-Martínez, N.D., Lozoya-Martínez, R., Carranza-Rosales, P., Guzmán-Delgado, N.E., Carranza-Torres, I.E., and Morán-Martínez, J. 2021. Transgenerational effects in DNA methylation, genotoxicity and reproductive phenotype by chronic arsenic exposure. *Scientific Reports*, **11**(1):1-16.

Nayak, P., and Solanki, H. 2021. Pesticides and Indian agriculture-a review. *International Journal of Research Granthaalayah*, **9**:250-63.

Nirankari, S., Kamal, R., and Dhawan, D.K. 2016. Neuroprotective role of quercetin against arsenic induced oxidative stress in rat brain. *Journal of Environmental & Analytical Toxicology*, **6**(2):359-364.

Nordby, K.C., Andersen, A., Irgens, L.M., and Kristensen, P. 2005. Indicators of mancozeb exposure in relation to thyroid cancer and neural tube defects in farmers' families. *Scandinavian Journal of Work, Environment & Health*, **31**(2):89-96.

Nurchi, V.K., Djordjevic, A.B., Crisponi, G., Alexandar, J., Bjorklund, and G., Aaseth. 2020. Arsenic toxicity: Molecular targets and therapeutic agents. *Biomolecules*, **10**:235.

Ogut, S., Kucukoner, E., Gultekin, F., and Gurbuz, N. 2015. A study of long-term pesticide application amongst agricultural workers: total antioxidant status, total oxidant status and acetylcholinesterase activity in blood. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, **85**(1):155-159.

Owumi, S.E., Nwozo, S.O., and Najophe, E.S. 2019. Quercetin abates induction of hepatic and renal oxidative damage, inflammation, and apoptosis in carbendazim-treated rats. *Toxicology Research and Application*, **3:**2397847319849521.

Panaullah, G.M., Alam, T., Hossain, M.B., Loeppert, H.R., Lauren, J.G., Meisner, C.A., Ahmed, Z.U., and Duxbury, J.M. 2009. Arsenic toxicity to rice (*Oryza sativa* L.) in Bangladesh. *Plant and Soil*, **317**(1):31-39.

Paru, R., Tiboni, G.M., Buccione, R., Rossi, G., Cellini, V., Canipari, R., and Cecconi, S. 2012. The fungicide mancozeb induces toxic effects on mammalian granulosa cells. *Toxicology and Applied Pharmacology*, **260**:155-161.

Pavlovic, V., Cekic, S., Kamenov, B., Ciric, M., and Krtinic, D. 2015. The effect of ascorbic acid on mancozeb induced toxicity in rat thymocytes. *Folia Biologica*, **61**:116-123.

Peng, J., Peng, L., Stevenson, F.F., Doctrow, S.R., and Andersen, J.K. 2007. Iron and paraquat as synergistic environmental risk factors in sporadic Parkinson's disease accelerate age-related neurodegeneration. *Journal of Neuroscience*, **27**(26), 6914-6922.

Peruru, R., Rani, R.U., Thatiparthi, J., Sampathi, S., Dodoala, S., and Prasad, K.V.S.R.G. 2020. Devil's claw (*Harpagophytum procumbens*) ameliorates the neurobehavioral changes and neurotoxicity in female rats exposed to arsenic. *Heliyon*, **6**(5):1-8.

Prakash, B., Surendran, A., Radhakrishnan Chandraprabha, V., Pettamanna, A., and Nair, H.N. 2018. Betulinic acid, natural pentacyclic triterpenoid prevents arsenic-induced nephrotoxicity in male Wistar rats. *Comparative Clinical Pathology*, **27**(1):37-44.

Prakesh, C., Soni, M., and Kumar, V. 2016. Mitochondrial oxidative stress and dysfunction in arsenic neurotoxicity: A review. *Journal of Applied Toxicology*, **36**: 179-188.

Prakesh, C., Soni, M., and Kumar, V. 2016. Mitochondrial oxidative stress and dysfunction in arsenic neurotoxicity: A review. *Journal of Applied Toxicology*, **36**:179-188.

Preeti, S., Aksha, S., Nakuleshwar, J.D., Nidhi, S., and Suresh, J.C. 2015. A Review on Toxicological Effects of Fungicides. *Research Journal of Pharmaceutical, Biological and Chemical Sciences,* **6**(2):348.

Raeeszadeh, M., Karimfar, B., Amiri, A.A., and Akbari, A. 2021. Protective effect of nano-vitamin C on infertility due to oxidative stress induced by lead and arsenic in male rats. *Journal of Chemistry,* **2021**:1-12.

Rahaman, M.S., Rahman, M.M., Mise, N., Sikder, M.T., Ichihara, G., Uddin, M.K., and Ichihara, S. 2021. Environmental arsenic exposure and its contribution to human diseases, toxicity mechanism and management. *Environmental Pollution*, **289**:117940.

Ramadan, S.S., Almeer, R., Albasher, G., and Abdel Moneim, A.E. 2022. Lycopene mitigates arsenic-induced nephrotoxicity with activation of the Nrf2 pathway in mice. *Toxin Reviews*, **41**(2):446-456.

Ramos-Treviño, J., Bassol-Mayagoitia, S., Hernández-Ibarra, J.A., Ruiz-Flores, P., and Nava-Hernández, M.P. 2018. Toxic effect of cadmium, lead, and arsenic on the sertoli cell: Mechanisms of damage involved. *DNA and Cell Biology*, **37**(7):600-608.

Rana, T., Bera, A.K., Das, S., Pan, D., Bandyopadhyay, S., Bhattacharya, D., and Das, S.K. 2010. Effect of ascorbic acid on blood oxidative stress in experimental

chronic arsenicosis in rodents. *Food and Chemical Toxicology*, **48**(4):1072-1077.

Rao, A.V., and Balachandran, B. 2002. Role of oxidative stress and antioxidants in neurodegenerative diseases. *Nutritional Neuroscience*, **5**(5):291-309.

Ratnaike, R.N. 2003. Acute and chronic arsenic toxicity. *Postgraduate Medical Journal*, **79**(933):391-396.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., and Rice-evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, **26**(9-10):1231-7.

Renu, K., Madhyastha, H., Madhyastha, R., Maruyama, M., Vinayagam, S., and Gopalakrishnan, A.V. 2018. Review on molecular and biochemical insights of arsenic-mediated male reproductive toxicity. *Life Sciences*, **212**, 37-58.

Renu, K., Saravanan, A., Elangovan, A., Ramesh, S., Annamalai, S., Namachivayam, A., and Gopalakrishnan, A.V. 2020. An appraisal on molecular and biochemical signalling cascades during arsenic-induced hepatotoxicity. *Life Sciences*, **260**:118438.

Rizwan, S., Naqshbandi, A., Farooqui, Z., Khan, A.A., and Khan, F. 2014. Protective effect of dietary flaxseed oil on arsenic-induced nephrotoxicity and oxidative damage in rat kidney. *Food and Chemical Toxicology*, **68**:99-107.

Rossi, G., Buccione, R., Baldassarre, M., Macchiarelli, G., Palmerini, M.G., and Cecconi, S. 2006. Mancozeb exposure in vivo impairs mouse oocyte fertilizability. *Reproductive Toxicology*, **21**(2):216-219.

Runkle, J., Flocks, J., Economos, J., and Dunlop, A.L. 2017. A systematic review of Mancozeb as a reproductive and developmental hazard. *Environment International*, **99**:29-42.

Runkle, J.D., Tovar-Aguilar, J.A., Economos, E., Flocks, J., Williams, B., Muniz, J.F., and McCauley, L. 2013. Pesticide risk perception and biomarkers of exposure in Florida female farmworkers. *Journal of Occupational and Environmental Medicine*, **55**(11):1286-1292.

Saber, T.M., Abo-Elmaaty, A.M., and Abdel-Ghany, H.M. 2019. Curcumin mitigates mancozeb-induced hepatotoxicity and genotoxicity in rats. *Ecotoxicology and Environmental Safety*, ***183*:**109467.

Saber, T.M., and El-Aziz, R.M.A. 2016. Curcumin ameliorates mancozeb-induced neurotoxicity in rats. *Japanese Journal of Veterinary Research*, **64**:S197-S202.

Saddein, E., Haghpanah, T., Nematollahi-Mahani, S.N., Seyedi, F., and Ezzatabadipour, M. 2019. Preventative effects of vitamin E on testicular damage and sperm parameters in the first-generation mice pups due to pre-and postnatal mancozeb exposure. *Journal of Toxicology*, **2019**:4763684.

Sadowska-Woda, I., Popowicz, D., and Karowicz-Bilińska, A. 2010. Bifenthrin-induced oxidative stress in human erythrocytes in vitro and protective effect of selected flavonols. *Toxicology in Vitro*, **24**(2):460-464.

Sakr, S., EL-Kenawy, A., and EL-Sahra, D. 2013. Metiram induced nephrotoxicity in albino mice: Effect of licorice aqueous extract. *Environmental Toxicology*, **28**(7):372-379.

Sakr, S.A. 2007. Ameliorative effect of ginger (*Zingiber officinale*) on mancozeb fungicide induced liver injury in Albino rats. *Australian Journal of Basic and Applied Sciences*, **1**(4):650-656.

Salam, S., Arif, A., and Mahmood, R. 2020. Thiram-induced cytotoxicity and oxidative stress in human erythrocytes: an in vitro study. *Pesticide Biochemistry and Physiology*, **164**:14-25.

Samarghandian, S., Azimi-Nezhad, M., and Farkhondeh, T. 2017. Catechin treatment ameliorates diabetes and its complications in streptozotocin-induced diabetic rats. *Dose-Response*, **15**(1):1559325817691158.

Santra, A., Maiti, A., Das, S., Lahiri, S., Charkarborty, S.K., and Mazumder, D.N.H. 2000. Hepatic damage caused by chronic arsenic toxicity in experimental animals. *Clinical Toxicology*, **38** (4):395-405.

Saraiva, M.A., da Rosa Ávila, E., da Silva, G.F., Macedo, G.E., Rodrigues, N.R., de Brum Vieira, P., and Posser, T. 2018. Exposure of Drosophila melanogaster to mancozeb induces oxidative damage and modulates Nrf2 and HSP70/83. *Oxidative Medicine and Cellular Longevity*, **2018**:5456928.

Sardoo, M.M., Nabiuni, A.M.M., Mahani, S.N.M., and Amirheidari, B. 2018. Mancozeb induces testicular dysfunction through oxidative stress and apoptosis: protective role of N-acetylcysteine antioxidant. *Toxicology and Industrial Health*, **20** (10):1-14.

Sastry, K.V.H., Moudgal, R.P., Mohan, J., Tyagi, J.S., and Rao, G.S. 2002. Spectrophotometric Determination of Serum Nitrite and Nitrate by Copper-Cadmium Alloy. *Analytical Biochemistry*, **306**:79-82.

Satyendra, R.V.R., Vishnumurthy, K.A.A., Vagdevi, H.M., Rajesh, K.P.G., Manjunatha, H., and Shruthi, A.G. 2012. *In vitro* antimicrobial and molecular docking of dichloro substituted benzoxazole derivatives. *Medicinal Chemistry Research*, **21**(12):4193-4199.

Selvi, M., Çavaş, T., Cağlan Karasu Benli, A., Koçak Memmi, B., Çinkılıç, N., Dinçel, A.S., and Erkoç, F. 2013. Sublethal toxicity of esbiothrin relationship with total antioxidant status and *in vivo* genotoxicity assessment in fish (*Cyprinus carpio*) using the micronucleus test and comet assay. *Environmental Toxicology*, **28**(11):644-651.

Shafiq-Ur-Rehman. 1984. Lead-induced regional lipid peroxidation in brain. *Toxicology Letters*, **21**:333-337.

Shahid, F., Rizwan, S., Khan, M.W., Khan, S.A., Naqshbandi, A., and Yusufi, A.N.K. 2014. Studies on the effect of sodium arsenate on the enzymes of carbohydrate metabolism, brush border membrane, and oxidative stress in the rat kidney. *Environmental Toxicology and Pharmacology*, **37**(2):592-599.

Sharma, A., Kshetriyanum, C., Patel, B., Kashyap, R., Sadhu, H.G., and Kumar, S. 2021a. Prevention of Arsenic Induced Testicular Oxidative Stress and DNA Damage by Coenzyme Q_{10} and Vitamin E in Swiss Albino Mice. *Journal of Infertility and Reproductive Biology*, **9**(2):93-99.

Sharma, B., and Sharma, P.M. 2013. Arsenic toxicity induced endothelial dysfunction and dementia: Pharmacological interdiction by histone deacetylase and inducible nitric oxide synthase inhibitors. *Toxicology and Applied Pharmacology*, **273**:180-188.

Sharma, P., Verma, P.K., Sood, S., Pankaj, N.K., Agarwal, S., and Raina, R. 2021b. Neuroprotective potential of hydroethanolic hull extract of *Juglans regia L*. on isoprenaline induced oxidative damage in brain of Wistar rats. *Toxicology Reports*, **8**:223-229.

Simakani, P., Abolhasani, M. H and Hoseini, S.M. 2018. Determination of mancozeb toxicity and biochemical effects in common carp (*Cyprinus carpio*). *International Journal of Aquatic Biology*, **6**(3):157-161.

Singh, A.P., Goel, R.K and Kaur, T. 2011. Mechanisms pertaining to arsenic toxicity. *Toxicology International*, **18**(2):87-93.

Singh, N., Gupta, V.K., Kumar, A., and Sharma, B. 2017. Synergistic effects of heavy metals and pesticides in living systems. *Frontiers in Chemistry*, **5**:70.

Singh, P., Verma, P.K., Raina, R., Sood, S., and Sharma, P. 2020. Maximum contaminant level of arsenic in drinking water potentiates quinalphos-induced renal damage on co-administration of both arsenic and quinalphos in Wistar rats. *Environmental Science and Pollution Research*, **27**(17):21331-21340.

Sodhi, K.K., Kumar, M., Agrawal, P.K., and Singh, D.K. 2019. Perspectives on arsenic toxicity, carcinogenicity and its systemic remediation strategies. *Environmental Technology & Innovation*, **16**:100462.

Souza, A.C.F., Bastos, D.S.S., and Marcela, F.S.S. 2018. Arsenic aggravates oxidative stress causing hepatic alterations and inflammation in diabetes rats. *Life sciences*, **209**:472-480.

Souza, A.C.K., Marchesi, S.C., Lima, G.D.A., and Neves, M.M. 2017. Effects of arsenic compounds on microminerals content and antioxidant enzyme activities in rat liver. *Biological Trace Element Research*, **183**(2):305-313.

Srivastava, A.M., Ali, W., Singh, R., Bhui, K., Tyagi, S., Al-Khedhairy, A.A., Srivastava, P.K., Masarrat, J., and Shukla, Y. 2012. Mancozeb-induced genotoxicity and apoptosis in cultured human lymphocytes. *Life Sciences*, **90**: 815-824.

Susan, A., Rajendran, K., Sathyasivam, K., and Krishnam, U.M. 2019. An overview of plant-based interventions to ameliorate arsenic toxicity. *Biomedicine & Pharmacology*, **109**:838-852.

Sutcu, R., Altuntas, I., Buyukvanli, B., Akturk, O., Ozturk, O., Koylu, H., and Delibas, N. 2007. The effects of diazinon on lipid peroxidation and antioxidant enzymes in rat erythrocytes: role of vitamins E and C. *Toxicology and Industrial Health*, **23**(1):13-17.

Tandon, N., Roy, M., Roy, S., and Gupta, N. 2012. Protective effect of *Psidium guajava* in arsenic-induced oxidative stress and cytological damage in rats. *Toxicology International*, **19**(3):245.

Thakur, M., Rachamalla, M., Niyogi, S., Datusalia, A.K., and Flora, S.J.S. 2021. Molecular mechanism of arsenic-induced neurotoxicity including neuronal dysfunctions. *International Journal of Molecular Sciences*, **22**(18):10077.

Thangapandiyan, S., Ramesh, M., Miltonprabu, S., Hema, T., Jothi, G.B., and Nandhini, V. 2019. Sulforaphane potentially attenuates arsenic-induced nephrotoxicity via the PI3K/Akt/Nrf2 pathway in albino Wistar rats. *Environmental Science and Pollution Research*, **26**(12):12247-12263.

Tripathi, N., Kannan, G.M., Pant, B.P., Jaiswal, D.K., Malhotra, P.R., and Flora, S.J.S. 1997. Arsenic-induced changes in certain neurotransmitter levels and their recoveries following chelation in rat whole brain. *Toxicology Letters*, 92(**3**):201-208.

Tsang, M.M., and Trombetta, L.D. 2007. The protective role of chelators and antioxidants on mancozeb-induced toxicity in rat hippocampal astrocytes. *Toxicology and Industrial Health*, **23**(8):459-470.

Turk, E., Kandemir, F.M., Yildirim, S., Caglayan, C., Kucukler, S., and Kuzu, M. 2019. Protective effect of hesperidin on sodium arsenite-induced nephrotoxicity and hepatotoxicity in rats. *Biological trace element research*, **189**(1):95-108.

Upadhyay, M.K., Shukla, A., Yadav, P., and Srivastava, S. 2018. A review of arsenic in crops, vegetables, animals and food products. *Food Chemistry*, **276**:608-618.

Uzun, F.G., and Kalender, Y. 2013. Chlorpyrifos induced hepatotoxic and hematologic changes in rats: the role of quercetin and catechin. *Food and Chemical Toxicology*, **55**:549-556.

Uzun, F.G., Demir, F., Kalender, S., Bas, H., and Kalender, Y. 2010. Protective effect of catechin and quercetin on chlorpyrifos-induced lung toxicity in male rats. *Food and Chemical Toxicology*, **48**(6):1714-1720.

Vieira, R., Venancio, C.A.S., and Felix, L.M. 2020. Toxic effects of mancozeb-containing commercial formulation at environmental relevant concentrations on zebrafish embryonic development. *Environmental and Pollution Research*, **27**(17):21174-21187.

Voss, G., and Sachsse, K. 1970. Red cell and plasma cholinesterase activities in microsamples of human and animal blood determined simultaneously by a

modified acetylthiocholine/DTNB procedure. *Toxicology and Applied Pharmacology*, **16**:764-72.

Wan, F., Zhong, G., Wu, S., Jiang, X., Liao, J., Zhang, X., and Hu, L. 2021. Arsenic and antimony co-induced nephrotoxicity via autophagy and pyroptosis through ROS-mediated pathway in vivo and in vitro. *Ecotoxicology and Environmental Safety*, **221**:112442.

Witko-sarsat, V., M. Friedlander, C., Capeillère-blandin, T. Nquyen-Khoa, A.T.,Nquyen, J., Zingraff, P., Jungers, B., and Descamps-Latscha. 1996. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney International*, **49**(5):1304-1313.

Wongmekiat, O., Peerapanyasut, W., and Kobroob, A. 2018. Catechin supplementation prevents kidney damage in rats repeatedly exposed to cadmium through mitochondrial protection. *Naunyn-Schmiedeberg's Archives of Pharmacology*, **391**(4):385-394.

Wu, S., Zhong, G., Wan, F., Jiang, X., Tang, Z., Hu, T., and Hu, L. 2021. Evaluation of toxic effects induced by arsenic trioxide or/and antimony on autophagy and apoptosis in testis of adult mice. *Environmental Science and Pollution Research*, **28**(39):54647-54660.

Yadav, R.S., Shukula, R.K., Sankhwar, M.L., Patel, D.K., Ansari, R.W., Pant, A.B., Islam, F., and Khanna, V.K. 2010. Neuroprotective effect of curcumin in arsenic-induced neurotoxicity in rats. *Neurotoxicology*, **31**:533-539.

Yahia, D., El- Amir, Y.O., and Rushdi, M. 2019. Mancozeb fungicide-induced genotoxic effects, metabolic alterations, and histological changes in the colon and liver of Sprague Dawley rats. *Toxicology and Industrial Health*, **35**(4):265-276.

Zargari, F. 2019 Arsenic Toxicity, Vitamin C and PON1. Toxicology Open Access **5**: 139. DOI: 10.4172/2476-2067.1000139

Zeng, Q., Yi, H., Huang, L., An, Q., and Wang, H. 2019. Long-term arsenite exposure induces testicular toxicity by redox imbalance, G2/M cell arrest and apoptosis in mice. *Toxicology*, **411**:122-132.

Zhang, M., Swarts, S.G., Yin, L., Liu, C., Tian, Y., Cao, Y., and Okunieff, P. 2011. Antioxidant properties of quercetin. In *Oxygen Transport to Tissue XXXII*. Springer, Boston, MA. pp 283-289.

Zhang, Y., Wen, R., Bao, J., Gong, Y., Liu, T., and Wang, X. 2022. Transcriptomics and Metabolomics Reveal Mancozeb-Induced Nephrotoxicity in Mice. *Available at SSRN 4033138*.

Zhao, H., Wang, Y., Guo, M., Liu, Y., Yu, H., and Xing, M. 2021. Environmentally relevant concentration of cypermethrin or/and sulfamethoxazole induce neurotoxicity of grass carp: involvement of blood-brain barrier, oxidative stress and apoptosis. *Science of The Total Environment*, **762**:143054.

Zhao, P., Guo, Y., Zhang, W., Chai, H., Xing, H., and Xing, M. 2017. Neurotoxicity induced by arsenic in *Gallus gallus*: Regulation of oxidative stress and heat shock protein response. *Chemosphere*, **166**: 238-245.

Zhou, Y., Shie, F.S., Piccardo, P., Montine, T.J., and Zhang, J. 2004. Proteasomal inhibition induced by manganese ethylene-bis-dithiocarbamate: Relevance to parkinson′ s disease. *Neuroscience*, **128**(2): 281-291.

Zizza, M., Di Lorenzo, M., Laforgia, V., Furia, E., Sindona, G., Canonaco, M., and Facciolo, R.M. 2018. Orexin receptor expression is increased during mancozeb-induced feeding impairments and neurodegenerative events in a marine fish. *Neurotoxicology*, **67**:46-53.

Zubrod, J.P., Bundschuh, M., Arts, G., Brühl, C.A., Imfeld, G., Knäbel, A., and Schäfer, R.B. 2019. Fungicides: an overlooked pesticide class? *Environmental Science and Technology*, **53**(7):3347-3365.

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CERTIFICATE – IV

Certified that all necessary corrections as suggested by the external examiner and advisory committee have been duly incorporated in the thesis entitled **"SUBACUTE TOXICITY OF MANCOZEB ALONE AND IN-COMBINATION WITH ARSENIC IN WISATR RAT"**, submitted by **Ms. Rasia Yousuf**, Registration No. **J-20-MV-642**.

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