Protective Effects of Kisspeptin-10 and N-Acetyl Cysteine against Sodium arsenite induced Toxicity in Liver and Kidney of Adult Male Mice

BY

Semab Khadam

Department of Zoology

Faculty of Biological Sciences

Quaid-i-Azam University

Islamabad, Pakistan

2023

Protective Effects of Kisspeptin-10 and N-Acetyl Cysteine against Sodium arsenite induced Toxicity in Liver and Kidney of Adult Male Mice

A thesis submitted in the partial fulfillment of the requirements for the degree

of

MASTER OF PHILOSOPHY

IN

PHYSIOLOGY

BY

Semab Khadam

Department of Zoology

Faculty of Biological Sciences

Quaid-i-Azam University

Islamabad

2023

“In the name of ALLAH, the Most Gracious, the Most Merciful”

DECLARATION

I hereby declare that the work presented in the following thesis is my own effort and the material contained in this thesis is my original work. I have not previously presented this work elsewhere for any other degree.

Semab khadam

DEDICATION

This research work is dedicated to my beloved parents, who realized the importance of education and made me capable of reaching this level. At the same time, it is dedicated to my dearest brothers and sisters, who supported and guided me in every field of life. It is their love and support that enabled me not only to complete this task but also to walk every step of life with confidence and commitment.

ACKNOWLEDGMENTS

I owe my gratitude to the One who is the Most Beneficent, Altruistic and Merciful, ***Almighty Allah****, Who puts the sun's seal on the tablets of the flowing waters and throws clouds to the skies, Who distills the waters of the clouds over the seas to conceive the pearl in the womb of the oyster, Who creates fire in every stone, colour in the fire, radiance in the colour, Who gives voices to the dust, word to the voices, and life to the world, Who created us as a Muslim and blessed us with knowledge to differentiate between right and wrong. All prays to Him as He blessed us with the* ***Holy Prophet, Hazrat Muhammad*** *(SAW) for whom the whole universe is created and who enabled us to worship only one God. He (SAW) brought us out of darkness and enlightened the way of heaven.*

Then I am grateful to many people who made it possible for me to complete my dissertation. First, I cannot thank my kind supervisor, ***Prof. Dr. Irfan Zia Qureshi****, for making my M.phil an intense and deep learning experience for me. Thank you for being a wonderful teacher and a great mentor. Thank you for telling me all the time how to have research work and writing. He not only made me work in a better way, but also worked hard with me patiently. I am also grateful to* ***Dr. Amina Zubairi,*** *Chairperson, Department of Zoology for making Departmental facilities available to use for my research work.*

I would like to pay special thanks to my respected seniors ***Iffat Fatima and Tariq Aziz for*** *always guiding me in my research. Thank you so much for always supporting and helping me. I would like to extend my gratitude to my seniors* ***Halima Sadia, Sumeria Hussan, and Ayesha Razzaq*** *for their affectionate efforts, support, encouragement and cooperation during my research work. I am deeply indebted to* ***Nazar Hussain, Izhar Ahmad, Taimoor Ahmad, Hina Affaqi, Shanza shabbir*** *and* ***Momina Shakoor*** *for their cooperation. The completion of my thesis would not have been possible without their emotional and instrumental support.*

I also appreciate the lively and supporting company of my senior and junior lab fellows ***Zainab Ashraf, Nagina Saeed, Momina, Shafqat, Bakhtawar, and Ruqqya.*** *The time spent in the company of these people has become a memorable part of my life.*

Furthermore, I truly appreciate the endless love and courage of my friends ***Sonia, Iqra, Tahira Iram, Hira, Aleena Azhar and Iqra Ahmad*** *who made sure that I do not become a recluse while*

working. on this dissertation., thanks for believing in me with your love and support and accompanying me through thick and thin.

Last, but not the least, My best friend, My Roomie, ***Qudsia Rehman!*** *You are not just a roommate but a fantastic friend. Thank you for giving me the beautiful memories we have built over the last two years. Whenever life gets hard and I don't know where to turn, I feel so thankful that I always have your shoulder to lean on. Thanks for all the laughs, advice, and sweet (and naughty) memories throughout time. Thank you so much for always being around. I cherish the moments we spent in hostel days.*

I don't have enough words to express my feelings for my parents and family. It is pleasure to pay tribute to my ***Parents,Brothers,****and* ***Sisters*** *for providing me with technical and financial support. Lastly, I offer my regards and blessings to all who supported me in any respect during the completion of this thesis.*

Semab Khadam

TABLE OF CONTENTS

List of abbreviations

Symbols	Abbreviations
AA	Arachidonic acid
AChE	Acetylcholinesterase
AKI	Acute kidney injury
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMPA	a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ASA	Acetyl salicylic acid
AST	Aspartate aminotransferase
BSA	Bovine serum albumin
CAT	Catalase
DPX	Dibutylpthalate Polystyrene Xylene
DNTB	Dithiobis nitrobenzoic acid
DEPPD	Diethyl para phenyldiamine
ChAT	Choline acetyltransferase
CKD	Chronic kidney disease

EDTA	Ethylenediaminetetraacetic acid
NADH	Nicotinamide adenine dinucleotide hydrogenase
NAC	N-Acetyl cysteine
MOS	Mitochondrial oxidative stress
LOX	Lipoxygenase
MDA	Malondialdehyde
FDA	Food and Drug Administration
GSH	Reduced glutathione
NBT	Nitro blue tetrazolium
NF-KB	Nuclear factor kappa-light-chain-enhancer of B cells
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
POD	Peroxide dismutase
PMSF	Phenyl-methyl-sulfonyl fluoride
PBS	Phosphate buffer saline
NMDA	N-methyl-D-aspartate
PGs	Prostaglandins

SDS	Sodium dodecyl sulfate
TBA	Thiobarbutiric acid
TBARS	Thiobarbituricacid reactive substances
TCA	Trichloroacetic acid
TNF	Tumor necrosis factor
TxA	Thromboxane
JNK	c-Jun N-terminal kinase
MAPKK	MAPKK mitogen-activated protein kinase
SOD	Superoxide dismutase
WHO	World Health Organization
NaAsO2	Sodium arsenite
H & E	Hematoxylins and eosin
KP-10	Kisspeptin-10
LH	Luteinizing hormone
FSH	Follicle stimulating hormone

LIST OF FIGURES

ABSTRACT

Arsenic, a heavy metal, can exist in both organic and inorganic forms and is often present in soil and water that has become contaminated. The inorganic form of arsenic is more dangerous and has been linked to a range of adverse health effects, including damage to the kidneys and liver, cardiovascular problems, neurological abnormalities, and malfunctions of the nervous system. N-Acetyl-L-Cysteine (NAC), a strong antioxidant, has been utilized for the treatment of numerous diseases and toxicities. Additionally, kisspeptin, a hypothalamic peptide recently discovered, plays a vital role in the central regulation of reproductive processes. A recent study evaluated the effect of kisspeptin-10 and NAC on liver and kidney function in male mice exposed to low and high doses of sodium arsenite in drinking water. The study divided the mice into various groups: tap water control, sodium arsenite only, kisspeptin-10 only, NAC only, and combinations of sodium arsenite with kisspeptin-10 and NAC. The kisspeptin-10 was administered once a week and NAC was given every alternate day. The study aimed to examine the potential benefits of these treatments in counteracting the toxic effects of arsenic. The results of the study showed that exposure to sodium arsenite caused a significant increase in oxidative stress markers (ROS and TBARS) in the liver and kidney, compared to the control group. The levels of antioxidant enzymes (CAT, SOD, POD, and GSH) were significantly decreased in the sodium arsenite-treated group. The total protein content increased in the liver and kidney. The study also found that the arsenic-treated groups had significantly higher levels of AST, ALP, ALT, and creatinine in the liver and kidney tissue. Additionally, the levels of triglycerides and cholesterol were also analyzed. The study found that exposure to arsenic altered the normal histological structure of the kidney and liver. The kidney tissue showed a shrunken glomerulus, increased Bowman's space, decreased glomerulus diameter, leukocyte infiltrations, and vacuole formation. The liver tissue showed an increased sinusoidal space, irregular hepatocyte distribution, and nuclear cell infiltrations. Additionally, the study reported a non-significant decrease in organ mass index in the arsenic-treated group. The results showed that both kisspeptin and NAC supplementation were effective in preventing kidney and liver damage caused by sodium arsenite. When combined with sodium arsenite exposure, both kisspeptin and NAC prevented significant tissue damage. The combination of kisspeptin and NAC with sodium arsenite exposure improved the oxidative stress markers, antioxidant enzymes, biochemical effects, and histoprotective effects better than kisspeptin-10 supplementation alone. This indicates the synergistic effect of kisspeptin and NAC in protecting against arsenic-induced toxicity in the liver and kidney of adult male mice.

INTRODUCTION

1.1 Heavy metals

Metals are lustrous, malleable substances with excellent electrical conductivity that lose their electrons to generate cations voluntarily (Khlifi and Hamza, 2010). The earth's crust contains naturally occurring metallic elements, whose compositions vary depending on their sources and geographic locations (Khlifi and Hamza, 2010). Environmental toxins typically comprise pesticides and heavy metals and pose a major threat to the ecosystem, seriously impairing its structure and function (Chin, 2010). The two main categories of heavy metal sources identified by scientists are natural and anthropogenic sources.

Volcanic eruptions, sedimentary rocks, rock weathering, and soil formation, are examples of natural sources, whereas domestic effluents, industry, mining, and agriculture are examples of anthropogenic sources (Ozbahani *et al.*,2015). The geochemical cycle of heavy metals has significantly changed because of anthropogenic activity (Rai *et al.,* 2019; Rahman and Singh, 2020). The most prevalent agricultural sources of heavy metals are sewage sludge, pesticides, and fertilizers (Alloway, 2013).

Among multilayered soil and environmental pollutants, heavy metals are regarded as one of the most dangerous toxicants. A great deal of attention has been captured by heavy metal pollution, even at low concentrations, due to its hazardous effects, long-term accumulation, and bio-magnification properties (Jiang *et al.*,2019; Xiao *et al.*,2019). The presence of heavy metals in the ecosystem raises the possibility of their potential consummation and then, accumulation in various organs of living organisms, for instance, the liver, kidneys, bones, etc. This accumulation leads to various damages to organ systems; the neurological, skeletal, endocrine, immunological, and circulatory systems (Lamas *et al*., 2016; Ma *et al*., 2016).

The effects of these metals on cellular organelles, like cell membranes, endoplasmic reticulum, nuclei, and several metabolic enzymes are well recognized (Tchounwou *et al.,* 2012). The extremely low quantities of these metals are quintessential for the maintenance of a variety of physiological and biochemical processes in living organisms, but when certain threshold concentrations are surpassed, these become noxious. Heavy metal exposure persists and is rising in many regions of the world, even though heavy metals are known for causing long-lasting adverse health impacts. The toxicity of heavy metals and its continual rise is a concern of growing significance especially for environmental, nutritional, ecological, and evolutionary reasons (Jaishankar *et al*., 2013; Nagajyoti *et al*., 2010).

Most heavy metals are cancer-causing agents (Tchounwou *et al.,* 2012). Almost all heavy metals cause numerous diseases and cancers, however, some of them like zinc (Zn), and copper (Cu), have DNA-binding domains and also function as enzymes that are crucial for intracellular functions (Fergusson,1990; Stern,2010; Hambidge and Krebs ,2007). Reactive oxygen species (ROS) generate oxidative stress, which is a well-known mechanism of damage caused by heavy metals (Tchounwou *et al.,* 2012; Bánfalvi, 2011). Heavy metals are used in many industrial items despite their extreme toxicity; they are used in vehicle emissions, paints, and batteries. In addition, heavy metals are utilized in pigments, which are, in turn, are used in consumer products like toys and jewelry for children. (Finch *et al*., 2015).

The bio-system, including the food chain, can magnify dissolved forms of hazardous heavy metals via circulation, which can then reach extremely high concentrations in humans (Tchounwou *et al.,* 2012; Bánfalvi, 2011; Worsztynowicz and Mill, 1995). According to the International Agency for Research on Cancer, category 1 heavy metals include nickel (Ni), chromium (Cr), arsenic (As) and cadmium (Cd) (IARC, 2012). Numerous reports have found that exposure to these compounds causes disruptions in damage repair mechanisms, in the expression of tumor suppressor gene, and enzymatic activities concerned in metabolism via oxidative damage (Bánfalvi, 2011; Ercal *et al.*, 2001). Some studies have demonstrated that the contamination source and the risk of exposure to heavy metals are interrelated (Harvey *et al.,* 2015; Gul *et al.,* 2015). For instance, studies revealed a higher risk of occupational disease and cancer among workers in heavy metal industrial zones (Grimsrud and Andersen ,2012; Grimsrud *et al.*, 2003). Exposure to heavy metals through various penetrating mechanisms, such as inhalation, swallowing, and dermal absorption result in adverse health impacts. Children experience more severe effects of heavy metals than adults (Yang and Massey, 2019). Almost all cells and tissues in the human body are susceptible to the effects of certain heavy metals (Oldewage and Marx, 2000).

The variety of contaminants has grown exponentially because of economic globalization, population growth, and the industrial revolution (Janani *et al.,* 2021). Cadmium (Cd), chromium (Cr), arsenic (As), lead (Pb), and mercury (Hg) are the heavy metals of most concern since higher concentrations of these non-threshold toxins have been reported in aerial, aquatic, and terrestrial systems (Rahman and Singh, 2020; ATSDR). According to recent estimates, 66 million individuals worldwide are believed to be in grave danger from various sources of Hg, Pb, Cr, and Cd (Rahman and Singh, 2020). More than 150 million

people globally have been affected by the water contamination caused by As alone (Ravenscroft *et al.,* 2011). These metals can be found as organic and inorganic compounds such oxides, hydroxides, and silicates. One of the well-known heavy metals that can be found in the environment in minute concentrations is arsenic. Its organic forms include monomethylarsonic acid, trimethylarsine oxide, and dimethylarsinic acid while inorganic forms are tetravalent and pentavalent ones (Wanibuchi *et al.*,2004).

1.2 Arsenic

The king of poisons, arsenic has great impact on the human population than any other element or poison. Currently, millions of people are exposed to increased levels of arsenic present in the air, food, water, and soil. Arsenic has been regarded as a frightful and bizarre element throughout the history of human progress. In both developed and developing countries, arsenic's toxic effects are highly prevalent. As a result of escalating contamination of air, water, and soil, arsenic toxicity has become a significant issue (Bhattacharya *et al*., 2002; Bundschuh and Garcia, 2007).

Ingestion of drinking water, consumption of food, and inhalation of air are the three main pathways of arsenic exposure in humans. Drinking water has been recognized as the primary source of arsenic exposure globally (Bhattacharya *et al.,* 2002; Bundschuh and Garcia, 2007). Arsenic compounds, because of their high solubility in water, can easily enter aquatic bodies,such as lakes, rivers, and ponds and by surface run off (Bundschuh and Garcia, 2007; Bhattacharya *et al.*, 2002).

In the 9th Report on Carcinogens, the US Department of Health and Human Services classified compounds of arsenic as human carcinogens. Arsenic exposure causes severe health like, for instance, cancers, diabetes mellitus, melanosis (hyper- or hypopigmentation), black foot disease (peripheral vascular disorder), ischemic heart disease, hyperkeratosis (hardened skin), gangrene, hypertension, etc. (Guha Mazumdar *et al.*, 2012; Rahman *et al.*,2006). The US EPA lowered the maximum permissible limit of arsenic in drinking water from 50 to 10 g/L in 2002 because of growing health concerns and the harmful effects of arsenic on humans (USEPA, 2001).

1.2.1 Origin and History

The origin of the word "arsenic "is from the Greek word "arsenikos", which means "potent". Since ancient times, arsenic has been known in its sulfide form. The two arsenic minerals; red colored realgar (As4S4) and bright yellow orpiment (As2S3) were known to the Greek philosopher, Theophrastus. The first person to obtain white arsenic (As2O3) by

heating arsenic sulfide was the Greek historian, Olympiodorus of Thebes (5th century AD). Albertus Magnus, a German philosopher in the 1200s, is credited with the discovery of "arsenic" and was the first one to report its metallic behavior.

In the 1500s, during the Ming dynasty, the Chinese scientist, Tsao Kan-Mu, studied the toxicity of arsenic compounds and described its use as pesticides in rice fields. Since ancient times, humans have used arsenic as a poison as well as a medicine. Arsenic trioxide, one of the most popular oxides of arsenic, is a tasteless, odorless, white powder which was used as a chemical warfare agent in the past. However, green colored copper acetoarsenate was traditionally used in wallpapers as a pigment. (Nriagu ,2002). Arsenic has not always had a negative reputation in human history due to its toxicity. (Süss-Fink and Arsenvergiftungen, 2012).

1.2.2 Chemistry

Arsenic, ranked 33rd in the periodic table, is a member of the nitrogen family and a part of the elements in Group 15. Its atomic number is 33 and its atomic weight is 74. 921. It is a brittle gray metal in its pure form, but in nature, it is found with other metals,like, copper, iron, silver, and nickel as oxides and sulfides. Arsenite is known to be more toxic and 25–60 times more mobile than arsenate (Dutre and Vanecasteele, 1995).

Arsenic is an element that has remained fascinating over the years in both its elemental and compounds forms (Schwarzmaier *et al.,* 2013). It has completely filled s orbital and half-filled p orbital in its fourth shell. That is why it shows four common redox states: −3, 0, +3, and +5 (Dutre and Vanecasteele, 1995). Its ability to readily change its oxidation state and bonding configuration results in its diverse chemical behavior in the environment, and the ability to form large numbers of organic and inorganic compounds (Bhattacharya *et al.,* 2002; Bundschuh, 2007).

The oxidation and reduction of arsenic potentially depends on the pH conditions and redox potential. The figure illustrates the stability of several arsenic species under different pH conditions, highlighting the significance of pH in the occurrence and formation of diverse forms of arsenic in natural water bodies (Dutre and Vanecasteele, 1995). Figure 1.1 shows

(1) Oxidation of arsenic under oxidizing and reducing conditions,

(2) Formation of acids by As (+3)/As (III) and As(+5)/As(V) under different pH conditions,

(3) Dissociation of acids to oxyanions under various sets of pH conditions.

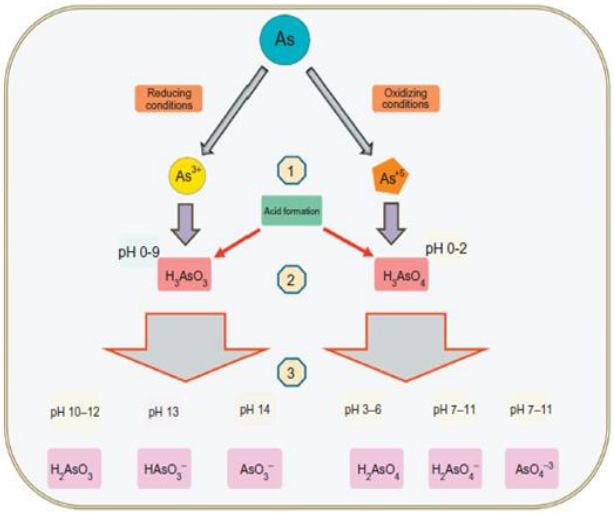

Fig.1.1Pictorial depiction showing different compounds formation of arsenic (Dutre and Vanecasteele, 1995)

1.2.3 Sources

Arsenic is a ubiquitous element with 20th, 14th and 12th ranking in terms of its abundance in the earth's crust, seawater and human body, respectively. Its terrestrial abundance is aroundc1.5–3.0 mg kg^{-1} while its sources in the environment are both natural and anthropogenic (Woolson, 1975).

Natural

Arsenic is a rare crystal element that makes only 0.00005% (about five hundred–thousandths of 1%) of the earth's crust. (Gulledge and Connor, 1973), with an average concentration of 2 mg kg^{-1} in igneous and sedimentary rocks (Pendias, 1984). There are more than 200 different mineral forms of arsenic that are found in nature, of which about 60% are arsenates, 20% are sulphides and sulfosalts, and remaining 20% arsenites, arsenides, oxides, silicates, and elemental arsenic (As) (Onishi,1969). The levels of arsenic in the soils range from 0.1 to 40 mg kg^{-1} (mean 6 mg kg^{-1}) (Bowen,1979), 1 to 50 mg kg−1(mean 6 mg kg^{-1}) (Backer,1975) and mean 5 mg kg^{-1} (Vinogradov, 1959), however, there is significant regional variation (Collourn *et al.*,1975). Soils have higher quantities of arsenic as compared to rocks (Peterson *et al.*,1981). The parent rock and human activities are the principal factors affecting the elemental concentrations in soils. The concentration of arsenic in soils is affected by various factors including the climate, the organic and inorganic soil components, and the soils' redox potential status (Tang, 1987). It has been reported that common fungi, bacteria, and yeasts can methylate arsenic to MMA, DMA, and other gaseous derivatives of arsenic and the resulting methylated arsenic is widely disseminated in soils (McBride and Wolfe, 1971; Cox

and Alexander, 1973; Woolson *et al.,* 19731; Woolson, 1977). The natural concentration of arsenic in sediments is typically lower than 10 mg kg^{-1}, dry weight (Crecelius,1974), although it varies significantly across the world. EPA and WHO propose 50 g l-1 of arsenic as the maximum allowable concentration in drinking water and the recommended value is 10g l−1 (WHO, 2001; EPA, 1975). Arsenic levels in seawater range from 0.001 to 0.008 mg l-1 (Johnson, 1972). The normal arsenic concentrations in the air range from 0.4 to 30 ng m^{-3} but human exposure to arsenic via air is generally very low. (WHO, 1996).

According to USEPA, the US's estimated average exposure is 6 ng As m^{-3}. According to current estimates, typical arsenic concentrations in the European region range from 0.2 to 1.5 ng/m^3 in rural areas, 0.5 to 3 ng/m^3 in urban areas, and no more than 50 ng/m^{-3} in industrial areas (EEC, 2000). A plant's arsenic content is almost solely determined by the amount of arsenic to which it is exposed. On a dry weight basis, its concentration ranges from less than 0.01 to 5 μg^{-1} (Feed Additive Compendium, 1975). Arsenic is reported to accumulate in marine animals to levels of between 0.005 and 0.3 mg kg^{-1} in coelenterates, crustaceans, and certain molluscs (Bowen,1966). Arsenic levels in some shellfish may exceed 100µg g^{-1}. Moreover, freshwater fish, as per total wet weight, have an average arsenic concentration of 0.54 µg g^{-1} whereas some values also increase up to 77.0 µg g^{-1} in the liver oil of freshwater bass (Whitacre and Pearse, 1972) Arsenic has been found to accumulate in certain ectodermic tissues of mammals, primarily nails and the hairs. (Smith,1964). The total human body content varies between 3 and 4 mg and tends to increase with age. Most body tissues, with the exception of hair, nails, and teeth, have dry weight contents of less than 0.3–147 µg g^{-1}. (WHO, 2001). Arsenic levels in hair typically range from 0.08–0.25 µgg^{-1} with 1.0 µg g^{-1} being a sign of excessive arsenic poisoning (Arnold *et al.,* 1990). As compared to the normal arsenic concentration in nails, which is (0.34± 0.25 µg g^{-1}; Mujumder *et al.,* 1988), the nail clippings of acute polyneuritis's patient caused by arsenic poisoning contain arsenic at 20–130µg g^{-1} (Itallie, 1932). Urine typically contains between 5 to 40µg of arsenic per day (total). Acute and sub-acute poisoning will produce an excess of 100 µg per day (Arnold *et al.,* 1990).

Anthropogenic

Man's utilization of natural resources releases arsenic into the air, water, and soil which, in turn, affects residue levels in plants and animals. Arsenic accumulation in the soil can be caused by several ways, such as dust from the burning of fossil fuels, the use of fertilizers and arsenical pesticides, and the disposal of industrial and animal wastes (Piver, 1983; Woolson,

1983). Around 90% of the world's production of arsenic was produced in China, France, Germany, USSR, Mexico, Namibia, Peru, Sweden, and the United States (Nelson, 1977). In the past, arsenic was frequently utilized for the preparation of pesticides and insecticides. Some 37,000 tonnes of white arsenic as produced globally in 1955, out of which 10,800 tonnes were produced in the United States, while domestic consumption exceeded 18,000 tons (Vallee *et al.,* 1960). Inorganic arsenicals, primarily sodium arsenite,are being widely used since 1890 as weed killers, particularly as non-selective soil sterilant (EPA, 1980). Arsenic acid has been used extensively as a cotton desiccant for many years. In 1964, 2500 tons of H_3AsO_4 were used as desiccants on 495,000 ha of U.S. cotton (Fordyce *et al.,* 1995). As wood preservatives, Wolman salts, Osmosalts, zinc, and chromium arsenate are also used (Lansche, 1965). Many arsenic compounds, including H_3AsO_4, 3-nitro-4-hydroxy phenylarsonic acid, 4-nitrophenylarsonic acid, etc., are used as feed additives. Under the Food Additives Law of 1958, all substituted phenylarsonic acids were utilized as feed additives (Feed Additive Compendium, 1975). The medicinal virtues of arsenic are known for some 2500 years. A significant amount of arsenic was consumed by Austrian peasants for beauty, cleanliness, and softness of skin, to give freshness to the complexion and plumpness to the figure, and also to overcome breathing problems. (Sollman, 1957). Common arsenic-containing medicinal preparations include Donovan's solution (arsenic and mercuric iodides), de Valagin's solution (liquor arsenii chloridi), Fowler's solution (potassium arsenite), sodium cacodylate, Asiatic pills (arsenic trioxide and black pepper), neoarsphenamine, arsphenamine (Salvarsan), oxophenarsine hydrochloride (Mapharsen), acetarsone, arsthinol (Balarsen), carbarsone and tryparsamide (Vallee *et al.,* 1960).Doak and Freedman(1970), Sullivan(1969) and (Bates *et al.,* 1992) provide detailed uses of arsenic compounds as medicines and drugs. Arsenic compounds are infamous as very potent poisons and are preferred to homicidal and suicidal agents. Even Napoleon Bonaparte's demise was supposed to have been caused by arsenic poisoning (Marcelet, 1913).

1.2.4 Significance

In addition to medicine, it has also been frequently used in various fields, for instance, livestock, agriculture, industry, electronics, and metallurgy (Nriagu *et al.,* 1990). Large quantities of arsenic are released into the biosphere through the application of pesticides and herbicides, long-term mining and smelting of the sulfide ores, burning of coal in thermal power plants and disposal of fly ash, and runoff from mine tailings. Moreover, arsenic is also utilized in the production of pesticides and herbicides, semiconductors, lead-acid batteries, in

the hardening of metal alloys, in the copper refining industry, and in the glass industry. Arsenic is frequently used to preserve wood, and its use has considerably increased in recent years (Nico *et al.,* 2004).

Insect, fungal, bacterial, and animal attacks can cause wood to deteriorate, however, it can be protected by impregnating with CCA with the composition CuO (18.5%), Cr_2O_3 (47.5%), and As_2O_3 (18.5%). Calcium arsenate, sodium arsenate, and lead arsenate were once used to eradicate ticks, fleas, and lice, manage aquatic weeds, and also as pesticides for debarking trees. However, because of the toxic effects of arsenic and later public awareness about food safety and environmental contamination, these applications have been banned (Smith *et al.,* 1998).

1.2.5 Metabolism

Humans are vulnerable to a variety of organic and inorganic arsenic species via water, food, and other environmental media. The kinetics and metabolism of arsenicals in animals and humans are complicated to investigate since each form of arsenic has unique physicochemical characteristics and bioavailability. For dust and fumes, the routes of arsenic intake are respiratory; but for arsenic in water, drinks, soil, and food, the routes are oral. Few investigations of dermal absorption rates for arsenicals have been undertaken. The available datasets show generally low absorption rates (less than10%); nevertheless, higher rates may also be observed for some types of arsenic (WHO, 2001).

The bioavailability of ingested inorganic arsenic will vary depending on the solubility of the arsenical compound itself, the matrix in which it is ingested (i.e., food, water, beverages, or soil), and the presence of other food constituents and nutrients in the gastrointestinal tract. Blood perfusion, membrane characteristics, tissue volumes, tissue affinities, and diffusion coefficients are some dependence factors of tissue distributions of arsenic. The fate of ingested arsenic in vivo depends on (1) redox reactions between iAsV and iAsIII in the plasma; and (2) consecutive methylation reactions in the liver. In vivo methylation of inorganic arsenic occurs in humans. Rapid reduction of arsenate results in arsenite, which was later partly methylated.

In mammals, inorganic arsenic compounds are methylated in the liver to $(CH3)_2As^{V}(O)OH$ (dimethylarsinic acid) and to $(CH3)_2As^{III}OH$ (dimethylarsinous acid) via the activity of methyltransferases. Methionine, in the form of S-adenosyl methionine, is the source of the methyl groups during the detoxification of inorganic arsenicals (Elke *et al.,* 2010). Overexposure to inorganic arsenic compounds inhibits the liver's methylation

capacity, causing an imbalance that has deleterious effects on various organs and leads to carcinogenesis.

In the metabolism of inorganic arsenic, bio-methylation plays a significant role. Its mechanism entails alternate phases of two-electron reduction followed by oxidative addition of a methyl group, or oxidative methylation (Healy *et al.*, 1999). The figure 1.2 shows the methylation of arsenic through alternate reduction and oxidative methylation.

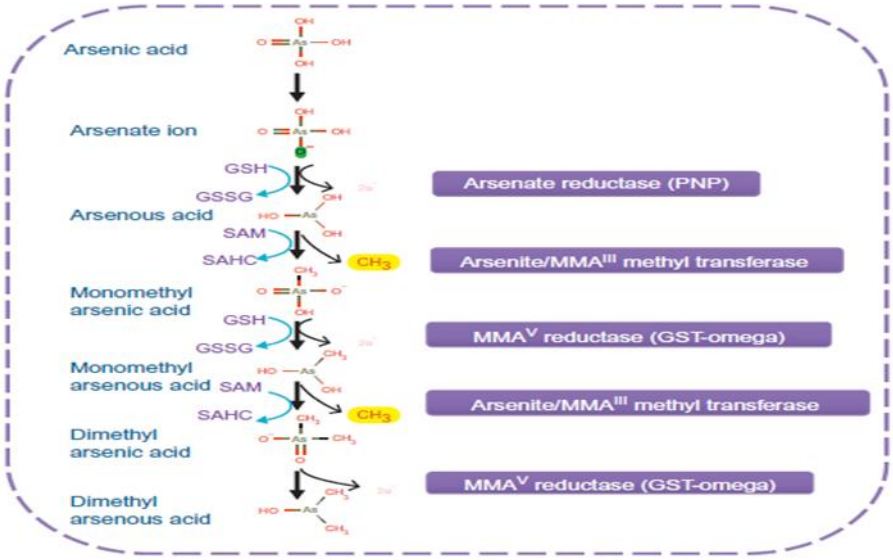

Fig1.2. Biotransformation of inorganic arsenic in the mammals.

According to studies conducted on humans, methylation may start acting on limiting at doses of about 0.2-1 mg kg^{-1} (0.003-0.015 mg As kg^{-1} per day) (Nagvi *et al.*, 1994; Raie, 1996). Data suggests the fact that arsenic accumulates in tissues with age which is completely congruent with observations in laboratory animals (Marafante *et al.*, 1982). In the majority of animal studies, DMA is the primary metabolite, while in humans, under normal circumstances, urine excretion consists of around 20% inorganic arsenic, 20% MMA, and 60% DMA, without excessive absorption of inorganic arsenic. Inorganic arsenic is methylated to MMA and DMA in vivo. The MMA is partly absorbed during circulation in plasma. Then, this absorbed MMMA is further methylated to DMA, while DMA is excreted primarily in an unchanged form (Vahter *et al.*, 1984; Buchet *et al.*, 1981). Both DMAV and MMAV are reported to be reduced to their trivalent analogues dimethylarsinous acid (DMAIII) and monomethylarsonous acid (MMAIII), respectively, before excretion through urine (Mandal *et al.*, 2001; Le *et al.*, 2000). About 60–70% of the daily-ingested inorganic arsenic is estimated to be excreted in the urine. (Buchet *et al.*, 1981). Arsenic can also be excreted via minor routes, including in sweat, other than in urine and feces, being the major

ones (WHO, 2001). Skin, hair, and nails also act as potentially minor excretory routes for arsenic because of its accumulation in keratin-containing tissues (Benko *et al.,* 1971). It can also be excreted in human milk, (WHO, 2001) even though its concentrations are small (Grandjean *et al.,* 1995; Concha *et al.,* 1998). However, urine levels are the best-suited indicators of recent arsenic exposure because of its rapid metabolization and excretion into the urine. The biomarkers of arsenic exposure used in recent times include total arsenic, inorganic arsenic, and the sun of arsenic metabolites (inorganic arsenic+ MMA+DMA) in urine.

1.2.6 Toxicological pathways

The common denominator in arsenic toxicity is oxidative stress, mediated by reactive oxygen species (ROS). Arsenic's toxic mechanisms are intricate and poorly understood. At a biochemical level, phosphate can be replaced by iAs V in various reactions. In proteins, Arsenite (iAs III) and trivalent organic (methylated) arsenicals react with thiols (-SH), and their activity is inhibited. Some other mechanisms include oxidative stress, autophagic defects, inflammation, and epigenetic alteration (Du *et al.,* 2018; Xu *et al.,* 2019). The superoxide radical anion ($O2^{\bullet)}$, hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), hydroperoxyl radical ($HOO^{\bullet}$), peroxyl radical (ROO•), and singlet oxygen (1O2) are among the ROS that are produced in biological systems during the reduction of molecular oxygen (Hrycay and Bandiera, 2015). Arsenic induces formation of 1O2, $O2^{-\bullet}$, h2O2, •OH, and $ROO^{\bullet}$.

(i) Mitochondria: In the electron transport chain, mitochondrial complexes I and III are responsible for the production of $O2^{-\bullet}$. Arsenic shows mitochondrial toxicity because it inhibits succinic dehydrogenase activity and also uncouples oxidative phosphorylation with the production of $O2^{-\bullet}$, which results in the formation of other forms of ROS (Corsini *et al.,* 1999).

(ii) Nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase (Nox): Nox is a membrane-associated enzyme involved in ROS generation in response to arsenic (Ellinsworth,2015).

(iii) Generation of ROS during the formation of intermediate arsine species (Yamanaka *et al.,* 1997; Yamanaka and Okada, 1994). For example, the metabolic processing of DMA generates dimethyl arsenic peroxyl radical (Kato *et al.,* 1994)

(iv) Redox-active iron released from ferritin caused by methylated arsenic species (Ahmad *et al.,* 2000)

(v) The formation of ROS by arsenic lay on the oxidation of arsenite to arsenate, under physiological conditions (Razo *et al.*,2001).

(vi) Endoplasmic reticulum (ER): ER is suggested to be a source of ROS caused by DMA III (Naranmandura *et al.,* 2012).

(vii) Indirect increase in ROS levels due to the interference with cellular antioxidants, for instance, superoxide dismutase (SOD), glutathione (GSH), catalase (CAT) (Nordenson and Beckman,1991), and GSH-related enzymes (Chouchane and Snow,2001; Styblo *et al.*,1997).

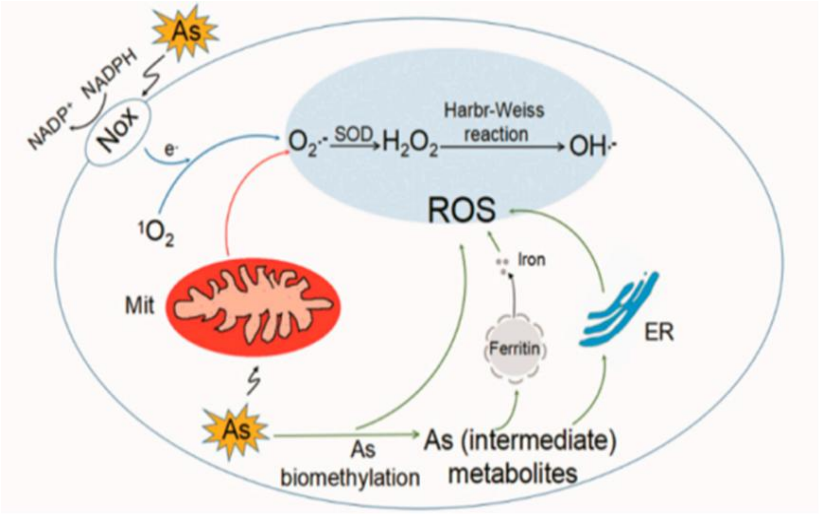

Fig1.3. Mechanisms of generation of reactive oxygen species (ROS) induced by arsenic.

1.2.7 Health Effects

Initial symptoms of acute arsenic poisoning include nausea, vomiting, severe diarrhea, and abdominal pain. (Ratnaike, 2003). There could be encephalopathy and peripheral neuropathy. A frequent symptom of iAs exposure is paresthesia in the limbs, which in certain circumstances can progress to extensive polyneuropathy (Tseng *et al.,* 2006). Cancer in the skin, liver, lungs, kidney, and bladder are only a few of the multiple detrimental health effects that chronic ingestion of inorganic arsenic causes. (IARC, 1980). Furthermore, the intake of arsenic-contaminated drinking water may cause growth retardation, stillbirth, spontaneous abortion, and infant mortality (Aschengrau *et al.,* 1989; Hopenhayn-Rich *et al.*, 2000).

Chronic oral exposure to inorganic arsenic leads to characteristic skin manifestation. It causes characteristic melanosis, squamous cell carcinoma, keratosis, and basal cell carcinoma (Maloney, 1996). Similarly, exposure to inorganic arsenic in crude and refined form causes emphysematous lesions leading to pulmonary insufficiency, trachea bronchitis, and rhino-pharyngo-laryngitis. (WHO, 1981). Some common complications of groundwater

arsenic toxicity include asthma, chronic cough, asthmatic bronchitis, and bronchopulmonary disease (Saha, 1995).

Inhaling inorganic arsenic for an extended period of time may damage blood vessels and result in cardiovascular disease, including arteriosclerosis (Zaldivar, 1974). Although leucopenia (decrease in WBC count), thrombocytopenia (decrease in thrombocytes), and Anaemia (decrease in RBC count and haemoglobin percentages due to haemolysis) are some common symptoms in inhabitants of arsenic-prone areas (Mizuta *et al.,* 1956), however, in underdeveloped countries like India, malnutrition is a major cause of anaemia. Damage to epithelial cells because of gastrointestinal toxicity leads to gastrointestinal irritation that might cause burning lips, thirst, painful swallowing, nausea, and several abdominal colic (Environmental Protection Agency, EPA, 1984; Goebel *et al.,* 1990). Renal damage, due to the clogging of nephrons with hemolytic debris, is secondary (Sittig, 1985). In addition, long-term exposure to inorganic arsenic was also linked to peripheral neuropathy, which is akin to the Guillain-Barre syndrome, (Goddard *et al.,* 1992), and cerebrovascular disease cerebral infarction (Chiou *et al.,* 1997).

Developmental abnormalities like physical, cognitive, psychological, and sensory deficits as well as speech disorders can result from chronic arsenic exposure(Brinkel *et al.,* 2009). Long-term ingestion of inorganic As can lead to respiratory problems such as bronchitis, coughing, chest sounds, and shortness of breath (Guha Mazumder *et al.,* 2000; Milton *et al.,* 2001, 2003; Islam *et al.,* 2007). It has been reported that in Karcag, Hungary, exposure to arsenic-containing drinking water with more than 100 lg/l arsenic content lead to an increase in unprompted abortion, perinatal mortality, and stillbirths. (Rudnai and Gulyas, 1998).

1.2.8 Arsenic and Hepatotoxicity:

The major target organ for many toxic substances in the body is the liver because of its role as the primary site of xenobiotic detoxification and metabolism due to the localization of xenobiotic-metabolizing enzymes in the hepatocytes (Jing and Teschke, 2018). Some early symptoms of liver diseases caused by arsenic exposure are jaundice, ascites, enlargement of the liver, and bleeding from esophageal varices. (Kapaj *et al.,* 2006). While in the case of severe arsenic toxicity in the liver, hepatic lesions coupled with other complications such as non-cirrhotic portal fibrosis, hepatic fibrosis, and cirrhosis with likely chances of liver failure may occur. (Kapaj *et al.,* 2006). In the population exposed to arsenic (0.05-3.2mg/L) in West Bengal, India, liver enlargement was also observed, in some cases, with increased levels of

globulin, alanine amino transferase, aspartate amino transferase, and alkaline phosphatase. (Mazumder *et al.,* 1998). Elevated levels of liver enzymes may also be indicated by blood analysis (Jomova *et al.,* 2011; Kapaj *et al.,* 2006).

On the other hand, increased ROS activity due to arsenic exposure may also induce lipid peroxidation, which, in turn, may cause hepatic cell damage and hepatic toxicity (Bashir *et al.,* 2006; Kokilavani *et al.,* 2005). Arsenic at low, medium, and high dosages of sodium arsenate may have a very potent hepatotoxic potential, according to a prior study by Fatoki *et al.* (2019a). In the study, various indicators of hepatotoxicity, for instance, activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltransferase (ɣ -GT) activities in the plasma were significantly increased when Wistar rats were exposed to 100, 150, and 200 ppm sodium arsenate for 4, 8, and 12 weeks. Meanwhile, evidence from earlier studies suggests that up-regulation of plasma ALT and AST activity is a highly sensitive indicator of hepatocyte cyto- plasmic and/or mitochondrial membrane damage (Crook, 2006).

Additionally, arsenic exposure has also been implicated in increasing ALP activity in liver and plasma cancer patients. The collective data obtained from all these researches suggested that arsenic might compromise the structural and functional integrity of the liver. The hepatic histological examination in the same study by Fatoki *et al.* (2019a) reported that different dose regimens of arsenic as sodium arsenate over a period of 1, 2, and 3 months degenerate cytoplasmic contents, enlarged hepatic sinusoids, collapse the central vein, and disrupt normal cytoarchitecture among other features that indicated major damage to the liver.

In the same vein, mice co-treated with 4 mg/kg arsenic trioxide (ATO) and 15 mg/kg antimony (Sb) concurrently for 60 days increased AST, ALT, and ALP activities (Zhong *et al.,* 2021). It caused hepatic congestion, autophagosomes, karyopyknosis, mitochondrial injury, and steatosis of liver tissues. While levels of the anti-apoptosis index (Bcl-2) decreased, pro-apoptotic biomarkers (Caspase-3, Caspase-9, Bax, P53, Cytc) and mitophagy biomarkers (LC3-B, P62, PINK1, Parkin) were significantly elevated. Summarily, coexposure causes abnormal liver energy metabolism and oxidative stress (Zhong *et al.,* 2021).

In vitro exposure of chicken hepatocyte cultures to arsenic trioxide (ATO) concentrations of 0, 0.6, 1.2, 2.4, and 4.8 μM was reported to increase apoptosis and reactive oxygen species (ROS), as well as accumulate and up-regulate antioxidant enzymes including

catalase (CAT) and super-oxide dismutase (SOD) along with increasing ATO concentrations. Dysregulation of the balance between antiapoptotic versus proapoptotic factors and G1-Phase arrest was also noted. Moreover, the upregulation of SOD-1, TRX, and HO-1 were consistent with ATO-induced oxidative damage. Substantial evidence proved that arsenic-induced liver injury is associated with apoptosis, oxidative stress, inflammation, and mitochondria dysfunction. (Akinboro *et al.,* 2022; Cordier *et al.,* 2021; Banerjee *et al.,* 2017; Bodaghi-Namileh *et al.,* 2017). Major pathways, including mTOR-mediated autophagy (Zhang *et al.,* 2021), and AKT2/NF-KB (Yang *et al.,* 2021), have been reported to be deregulated during arsenic provoked hepatic toxicity.

1.2.9 Arsenic and Nephrotoxicity

While hepatocytes are the major site of arsenic metabolism, renal cells are the major route of its excretion from the body system. As V is also converted into As III in the kidney (Prakash and Verma, 2021). Heavy metal-induced nephrotoxicity causes malfunction of proximal convoluted tubules. (Madden and Fowler, 2000). In addition, kidney capillaries and glomeruli may also be damaged by arsenic. (Rahman *et al.,* 2009). In various countries such as the US, the mortality rate due to kidney diseases resulting from exposure to high arsenic levels has increased. (Lewis *et al.,* 1999; Meliker *et al.,* 2007), Taiwan (Tsai *et al.,* 1999), Chile (Smith *et al.,* 2012a) and Bangladesh (Lokuge *et al.,* 2004). Histopathological studies have reported an association between arsenic induced tubular interstitial nephritis and glomerular atrophy and glomerular loss.

Furthermore, high urine Neutrophil gelatinase-assoicated lipocalin (NGal) levels (>300ug/mg creatinine/dL), tubular proteinuria, and high urinary arsenic were reported in CKDu cases (Jayasumana *et al.,* 2013). Increased levels of ROS activity and oxidative stress cause arsenic induced renal toxicity and a significant rise in the expression of several signaling pathways, for instance, Mitogen Activated Protein Kinase (MAPK), and Hemeoxygenase-1. These signaling pathways regulate transcription factors such as activator protein-1, activating transcription factor-2, and ETS domain containing protein ELK-1. Hence, elevated levels of ROS activity/oxidative stress also cause renal cell damage and lipid peroxidation ultimately leading to renal toxicity. (Parrish *et al.,* 1999; Sasaki *et al.,* 2007, El-Demerdash *et al.,* 2009; Kokilavani *et al.,* 2005).

According to data from earlier studies, intake of arsenic-containing drinking water and kidney disorders are positively correlated (Farkhondeh *et al.,* 2020). Similarly, Feng *et al.* (2013) observed a dose-dependent correlation between well-water arsenic levels and

kidney disorders among Taiwanese people. (Cárdenas-González *et al.*,2016) conducted a similar study in Bangladesh and reported that increased inflammation and a decreased estimated glomerular filtration rate (eGFR) are related to the intake of arsenic-containing drinking water. In complete agreement with these previous studies, another research by Fatoki *et al*. (2019a) indicated that the intake of inorganic arsenate via drinking water was characterized by increased creatinine and urea levels in the plasma of experimental animals. While the creatinine level in the blood is a reflection of renal functions and its excess indicates inefficient filtration by the kidney, (Curhan and Mitch, 2008), the circulating level of urea is a reflection of the balance between hepatic urea production and its excretion by the kidney. Therefore, elevated concentrations of urea in the blood are because of decreased excretion of urea due to renal disorders and reduced glomerular filtration rate (GFR) (Vasudevan and Sreeku, 2005).

1.3 N-acetylcysteine

It is a plant antioxidant and glutathione precursor. It is most found in Allium plant species, specifically in the onion (45 mg NAC/kg in Allium cepa) (Diniz *et al.,* 2006). Since 1960 it has been used as drug and is registered on WHO's Model List of Vital Medicines as a toxicity cure. Amino acid L-cysteine is a precursor for the NAC, which is then converted into the glutathione (GSH) antioxidant (Pieralisi *et al.,* 2016).

NAC in addition to being a dietary supplement, restore intracellular GSH. It is a nontoxic medicine in a wide variety of metabolic conditions, immunotoxicity, pulmonary infections, hepatotoxicity, and neurotoxicity for the glutathione deficiency. (Atkuri *et al.,* 2007) demonstrated the useful properties of oral NAC (2700 mg/day for 12 weeks) in cystic fibrosis of human. Furthermore, NAC is regarded as a safe and well tolerated drug that is used for a variety of medical disorders (Paintlia *et al.,* 2008).

It is broadly recognized for its protagonist as an antidote (oral dose of NAC/day 2.0–2.4 g) in overdose of acetaminophen (Green *et al.,* 2013). GSH, acts as a protecting factor and cleanses ROS both enzymatically and non-enzymatically. The group sulfhydryl (-SH) of NAC directly scavenges ROS (Radomska *et al.,* 2012), regulates the oxidation-reduction state of the amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (neurotransmitter effects) and N-methyl-D-aspartate (NMDA) (Guo *et al.,* 2013). Unlike glutathione, NAC has topical and oral bioavailability (Kang *et al.,* 2003).

1.3.1 Chemical structure of NAC

NAC (n-acetyl cysteine) is a thiol-containing compound which is soluble in water and alcohol and separated from the amino acid L-cysteine. NAC is also known as acetylcysteine, mercapturic acid, and n-acetyl-L-cysteine. This dynamic compound, with the chemical formula C5H9NO3S and a molecular weight of 163.195 g. mol-1, was developed in the 1950s (Ghafarizadeh *et al.*, 2021).

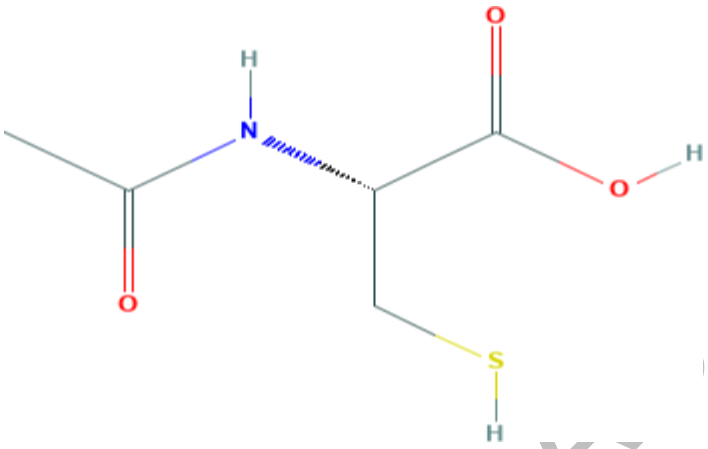

Fig1.4. Chemical structure of NAC (Ghafarizadeh *et al.*, 2021)

1.3.2 Clinical effect of NAC

From a pharmacological point of view, disulphides are formed in the plasma because of NAC injection, which has a six-hour duration from a few min. Previous research shown that NAC may play critical and specific role in glutathione synthesis, down-regulating and preventing the opposing properties of toxins on the liver and kidneys, refining psychiatric disorders and addictive behavior, lessening respiratory symptoms, providing brain health by replenishing glutathione and glutamate regularization, reducing fat cell inflammation and blood sugar fixation and lowering the risk of heart disease by inhibiting oxidative stress damage, refining function of immune system by improving levels of glutathione besides also increase fertility in men and women(Ghafarizadeh *et al.*, 2021).

NAC is also a cysteine metabolite that is used to treat acetaminophen poisoning and pulmonary fibrosis. Traditionally NAC has been used as a cough syrup (mucolytic) and has been shown to have anti-microbial properties. Similarly, NAC with contrast agents enhance contrast in radiography, is used as an accessory treatment in Helicobacter pylori, gentamicin inhibition of auditory damage and as a nutritive additive in dialysis patients (Ghafarizadeh *et al.*, 2021).

1.3.3 Mechanism of action

NAC does not need active diffusion to pass through the cell membrane, it is quickly hydrolyzed by glutathione synthetase enzymes and γ -GCS (γ -glutamyl-cysteine synthetase)

to form glutathione. The enzyme glutathione reductase converts intracellular glutathione to thiol, to reduce the ROS level non-enzymatically (Marmolino and Manto, 2010).

In the body NAC is converted into cysteine, which is the glutamate-cysteine anti-porter substrate, causing glutamate to return to the extracellular area and as a result decreasing glutamate discharge from synapses. One of the most vital antioxidants in cells is glutathione which readily detoxifies toxins containing xenobiotics (Ghafarizadeh *et al.*,2021). During periods oxidative stress, glutathione concentration decreases, recommending NAC to compensate for this scarcity by upregulating glutathione. Instead, NAC directly antagonizes free radicals, stabilizing them by supplying electron from capacity layer particularly in ROS (Ghafarizadeh *et al.*,2021).

Furthermore, this powerful antioxidant promotes the synthesis of nitric oxide by widening the arteries and has a variety of other therapeutic applications. NAC works as an inhibitor of neutrophil Infiltration and balances the state of antioxidants and oxidants, in addition to controlling inflammatory mediators and thus protecting tissues (Ghafarizadeh *et al.*,2021). NAC activates several mechanisms to inhibit endoplasmic reticulum (ER) stress via;

1. to reduce the glucose regulating protein (GRP78), which is a major chaperone at the ER level and induces apoptosis by activating caspase 12.
2. It decreases triggering of the signal of unfolded protein response (UPR), which activates ER dysfunction and in addition brings apoptosis in body cells (Ji *et al.*,2013).
3. NAC inhibits the proliferation of two key ER stress bases, JNK (c-Jun N-terminal kinase) and C/EBP homologous protein (CHOP). These elements are transcription factors that decrease Bcl2 (the cell's anti-apoptotic factor) expression, resulting in apoptosis (Ji et al., 2013). MAP kinase p38, JNK, NF-κB, SAPK/INK (stress-activated protein kinase) and c-fos pathways are all inhibited by NAC. All of which can control several anti-apoptotic genes (Marmolino and Manto, 2010; Ghafarizadeh *et al.*, 2021).

1.3.4 NAC as an antioxidant

The imbalance between antioxidants and oxidants causes oxidative stress. A broad word that signifies molecules resulting from O2 is ROS which remain active species or can certainly develop active species (Crespy and Williamson,2004). Superoxide, peroxides, hydroxyl radicals, alpha-oxygen and singlet oxygen are all components of certain ROS

(Hayyan *et al.*,2016). Superoxide is produced when molecular oxygen levels fall, and it is the precursor to the majority of ROS (Turrens,2003).

The dismutation of superoxide produces H2O2. Hydrogen peroxide can be reduced partially, producing hydroxyl radicals and hydroxide ions, or completely reduced to H2O (Hayyan *et al.*,2016). ROS are basically energetic chemical mediators that result from the partial regeneration of oxygen (Cotter *et al.*,2007). ROS molecules are transient and capable of inducing DNA oxidative injury, acting as a marker of critical oxidative stress (Cotter *et al.*, 2007). Antioxidants can prevent oxidation because of their chemical structure, which can be divided into two types: fat-soluble and water-soluble. In blood plasma and cell cytosol water soluble antioxidants react with oxidants or vice versa, whereas phospholipid membranes protected from lipid peroxidation by fat-soluble antioxidants (Vertuani *et al.*,2004).

Glutathione cysteine group are reducing mediator, that can be decreased and oxidized reversibly. Free radicals cause damage to many macromolecules, including nucleic acids, proteins and lipids. Because of the high fatty acid content of sperm membranes and germ cells, these cells are highly vulnerable to lipid peroxidation. ROS also stimulates apoptosis, decreases testosterone production, and causes DNA fragmentation in Sertoli and germ cells, thus disrupting germinal epithelium (Malmir *et al.*,2018).

1.3.5 NAC on hepato-renal oxidative damage

NAC is extensively used in the treatment of pulmonary fibrosis, contrast-induced nephropathy, and chronic obstructive pulmonary disorder. It protects the kidney and liver from oxidative stress and apoptosis by releasing L-cysteine and cysteine, which scavenges free radicals (Millea,2009).

NAC administration within 10–18 hours of an acetaminophen overdose and alcoholism prevented liver damage and significantly reduced mortality. Potential anti-toxicity mechanisms include improved blood flow in the liver, glutathione replenishment and free radical scavenging. NAC also replenishes glutathione levels in HIV-infected patients that have been depleted by acetaminophen overdose (Zafarullah *et al.*,2003).

1.3.6 Medical uses

Since the 1960s, NAC has been a well-known drug; it is on WHO's list of 40 Essential Medicines. It has traditionally been used to treat paracetamol overdose (Salamon *et al.*,2019) mucolytic (Slattery *et al.*,2015) and to prevent the toxicity of several substances that result in the production of carbon monoxide and free radicals (Pannu *et al.*,2004). NAC is also used to

help with the treatment of neuropsychiatric and neurological conditions (Radomska *et al.,* 2012;Slattery *et al.,* 2015).

Experimental findings have discovered the beneficial uses of NAC correspondingly in non-alcoholic steatohepatitis (Oliveira *et al.,* 2008), chronic bronchitis (Stey *et al.,* 2000), arterial hypertension of diabetic etiology (Martina *et al.,* 2008) and chronic-obstructive-pulmonary disease (COPD) (Pirabbasi *et al.,* 2016) and also substance abuse disorders (McClure *et al.,* 2014), male infertility (Safarinejad , 2009), recurrent unexplained pregnancy loss (Amin *et al.,* 2008), diabetic retinopathy, polycystic ovary syndrome (Fulghesu *et al.,* 2002), age-related macular deterioration , cataract and dry eye syndrome (Radomska *et al.,* 2012).

In April 2019, 300 NAC clinical trials were registered on Clinical Trials.gov. (ClinicalTrials.gov, 2019). Renal conditions (trials 48), with a focus on prevention of radio contrast nephropathy, reno-protection during surgery and chronic kidney disease; and neurological disorders (trials 36) and psychiatric, primarily with Parkinson's disease, bipolar disorder, cognitive conditions, and schizophrenia, were among the common conditions examined by recorded interventional trials with NAC (in the absence of currently active studies). Mitochondrial abnormalities increased oxidative stress and glutathione deficiency in the brain have all been linked to schizophrenia. General and negative signs in schizophrenia might be lowered once 8-24 weeks of adjunctive cure with NAC (Chen *et al.*,2016) in disorders of neuropsychiatric and debated in detail. (Castro *et al.,* 2017).

Addictive disorders (trials 23) contribute to a common target with cannabis, tobacco, cocaine, alcohol, and other types of dependence. The NMDA receptors controlled by NAC may be concerned (Hopf,2017) and three studies at least argue for the use of NAC in addiction disorders (Echevarria *et al.,* 2017; Tomko *et al.,* 2018). Among further investigated usage of NAC were usually applications in pulmonary diseases and gastrointestinal problems (Salmon *et al.,* 2019).

Pre-clinical studies suggest that NAC has a broad range of applications in human disease prevention and maintenance. Asthma, Alzheimer disease, intrauterine growth retardation, inflammatory bowel disease, influenza insulin resistance and obesity are some examples, as are heavy metal toxicity, ischemic cardiovascular disease, age-related memory impairment and diabetic neuropathy (Salamon *et al.,* 2019).

It is promising as an anti-microbial adjuvant medicine because its ability to damage biofilms and improve antibiotic permeability. Pre-clinical experiments have also confirmed

that NAC supplement results in life extension and reduced aging causes in human breast epithelial stem cells, mammals, invertebrates. Such outcomes must, however, be reproduced in humans. Similarly, this is due not only to the radical scavenging activity of NAC, but also to apoptosis inhibition and telomerase activation, as demonstrated by its ability to slow down oocyte ageing. Antioxidants, on the other hand, can shorten or lengthen lifespan dependent on the dosage and redox balance. NAC has also grown popularity as a sports supplement that can help athletes recover faster and reduce muscle fatigue (Salamon *et al.,* 2019).

1.3.7 NAC as a Nutritive supplements

NAC, like other antioxidants, has been found to be extremely effective as a dietary supplement, nutraceutical, and pharmaceutical market. According to the international market for NAC is expected to grow at a compound annual growth rate of nearly 22%, up from 490 million USD in 2017, over the next years. Despite insufficient scientific evidence, dietary supplement retailers make several allegations about the ability of NAC to defend against pollutants, environmental toxins and treat a variety of conditions, prolong life span and boost testosterone levels even in men. The effects of taking NAC as a dietary supplement by various consumers there is little to no consistent information (Šalamon *et al.,* 2019).

NAC can reduce the toxicity of a broad range of toxins, including metals, pesticides, and drugs. It scavenges ROS and other toxic free radicals and raises glutathione levels in various tissues. It also prevents mitochondrial membrane peroxidation and apoptosis. The current study hypothesized that NAC would be tested to assess its protective effect against sodium arsenite-induced tissue toxicity in the liver and kidney of male mice.

1.4 Kisspeptin

Kisspeptin was originally called metastin. It was first isolated as a potential metastasis suppressor gene from a human malignant melanoma cell line (Lee *et al.,*1996) in Hershey, Pennsylvania, the hometown of the famous chocolate Hershey's kisses. Kisspeptins have human and non-human genes, respectively known as KISS1 and Kiss1, and human and non-human receptors, known as KISS1R and Kiss1r. KISS1 and Kiss1 gene products are collectively referred to as kisspeptin (Gottsch *et al.,*2009).

The kisspeptin precursor, a 145 amino acid peptide, is encoded by the KISS1/Kiss1 gene. (West *et al.,* 1998). It is proteolyzed into fragments of varying lengths. (Muir *et al.,* 2001; Ohtaki *et al.,* 2001; Kotani *et al.,* 2001) The main fragment is kisspeptin-54, which has 54 amino acids (Ohtaki *et al.,* 2001). Other fragments include kisspeptin-10, kisspeptin-13, and kisspeptin-14. An RFamide motif at the carboxy terminal is shared by these fragments.

(Kotani *et al.,* 2001; Muir *et al.*,2001; Ohtaki *et al.*,2001) Kisspeptins are expressed in the hypothalamus, gonads, placenta, liver and pancreas (Kotani *et al.*,2001; Muir *et al.*,2001;Ohtaki *et al.*,2001;Lee *et al.*,1999) and bind to KISS1R/Kiss1r with equal efficacy(Kotani *et al.*,2001).

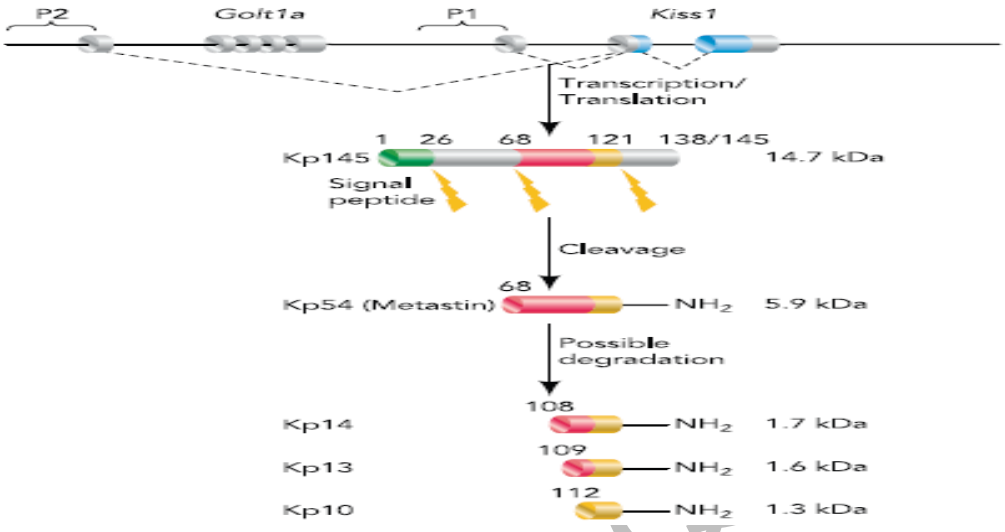

Fig1.5. (Xanvier and William, 2010)

Kiss1r, a G-protein coupled receptor (GPCR) with homology to the galanin family of receptors, was first discovered as an orphan receptor in the rat brain. It has approximately 45% sequence identity but no binding affinity to galanin (Lee *et al.*,1999). Later, KISS1R was discovered and was variably termed GPR54, AXOR12, hOT7T175, and HH8 (Kotani *et al.,* 2001; Muir *et al.*, 2001; Ohtaki *et al.,* 2001). The endogenous kisspeptin receptor, which is expressed in the pituitary gland, hypothalamus, pancreas, kidney, gonads, and placenta, was later identified as KISS1R/Kiss1r (Kotani *et al.*,2001; Muir *et al.*,2001; Lee *et al.*,1999; Messager *et al.*,2005; Irwig *et al.*,2004; Han *et al.*,2005; Parhar *et al.*,2004). KISS1R was discovered in 1999and it encodes a protein of 396 amino acids. It is a member of the rhodopsin family that has been mapped on chromosome 19p13.3 in humans.

Kisspeptin and its receptor are found in the hypothalamus of rodents at two neuronal sites, the arcuate nucleus (ARC) and the anteroventral periventricular nucleus (AVPV) (Gottsch *et al.*,2004). While it is primarily expressed in the infundibular nucleus of humans and primates, it is functionally equivalent to ARC in other mammalian species (Rometo *et al.*,2007). Female rodents have a higher population of kisspeptin neurons at the AVPV nuclei

than males, indicating sexual dimorphism (Clarkson and Herbison, 2006; Kauffman *et al.*, 2007). Similarly, in humans, such differential expression has been observed in the rostral periventricular area of the third ventricle (RP3V) and the infundibulum (Hrabovszky *et al.*, 2010; Hrabovszky *et al.*,2011).

KP10 and KP54 are the two most widely used native kisspeptins in human and animal research. In humans, KP10 has a terminal half-life of 4 minutes, whereas KP54 has a 28-minute terminal half-life (Tassigny *et al.*,2017; Jayasena *et al.*,2015; Dhillo *et al.*,2005). Kisspeptin functions as a neurotransmitter in some situations (for example, excitation of hypothalamic arcuate nucleus pro-opiomelanocortin (POMC) neurons; Fu and van den Pol, 2010), and as a hormone in others (e.g placental kisspeptin increasing insulin secretion during pregnancy; Bowe *et al.*,2019).

Dhillo *et al* conducted the first human kisspeptin study with KP-54 in 2005. They discovered that a 90-minute intravenous infusion of KP-54 significantly increased LH, FSH, and testosterone release with no side effects. LH secretion reached near-maximal levels, whereas FSH and testosterone showed less marked responses. Kisspeptins have been investigated as potential treatment targets for melanoma (Shircarcinoma *et al.*,2001), thyroid cancer (Ringel *et al.*,2002), bladder cancer (Carbayo *et al.*,2003), squamous cell carcinoma of the esophagus (Ikeguchi *et al.*,2004), gastric cancer (Dhar *et al.*,2004), hepatocellular carcinoma (Ikeguchi *et al.*,2003)and breast cancer(Lee *et al.*, 1996; Olbrich *et al.*,2010).

Kisspeptins are also described as excitatory neuromodulatory peptides for multiple homeostatic systems such as puberty initiation, anti-oxidative effect, regulation of prolactin release, glucose homeostasis, role in nutrition status and fertility, role in pregnancy, increased sensitivity of chemoreceptors on type 2 carotid bodies, anti-metastatic effect, hot flushes in postmenopausal women (Ohtaki *et al.*, 2001).

1.4.1 Structure

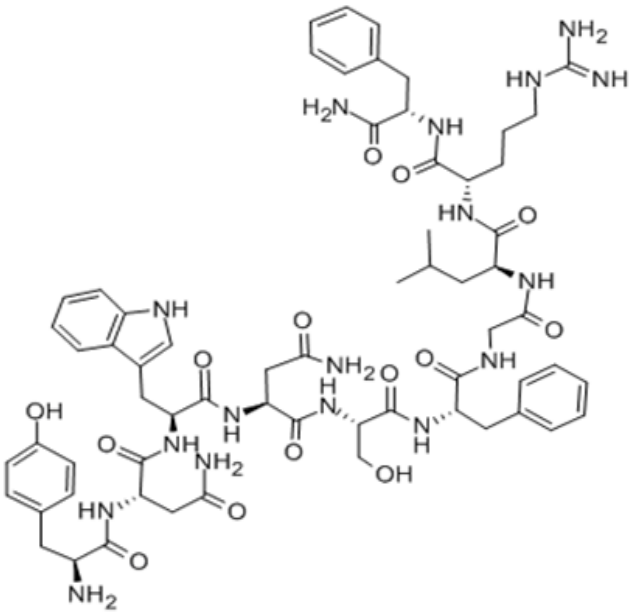

Fig1.6. (Olaniyan *et al.*,2013)

1.4.2 HPG AXIS

Kisspeptin has been identified to stimulate the hypothalamic-pituitary gonadal axis and to enhance the antioxidant system against oxidative damage in recent investigations (Milton *et al.*,2012; Akkaya *et al.*,2014). The reproductive axis is dependent on the dynamic interplay of neural and hormonal signals originating from three primary sources: the anterior hypothalamus, where GnRH is synthesized and secreted in pulses; the anterior pituitary, where GnRH pulses stimulate pituitary gonadotrophin (luteinizing hormone (LH) and follicle stimulating hormone (FSH)) secretion; and the gonads, which respond to the trophic actions of gonadotrophins by secreting sex steroids and producing gametes (Clarke *et al.*,2011; Navarro and Sempere, 2011). These sex steroids, in turn, 'feedback' to the GnRH neurons in the hypothalamus, regulating their activity (Smith ,2013), constituting the hypothalamic-pituitary-gonadal (HPG) axis. Kisspeptin is assumed to be active primarily as a 54 amino acid peptide, whereas in mice, it exists as 52 amino acids (Ohtaki *et al.*, 2001; Terao *et al.*, 2004).

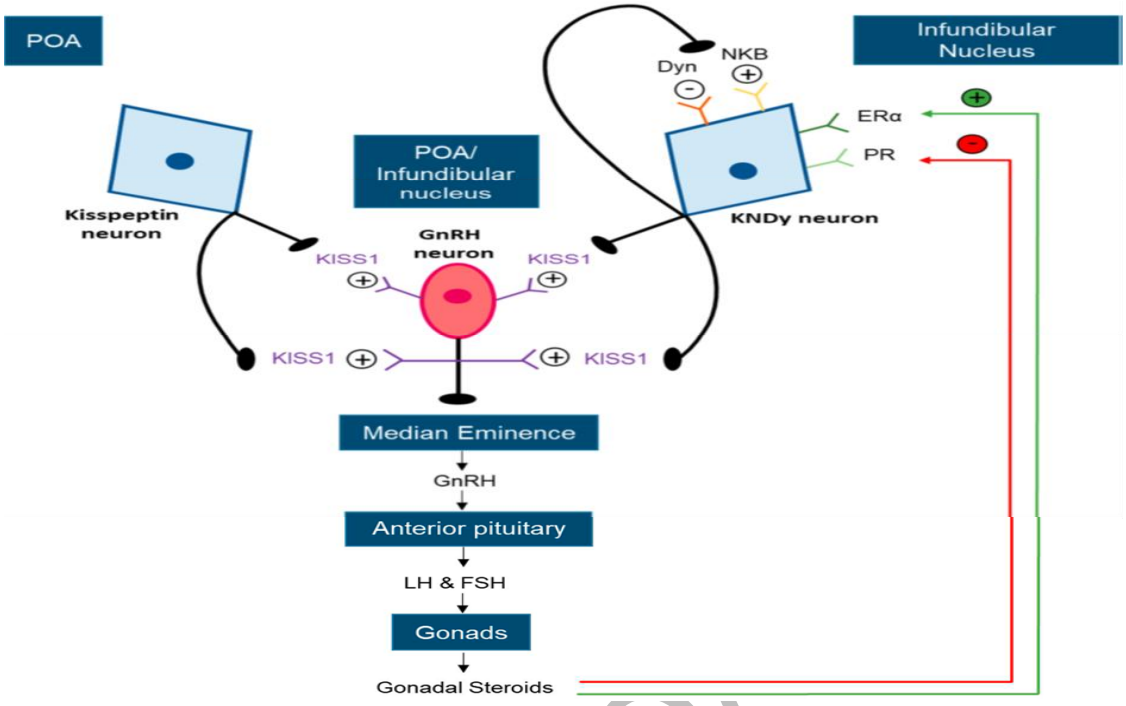

Fig1.7. Schematic diagram showing the neuroanatomy and regulation of the reproductive axis in humans (Yang,2020)

Neurokinin B and dynorphin receptor colocalization on kisspeptin neurons in the infundibular nucleus acts to autoregulate pulsatile kisspeptin secretion. Kisspeptin activates the downstream cascade of GnRH and gonadotrophin secretion by acting on kisspeptin receptors on GnRH neurons. KNDy neurons get both negative and positive input from gonadal hormones.

1.4.3 Mechanism of Action of Kisspeptin

When kisspeptin binds to its G-protein coupled receptors, it forms a composite that activates several physiological pathways. All of this results in the activation of phospholipase C (PLC-â) by the release of intracellular calcium reserves. Other intracellular pathways that occur after kisspeptin receptor activation include accumulations of inositol-1,4,5-triphosphate, hydrolysis of phosphatidyl inositol 4,5-bisphosphate, release of arachidonic acid, and phosphorylation of extracellular signal-regulated kinases 1/2 and p38 mitogen-activated protein kinases (MAPK) (Kotani *et al.*,2001; Castellano *et al.*,2006) calcineurin and nuclear factor-kappa B (NF-kB) (Castellano *et al.*,2006).

Furthermore, KISS1 has been shown to activate other kinases in the cytosol, including myeloid cell leukaemia 1, calcium/calmodulin-dependent kinases, and tyrosine kinases (Becker *et al.*, 2005), resulting in the breakdown of many proteins in an array, causing a very small amount of the hormone (kisspeptin) to have a strong effect on the target organ (Xanvier and William, 2010). DAG also promotes depolarization of GnRH neurons by activating a nonselective cation channel (TRPC) and inhibiting an inwardly rectifying potassium channel (Xanvier and William, 2010). Kisspeptins therefore operate directly on GnRH neurons to cause persistent depolarization and enhance the rate of action potential firing (Xanvier and William, 2010).

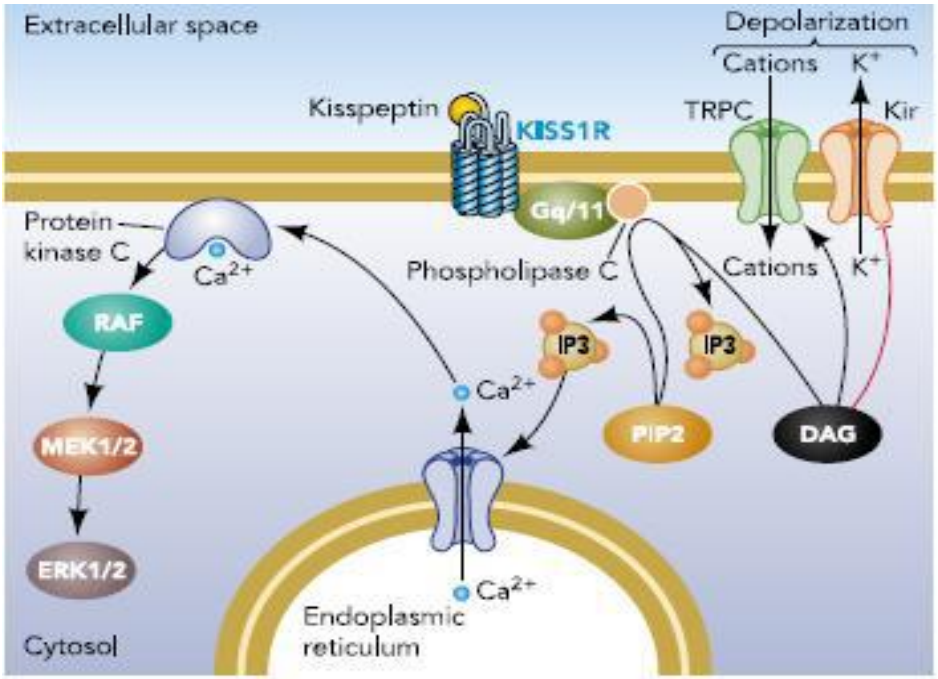

Fig1.8. Mechanism of kisspeptin receptor action and GnRH neuron depolarization (Xanvier and William, 2010).

1.4.4 Kisspeptin and body weight

Although more research is needed to understand kisspeptin's physiological involvement in body weight regulation, attention has also been drawn to kisspeptin's pharmacological activities. These studies show that the duration and route of administration of kisspeptin affect its effect on body weight. Kisspeptin-10 i.e. dosages ranging from 0.3 to 3 nmol had no effect on body weight in male rats 24 hours after administration (Thompson *et al.*,2004). In male rats, chronic peripheral administration of kisspeptin-54 (50 nmol/day subcutaneously for 13 days) had no impact on body weight (Thompson *et al.*,2006).

However, species-specific effects have been seen, with intraperitoneal treatment of sea cucumber kisspeptin-10 on alternate days for 40 days causing intestinal atrophy and weight loss in both male and female sea cucumbers (Wang *et al.*, 2020).

1.4.5 Kisspeptin and appetite/food intake regulation

Kisspeptin appears to be involved in the complicated regulation of appetite and food intake, according to in vitro and in vivo research. Kisspeptin and POMC neurons have reciprocal connections in the hypothalamus arcuate nucleus of mice and sheep, as do kisspeptin and neuropeptide Y (NPY) neurons (Backholer *et al.*, 2010; Fu& van den Pol, 2010). Kisspeptin stimulates POMC neurons in a dose-dependent manner, which is inhibited by a kisspeptin receptor antagonist (Fu and van den Pol,2010). Kisspeptin also inhibits NPY neurons (Fu and van den Pol, 2010), indicating that it has anorexigenic characteristics (as POMC reduces appetite and NPY increases appetite).

1.4.6 Kp and stress

Heat stress can increase the generation of ROS because the pattern of gene expression of heat-stressed animals and that of oxidative stress is identical (Slimen *et al.*, 2014). ROS, which include hydrogen peroxide, hydroxyl radicals, and free radicals, play a crucial part in normal physiology, but their overproduction may be the cause of several clinical conditions**.** Scavenging enzyme systems, such as catalase and superoxide dismutase (SOD), are available to protect cells from ROS damage (Orabi *et al.*,2011).

The cell's homeostasis is regulated by the balance of oxidation and reduction processes. Oxidative stress also limits steroid function, resulting in decreased testosterone production and metabolism (Li *et al.*,2016; Aydilek *et al.*,2015). Lipids are vulnerable to oxidation, and lipid peroxidation products are possible indicators for oxidative stress level in vivo and associated stress (Niki,2008). Increased ROS generation in mitochondria during heat stress results in non-specific lipid alteration, terminating in bioenergetic malfunction (Slimen *et al.*,2014). Hepatocyte membranes are high in unsaturated fatty acids, which are sensitive to oxidative damage by ROS. As a result, greater levels of lipid peroxides and a deterioration in the antioxidant system may be linked to lower amounts of circulating testosterone and gonadotropins. Furthermore, it is now well demonstrated that peripheral and intracerebroventricular injection of Kisspeptin has a stimulatory action on testosterone and gonadotropin levels. (Castellano *et al.*,2006; Cerrato *et al.*,2006; Clarke,2002).

1.4.7 Kp and puberty

Kisspeptin is thought to play an important function in the initiation of puberty as well. Seminara and colleagues evaluated the genomes of these families to identify a genetic contributing to this disorder and revealed that affected people were homozygous for a 'L148S' mutation in the GPR54 gene - Kiss1r. (Seminara *et al.,*2003) developed a mouse model missing Kiss1r and observed that these animals possessed the same phenotype of hypo gonadotrophic hypogonadism, with smaller testes in male mice and small ovaries in females, as well as an impairment of follicular differentiation and a delayed in opening of the vagina. However, exogenous GnRH treatment to these mice recovered them to a more normal phenotype, demonstrating that kisspeptin works via increasing GnRH secretion (Seminara *et al.,* 2003).

Furthermore, the importance of peripherally expressed kisspeptin has been verified by pharmacological studies utilizing kisspeptin antagonists that prevented kisspeptin function in the rat ovary, limiting ovarian development (Ricu *et al.,* 2012). Kisspeptin injections straight into the ovaries reversed ovarian maturation (Fernandois *et al.,* 2017).

1.4.8 Kp and Reproduction

Kisspeptin's importance in reproduction was initially discovered in humans and mice with mutations in the Kiss1r (and later, Kiss1) gene, which result infertility, delayed puberty, and very low levels of gonadotropins and sex steroids (Tassigny *et al.,* 2007; Roux *et al.,* 2003; Funes *et al.,* 2003; Seminara *et al.,* 2003). KP54 and KP10 are new medications licenced by the US Food and Drug Administration (FDA) for the treatment of reproductive system problems. They are currently being examined in phase 1 or 2 clinical studies. (KP54, ClinicalTrials.gov Identifier: NCT02081924, NCT01667406;KP10, ClinicalTrials.gov Identifier: NCT00914823). In animal investigations, KP54 has greater blood-brain barrier penetration and longer-lasting pharmacokinetics than KP10 (Tassigny *et al.,* 2017). According to Aslan *et al.,*2017, oxytocin and kisspeptin enhanced SOD and GSH levels while decreasing MDA levels, which correlated with histopathologic tissue damage recovery in the ovary and uterine (Aslan *et al.,*2017). Pre-exposure of sperms to KP can prevent sperm quality, particularly motility and DNA integrity, from the damaging effects of the freeze-thaw cycle (Kermani *et al.,*2020).

Kisspeptin has recently been revealed to be involved in metabolism and energy balance control, in addition to its well-known involvement in driving reproduction. Kisspeptin neurons have been shown to be governed by metabolic factors and conditions,

along with their ability to integrate multiple peripheral metabolic cues (e.g., leptin, insulin, and ghrelin) both directly and indirectly elsewhere (Castellano and Sempere, 2016;Ziarniak and Sliwowska, 2018; Navarro, 2020;Sempere and Castellano, 2020; Talbi and Navarro, 2020).

1.4.9 Kp and Metabolism

KP receptor deletion mice had increased body weight and obesity, poor glucose homeostasis, and decreased energy expenditure (Tolson *et al.*,2014; Tolson *et al.*,2016). KP-10 may influence glucose homeostasis by its effects on cellular glucose absorption, insulin secretion, and the expression of enzymes involved in rate-limiting processes in glycolysis (Song *et al.,* 2014; Song and Zhao,2016; Liu *et al.*,2013). KP-10 enhanced lipogenesis in vivo but inhibited lipid accumulation in vitro by boosting lipolysis at the detriment of lipogenesis. KP-10 injections in Japanese quail raised triglyceride (TG) and total cholesterol (TC) levels in the blood and liver (Wu *et al.*, 2012). The daily injection of KP-10 into quails for 21 days raised the amount of TC in the liver, which was accompanied by an increase in the transcription of the cholesterol-7alphahydroxylase gene (Zuxiang *et al.*,2012).

Preclinical investigations have shown that KPs therapy is effective for Alzheimer's disease (Milton *et al.*, 2012) and oxidative stress-induced brain damage (Akkaya *et al.*, 2014). KP-10 enhanced superoxide dismutase and catalase activity while decreasing malondialdhyde levels in the hepatic tissues of young male rats (Aydin *et al.*,2010). It inhibited L-pro-apoptotic methionine's action in rats by lowering malondialdehyde levels, raising glutathione levels, and activating superoxide dismutase (Akkaya *et al.*,2014).

Exogenous KPs therapies have been found in rat models to work as antioxidants and reduce oxidative damage to the liver and brain (Aydin *et al.*, 2010; Akkaya *et al.*, 2014). After oxidative brain damage, KPs therapy decreased neuronal death by boosting the activities of superoxide dismutase (SOD) and catalase (Akkaya *et al.*, 2014).

1.4.10 Kp and kidney

Shoji et al. (Shoji *et al.*,2010) discovered that kisspeptin and Kiss1r are expressed in renal tubular cells, collecting duct cells, and vascular smooth muscle cells in the rat kidney, and that under conditions of chronic renal impairment, Kiss1r protein levels are greatly reduced, trying to imply a role for kisspeptin signaling in kidney function. In another study, (Yi *et al.*,2010) found that in the embryonic kidney of Kiss1r null mice, branching morphogenesis and glomerular formation are slowed, which presumably predisposes the adult kidney to a decrease in glomerular number. The scientists hypothesized that the

deficiency in the Kiss1r null mice arose from decreased expression of bone morphogenetic protein 7, which is needed for kidney organogenesis. Both data clearly imply that decreased kisspeptin signalling in the rat kidney can affect function of this essential organ, but more research is needed to discover if this occurs or whether the foetal phenotype culminates in primary hypertension and chronic kidney failure in the adult mouse.

1.4.11 Kp and liver

Kisspeptin administration altered the liver metabolite profile and then cured liver damage in heat-stressed rats. Numerous metabolites, including purine metabolism, lipid metabolism, and amino acid metabolism, were altered in the heat stress group, and were sometimes restored by kisspeptin intervention (Hou *et al.*,2017).

For the first time, Sies demonstrated kisspeptin's effects on oxidative stress. SOD and CAT are antioxidant scavengers that help to mitigate the negative effects of ROS (Sies,1997). A decrease in the activity of these enzymes causes oxidative stress due to an increase in the amount of ROS (Muthuvel *et al.*,2006). In his work, Aydin et al.,2010 define 'direct impact' as effects of kisspeptin exerted through GPR54 receptors, however 'indirect effect' refers to effects caused by GnRH stimulation of gonadotropins and sex hormones. High levels of SOD and catalase were only discovered in kisspeptin-treated mice, indicating that kisspeptin's antioxidant effects are primarily indirect (Aydin *et al.*,2010). Previous research has found a link between oxidative stress and go(Murugesan ,2006; Muthuvel *et al.*,2006;Murugesan *et al.*, 2007; Hurtado *et al.*,2007). The generation of oxidative stress is further aided by a reduction in LH and FSH production caused by hypothalamic dysfunction. It has already been demonstrated that aroclor 1254 (a polychlorinated biphenyl) causes oxidative stress in the anterior pituitary, which is reversed by therapy with an antioxidant, vitamin C, leading to normal testosterone synthesis (Muthuvel *et al.*,2006; Murugesan *et al.*,2007).

The activities of catalase, superoxide dismutase (SOD), xanthine oxidase (XO), adenosine deaminase (AD), and malondialdehyde were tested in liver tissue. Kisspeptin was found to boost the activity of SOD and catalase (Aydin *et al.*,2010). SOD is an oxido-reductase that catalyses the dismutation of superoxide anion into molecular oxygen and hydrogen peroxide. CAT detoxifies hydrogen peroxide, CAT is a well-known antioxidative enzyme that has been involved in hydrogen peroxide protection, and their location is restricted to the peroxisome (Aydin *et al.*, 2010).

Since KP-10 is an endogenous hormone that is safe to administer in animal studies and even people, it has been suggested as a possible therapeutic treatment (Abbara *et al.*,

2020; Qureshi and Fatima, 2020). The studies illustrate that exogenous peripheral kisspeptin administration can have antioxidant effects. Kisspeptin's metastasis suppressor function is hypothesized to have a protective impact on the liver and kidney. In this research, I will investigate the effects of kisspeptin and n acetyl cysteine alone and in combination on many oxidative stress parameters, as well as other enzyme activities involved in liver and kidney toxicity, histological abnormalities, and serum lipid parameters against arsenic in adult male albino mice.

1.5 AIM AND OBJECTIVES

The aim of the current study was to evaluate the protective effects of Kisspeptin-10 and N-Acetyl Cysteine against sodium arsenite induced toxicity in liver and kidney of adult male mice.

Objectives

- To evaluate biochemical alterations induced by sodium arsenite.
- To evaluate histological alterations induced by sodium arsenite.
- Comparative assessment of biochemical, and histoprotective effects of Kisspeptin-10 and N-acetyl cysteine against sodium arsenite-induced toxicity in kidney and liver of adult male mice.

MATERIALS AND METHODS

2. 1.1 Animals and Maintenance

Male albino mice (BALB/c, n = 66) were purchased from the National Institute of Health (NIH), Islamabad, weighting of 28-35 gram. They were housed in the Animal House Facility of Quaid-i-Azam University Department of Zoology, in Islamabad. The animals were kept in polycarbonate rodent cages. Standard laboratory conditions were followed, including a 12:12 light: dark photoperiod, a relative humidity of 35-40% and a temperature of 25 ± 4ºC. The animals were provided fresh drinking water ad libitum and standard rodent chow. By keeping six animals per cage, stress caused by overcrowding was avoided. Animals were acclimated for one week prior to the start of the experiment.

2.1.2 Bioethical approval

The research was performed in the Animal and Human Physiology Laboratory of the Department of Zoology at Quaid-i-Azam University, Islamabad. Animal handling and preliminary experimental procedures were carried out in accordance with the guidelines established by the "Bioethics-committee of the Department of Biological Sciences, Quaid-i-Azam University, Islamabad" for the care and use of animals in scientific research.

2.1.3 Chemicals

Chemical were obtained from Sigma-Aldrich (Sigma, St. Louis, Missouri, USA). KP-10 (KiSS-1; 112-121; H-YNWNSFGLRF-NH2) was obtained as lyophilized powder from Calbiochem (La Jolla, CA, USA). Sodium pentobarbital (Sigma, Germany) and Heparin (Kota Bharu, Kelantan, Malaysia) were purchased locally. Inorganic sodium arsenite (NaAsO2) and N acetyl cysteine (NAC) were purchased from Sigma-Aldrich (Germany). Formaldehyde, chloroform, NBT, ferrous sulphate, comassive blue, Triton-x100, BSA, Sodium chloride, Potassium chloride, SDS, PMSF, DEPPD, sodium azide, sodium hydroxide, hydrogen peroxide, Tris-HCL, TBA, TCA, riboflavin, potassium phosphate monobasic, L-methionine, potassium phosphate dibasic, sodium phosphate dibasic, guaicol, sodium phosphate monobasic, Tri-sodium Citrate, Eosin, DTNB, phosphoric acid, Hematoxylin, Methanol, etc.

2.1.4 Experimental design

Mice (n=66) were selected randomly and allocated to eleven groups; each contained six animals. Experimental groups were:

1. Control having free access to drinking tap water without any treatment
2. KP alone+ drinking tap water
3. NAC alone + drinking tap water

4. NaAsO2 alone 4 ppm in drinking water
5. NaAsO2 4 ppm orally in drinking water + KP
6. NaAsO2 4 ppm orally in drinking water + NAC
7. NaAsO2 4 ppm orally in drinking water + KP+NAC
8. NaAsO2 alone 10 ppm in drinking water
9. NaAsO2 10 ppm ppm orally in drinking water + KP
10. NaAsO2 10 ppm + NAC
11. NaAsO2 10 ppm + KP + NAC

2.1.5 Measurement of the weight

Prior to the start of the experiment, body weights of mice were measured in g. With a two-day intermission each week, animals were weighed to administer the proper dose based on b/w and to observe weight loss or gain because of the dose administered.

2.1.6 Dose selection

Doses of NaAsO2, NAC and KP-10 for current experiment were selected following (Reddy et al., 2011, Ayturk et al., 2017; Guvenc et al., 2018; Fatima and Qureshi, (2021), Regimen for KP-10 treatment was limited to 'once a week intraperitoneal administration' at the dose of 50nmol/once a week.

2.1.7 Preparation of Dose

Doses of KP-10 and NaAsO2 (4ppm and 10ppm) were prepared from stock solution. All doses were freshly prepared before use. For NaAsO2, 100 ppm stock solution was prepared in distilled water. This was further diluted with drinking tap water to obtain the required concentrations of low (4 ppm) and high doses (10 ppm). For KP-10, 1 mg lyophilized powder was used to make stock solution in normal saline, and final injectable dose of 50 nmol/day was worked out in distilled water. NaAsO2 was given orally in drinking water while, KP-10 was administered as intraperitoneal (i.p) injections. Considering the outcomes of our previous study (Fatima and Qureshi, 2021), regimen for KP-10 treatment was also limited to 'once a week intraperitoneal administration' at the dose of 50nmol/day Whereas, NAC was intraperitoneally administered to experimental mice on 5 alternative days at the dose of 75 mg/kg b.w.

2.1.8 Dosing time

The dosing time was kept constant throughout the experiment to avoid any possible stress or resistance from the animal. Dose synchronization with specific time is critical because it prepares the animal physiologically and psychologically for the dose.

2.1.9 Dissections or Necropsy

At the end of experimental duration of 35 days, mice were fasted overnight and weighed next morning. Then, these were humanely sacrificed by using an overdose of sodium pentobarbital (80 mg/kg b.w.). With 3 ml heparinized syringes, blood was drawn directly from the heart. For serological analysis, 3 ml of blood was transferred to gel tubes (BIOTUBE BT, Vacuumed Tube Sterile, Gel Clot Activator) and allow to clott at room temperature for 30 min. Gel tubes were then centrifuged at 12204 g for 5 mints to separate the serum. After centrifugation, serum was transferred the 1.5ml autoclaved Eppendorf tubes via micropipette and stored at -20°C until analyzed. Liver and kidneys were removed using saline-rinsed sterilized surgical instruments. Half of the organs were wrapped in aluminum foil and stored at -20°C for biochemical analysis and half of them preserved in 10% buffered formalin for histological analysis. Following formula was used to calculate relative organ weight:

Weight of the organ (g)÷Weight of the animal body (g)×100

2.2.10 Biochemical parameters

Serum samples were processed on a UV-VIS spectrophotometer (Agilent-8453, California, U.S.A.) using commercial kits (Lab kit, Barcelona Spain) to analyze biochemical parameters. The total lipid profile, renal functional test and liver function tests were carried out in accordance with the standard protocols provided in the kits. The temperature for all enzymatic activities was kept at 37ºC. The results are presented in units as specified by the manufacturer. All the above-mentioned biochemical parameters were determined using commercially available kits.

2.3 Serum biochemistry

2.3.1 Aspartate amino transferase (AST/GOT)

Reagent composition

Reagent 1 (R1): R1 contained L-Aspartate 200 mmol/L, Tris 80 mmol/L of pH 7.8, Lactate dehydrogenase (LDH) 800U/L and Malate dehydrogenase (MDH) 600 U/L Reagent 2 (R2): R2 contained NADH 0.18 mmol/L and alpha-Ketoglutarate.

Principle

The enzyme aspartate-aminotransferase (AST) catalyses the reversible transamination of aspartate to form alpha-ketoglutarate, which results in the formation of oxaloacetate and glutamate. Oxaloacetate is converted to malate in the presence of malate dehydrogenase (MDH) and NADH. The concentration of NADH in the sample is proportional to the activity of the AST catalytic concentration.

Procedure

Working reagent (WR) was made by combining R1 and R2 in a 4:1 ratio. 100 µL of distilled water and 100 µL of sample were mixed with 1000 µl of WR for the blank and sample, respectively. For 1 minute, the contents were incubated at 37°C. At 340 nm, there was a change in absorbance. Final calculations for the test were done using the formula as followed: AST/GOT U/L = ΔA/min x 1750

2.3.2 Alanine-aminotransferase (ALT-GPT)

Reagent composition

Reagent R1: R1 contained L-alanine 750 mmol/L, Tris buffer 150 mmol/L of pH 7 and Lactate dehydrogenase > 1.350 U/L. Reagent R2: R2 contained 2-oxoglutarate 75 mmol/L, NADH 1.3 mmol/L.

Principle

"The enzyme ALT catalyzes the transfer of an amino group to oxoglutarate, as a result the formation of glutamate and pyruvate. In the presence of nicotinamide-adenine-dinucleotide (NADH), pyruvate reduce to lactate through the enzyme, lactate dehydrogenase also reduced and NADH is also oxidized to NAD+".

Procedure

Working reagent (WR) was made by carefully stirring together 4 parts R1 and 1-part R2. After incubation at 37ºC in an incubator, distilled water (100 µl) and serum (100µl) from each sample were combined with 1000 µl of WR to run the blank and sample, respectively. At 340nm, the absorbance per minute (ΔA/min) was measured. The following formula was used to calculate the final ALT quantity: ΔA/min x 1746 = ALT/GPT activity [U/L] at 37ºC.

2.3.3 Alkaline phosphatase (ALP/DEA)

Reagent composition

Reagent 1 (R1): R1 contained DEA buffer Magnesium Chloride 0.6mmol/L, 1.25mol/Lof pH 10.2; Reagent 2 (R2): R2 contained 4-Nitrophenylephosphate 50 mmol/L

Principle

The enzyme ALP catalyses the hydrolysis of 4-nitrophenylephosphate (4-NPP), resulting in the formation of 4-nitrophenol and free inorganic phosphate. The alkaline buffer acts as a phosphate group acceptor. Absorbance was measured at 405nm. ALP activity in the sample is equivalent to the formation of 4-nitrophenol.

Procedure

R1 and R2 were mixed in a 4:1 ratio to make the working reagent. Following that,1000µl of working reagent and 20 µl of samples were gently mixed. After 1 min of incubation at 37ºC, absorbance was measured at 405 nm. To calculate the average value,

readings were taken in triplicate after 1min intervals. The following formula was used to calculate the final concentration of ALP: ΔA/min x 2764 = ALP activity (U/L)

2.3.4 Renal Function Test (RFT's)

Creatinine

Reagent's composition

Reagent 1 (R1): Picric reagent contained 17.5 mmol/L of Picric Acid Reagent 2 (R2): Alkaline reagent contained 0.29 mol/L of Sodium Hydroxide creatinine calibrator: Creatinine aqueous primary calibrator, 2 mg/dl.

Principle

This test is based on the reaction of creatinine with sodium picrate (Alkaline picrate),which leads to the formation of a red complexion. Color strength equates to creatinine concentration in the sample.

Procedure

R1 (Picric reagent) and R2 (Alkaline reagent) were carefully mixed in equal parts. To measure the absorbance of the standard, blank and sample, 100 µl of standard, distilled water and serum were mixed with 1000 µl of working reagent. For 1 min, samples were incubated at 37ºC. At a wavelength of 492 nm, absorbance was measured after 30 seconds and again after 90 seconds ΔA (A2-A1) was discovered. The following formula was used for the calculation of final concentration: Creatinine (mg/dL) = (ΔA Sample) / (ΔA) Calibrator x 2(Calibrator conc.)

2.3.5 Lipid profile

2.3.6 Total cholesterol

Working solution: It contained phosphate buffer 30.0 mmol/l of pH 7, 4-aminoantipyrie 0.25 mmol/l, 25.0 mmol/l of phenol, > 5.0 of KU/l peroxide, > 150.0U/l of cholesterol esterase, > 100.0 U/L of cholesterol oxidase, < 0.01 % of sodium azide and standard 200 mg/dl.

Principle

Cholesterol concentration is determined by the oxidation and enzymatic hydrolysis processes. The reaction of hydrogen peroxide with 4-aminoantipyrine produced red dyestuff, in the presence of phenol and peroxidase. The concentration of the formed colour is proportional to the concentration of cholesterol.

Procedure

Take a 10 µl of serum and standard were mixed with 1000 µl of WR from each sample (working reagent). The contents were then incubated in an incubator for 5 min at

37ºC. The samples' absorbance was measured at a wavelength of 500 nm. The following formula was used to perform the final calculation: c = 200 x δA (sample) / δA (standard) (mg/dl)

2.3.7 Estimation of Triglyceride concentration

Reagents (R):

Pipes buffer of 40 mmol/l of pH 7.0, 5 mmol/l of 4-Chlorophenol, ATP 1 mmol/l, 5 mmol/l of Magnesium ion, ≥ 1 U/ml of Peroxidase, ≥ 1 U/ml of Glycerol kinase, 0.4 mmol/l of 4-aminoantipyrine, ≥ 3.5 u/ml of Glycerol-3-phosphate oxidase, 0.05 % of Sodium azide and 200mg/dl or 2.28 mmol/l of Standard.

Principle

Triglyceride hydrolysis is caused by the enzymatic activity of the enzyme lipoprotein-lipase. Under the influence of peroxidase catalytic activity, a colored phenazone is formed from 4-aminoantipyrine, hydrogen peroxide and 4-chlorophenol.

Procedure

For this assay, 10 µl of blank, standard and serum were mixed with 1000 µl of working solution. Contents were incubated for 5 min at 37ºC. The final absorbance was taken three times at a wavelength of 500 nm within 60 sec. Using the followed formula, the final values were calculated:

c = δA (sample)/ δA (standard) (mg/dl)

2.4 Biochemical analysis of tissue

"The biochemical analyses of kidney and liver, tissue was performed to analyze the activity of ROS, indirect measurement of Lipid peroxidation through thiobarbituric-acid-reactive substances (TBARS), enzymes of antioxidant, the peroxide dismutase (POD), Catalase (CAT), superoxide dismutase (SOD) and non-enzymatic reduced Glutathione (GSH) and total protein concentration".

2.4.1 Preparation of Extract buffer (Lysis buffer)

For the preparation of extract buffer, 5.95 g of HEPES, 0.1 g of Sodium azide, 0.5 g of SDS, 4.38 g of NaCl were added into 495 ml of distilled water and 5 ml of Triton-x-100 was then added to take the final volume of extract buffer up to 500 ml.

2.4.2 Method of preparation of tissue homogenate

Kidney and liver tissues were weighed (100 mg) and minced in frosted petri dishes using a hand-held manual homogenizer (GPE limited, UK). Tissues were homogenized in 1ml of extract buffer (Lysis buffer, pH 7.0), which also contained 0.1mg of PMSF. The

homogenate was then centrifuged at 5031 g for 10 min to separate the supernatant and taken into the labelled autoclaved 1.5 ml Eppendorf tubes and stored at -20°c for biochemical studies.

2.5 Oxidative Profile Parameters

2.5.1 Estimation of Reactive oxygen species

Protocol

The ROS concentration in tissue homogenates was determined using the protocol of(Hayashi et al., 2007). To make 0.1M sodium acetate buffer, 4.1 g of sodium acetate was first dissolved in 500 ml of distilled water (PH-4.8). To make Reagent 1, 10 mg of N.N-Diethylpara phenyldiamine sulphate (DEPPD) was dissolved in 100 ml of buffer. 50 mg of ferrous sulphate dissolve in 10 ml of sodium-acetate buffer to prepare stock solution of FeSo4 and 62.5 µl of FeSO4 from the stock solution was dissolved in 125 ml of sodium acetate buffer to prepare Reagent 2. Both Reagent 1and Reagent 2 were mixed in a 1:25 ratio and left in the dark for nearly 2 min. In a 3 ml cuvette, 1200 µl of sodium acetate buffer, 1680 µl of reagent mixture and 60 µl of homogenate sample were placed. A (UV-visible spectrophotometer) was used to measure absorbance at 505 nm (Agilent 8453, USA). Then three readings were taken

at the interval of 30 second for each sample.

2.5.2 Analysis of lipid peroxidation assay (TBARS)

Protocol

Malondialdehyde (MDA) was determined in the homogenate using the method described by Iqbal et al., (1996) by reacting it with thiobarbituric acid (TBA). This method indirectly determined oxidative stress caused by lipid peroxidation. A reaction mixture of 0.1 ml ascorbic acid (1.5 mM), 0.1 ml 50 mM Tris-HCL, 0.1 ml FeSO4 (1mM), 0.6 ml distilled water and 0.1 ml tissue homogenate was prepared in a 15 ml falcon tube. The mixture was vortexed and the incubated for 15 min at 37°C. Following that, 1ml of trichloroacetic acid (10 %) and 1 ml of Thiobarbituric acid (0.375 %) were added to the reaction mixture and at 90°C for 15 mints boiled in water bath. The above mixture was centrifuged at 3000 rpm for 10 min. The supernatants were then transferred to a cuvette and absorbance at 532 nm was measured. Then three readings were taken at the interval of 30 second for each sample.

2.5.3 Antioxidant enzymes.

Enzymatic antioxidants are CAT, SOD, POD and GSH.

2.5.4 Catalase Assays (CAT)

Protocol

The activity of CAT in tissues was determined using a protocol modified slightly from (Aebi, 1984). In 3 ml of cuvette, 1000 µl of H2O2 (5.9 mM), 50 mM of potassium phosphate buffer (1.99 ml) pH: 7.00 and 0.1 ml of homogenate sample were mixed. Absorbance at 240 nm was measured for each sample after every 30 seconds and three readings were taken and then averaged.

2.5.5 Superoxide dismutase (SOD)

Protocol

SOD activity was determined by using the (Kakar et al., protocol 1984). 4.5 mL of 9.9 mM L-Methione, 2.25 mL of Triton X-100 (0.025 percent) and 3 mL of 57 µM Nitroblue Tetrazolium were combined to make the reagent (NBT). By adding 50 mM Phosphate buffer saline, the final volume was increased to 90 ml (PBS) The pH is 7.8. One ml of the above mixture was transferred to a cuvette and 20 µl of sample was then added to each cuvette. These were then exposed to a fluorescent lamp for 7 min before being incubated at 37°C for 5 min. Later, 10 µl of chilled Riboflavin was added to the reaction mixture to start the process and the contents were then incubated at 40°C for 8 min. Then, at 1-minute intervals, three readings at 560 nm were taken.

2.5.6 POD Assay

Protocol

The concentration of POD in the tissue homogenate was determined using the (Chance and Maehly method 1955). 2.5 ml of 50 mM phosphate buffer, 0.1 ml of enzyme extract and 0.1 ml of 20 mM guaiacol were added to the reaction mixture. The contents were vigorously mixed to form a homogeneous solution and 0.3 ml of 40 mM H2O2 was added to the reaction mixture. After one minute, there was a change in absorbance of the reaction mixture at 470 nm.

2.5.7 Determination of Reduced glutathione (GSH)

Protocol

Reduced Glutathione was determined using the method defined by (Jollow et al.,1974). 1 ml of disodium phosphate buffer (0.4 M), 0.1 ml of tissue homogenate and 0.5 ml of DTNB were combined to make the reagent mixture. DTNB (Ellman's reagent) was created by dissolving 40 mg of DTNB in 100 ml of 1% trisodium citrate. At 412 nm, the absorbance of the yellow colour appeared was measured.

2.5.8 Estimation of total protein

By the standard Bradford assay, total Protein was quantified in kidney and liver.

Protocol

25 mL methanol, 50 mL H3PO4, 100 mL distilled water and 50 mg comassive blue were combined to make the stock solution. The solution was then stored until used it at 4°C in dark. Stock solution was diluted in a 1:4 ratio with distilled water to make the working solution. Similarly, 10 mg of BSA dissolve in 10 ml of phosphate buffer saline (PBS) to prepare stock solution of BSA. BSA dilutions of 100x, 50x, 25x, 12.5x and 6.25x were prepared from the stock solution. 2900 µl of working solution was combined with 100 µl of BSA serial dilutions in a cuvette to generate the standardization curve and the absorbance change was measured at 595 nm. For protein estimation, 100 µl of tissue homogenate was mixed with 2900 µl of working solution and a change in absorbance at 595 nm was observed. Within a minute, three readings were taken.

2.5.9 Calculation of Organ Mass Index

The organs kidney and liver were weighed on the day of dissection and mass index was calculated by using the formula developed by Reddy et al., (2011). Organ Mass Index = Organ weight/ Body weight x 100.

2.6 Histopathology

The conventional eosin and hematoxylin (H and E) staining method was used for tissue histology. Tissues from the kidney and liver were excised shortly after dissection, rinsed in fresh 0.9 % saline, and preserved overnight in 10% buffered formalin. Processing (fixation and dehydration), microtomy (tissue embedding and sectioning) and slide preparation are all steps in tissue histology (mounting, staining and microscopy).

2.6.1 Reagent preparation

Harris's Hematoxylin Solution

1 gram of Hematoxylin was dissolved in 10 mL of absolute ethanol and 20 g of potassium alum was mixed in 200 mL of distilled water (dH2O). Hematoxylin and alum solution were thoroughly mixed and boiled on a hot plate before adding 0.5 g mercuric oxide. After adding 8 mL of glacial acetic acid to the staining solution, it was allowed to cool. After that, the staining solution was filtered through 0.45µm filter paper and stored at room temperature.

2.6.2 Preparation of Eosin solution

1 g of eosin was mixed with 0.05 ml of acetic acid and then dH2O was added to bring the final volume to 100 ml. This staining solution was filtered through 0.45 m filter paper.

2.6.3 Preparation of Acid alcohol

To make acid alcohol, 1 mL of concentrated HCL was mixed with 99 mL of 70%ethyl alcohol.

2.6.4 Light microscopy

Fixed tissues were cut into appropriate sizes and dehydrated for 1-2 h in an ascending series of ethanol. Tissues were then immersed in xylene 1 and xylene II, in that order. Tissues were then fixed in paraffin wax that had been preheated in a wax dispenser to 59°C (TBS, Triangle Biomedical science, Durham, NC USA). A rotary microtome was used to cut 5 μm thick sections (Shandon, Finesse, Italy). Tissue-containing sections were immediately transferred to a water bath (Boekel Scientific, Japan). Tissue sections then transferred to new glass slides and placed on a glass slide warmer overnight. Tissue sections were deparaffinized in xylene twice for 5 min each time. Each section was then soaked in a descending series of alcohol concentrations of 100 %, 90 %, 80 %, 70 % and 50 % for 3 min, gently washed in running tap water and stained.

2.6.5 Hematoxylin and eosin staining

Tissue sections were stained in Harris' hematoxylin solution for 5 min, then gently washed for 2 min in running tap water, immersed for 5 seconds in acid alcohol, and washed again for 45 seconds in running tap water. Staining was done in a 1% working solution of eosin for 2 min, rinsed in running tap water for 45 seconds and dehydrated in a rising series of alcohol (50 percent, 70%, 80%, 90% and 100%) for 3 min each. After that, the tissues were immersed in xylene for 3 min. After that, the sections were mounted in DPX. Sections were examined at 10X and 40X magnifications. A photomicroscope (Olympus Japan) was used to magnify and analyze the photographed data. Adobe Photoshop was used to create image panels (Version 7.0, Microsoft Inc. USA).

2.7 Statistical Analysis

The results are given as Mean ± SEM. Graph pad prism software 8 and Sigma plot 12.0 used for one-way analysis of variance (ANOVA). The level of significance was set at $p < 0.05$. The Tukey's test was used to compare all groups with each other.

RESULTS

3.1 Biochemical parameters

3.1.1 Markers of Oxidative stress

ROS

3.1.2 Kidney

Mice treated with sodium arsenite (Group-IV and Group-V) showed significant increase in ROS level ($p < 0.001$), while significant decrease occurred in ROS in NAC+KP+sodium arsenite(4ppm) (Group-VI), and NAC+KP+sodium arsenite(10ppm) (Group-VII) as compared to Group IV and Group V.

3.1.3 Liver

The activity of ROS increased significantly in the liver tissue of mice treated with sodium arsenite (Group-IV and Group-V) ($p< 0.001$), while KP alone (Group-1I) and NAC-Alone groups have no significant difference as compared to control. ROS value was significantly restored by treatment groups.

3.2 Activity of TBARS

3.2.1 Kidney

The activity of TBARS increased significantly in the kidney tissue of mice treated with Sodium arsenite (Group-IV and V) while KP alone (Group-II) and NAC-alone groups have no significant difference as compared to control. TBARS value was significantly restored by Treatment of NAC and KP.

3.2.2 Liver

The activity of TBARS increased significantly in the liver tissue of mice treated with Sodium arsenite (Group-IV and V) while KP alone (Group-II) and NAC-alone groups have no significant difference as compared to control. TBARS value was significantly restored by Treatment of NAC and KP .

3.3 Activity of SOD

3.3.1 Kidney

In kidney, the activity of SOD decreased significantly in sodium arsenite treated group (Group-IV and Group V, $p < 0.001$) While in Group-III,and Group-II have does not significantly changed occurred as compared to control. In Group-VII and Group-VI Significantly restored the SOD levels as compared to alone treatment of KP and NAC.

3.3.2 Liver

In liver, the activity of SOD decreased significantly in sodium arsenite treated group (Group-IV,V, $p < 0.001$) While in group Group-III, and Group-II does not significantly change occurred

as compared to control. In group Group-VII and Group- VIII significantly restored the SOD level.

3.4 Activity of POD

3.4.1 Kidney

The activity of POD in kidney decreased significantly in Group-IV and Group-V ($p < 0.001$), While significantly restored the POD level in treatment groups. In Group-IV and V significantly difference occurred as compared to control while Group-II and Group-III does not.

3.4.2 Liver

In liver, the activity of POD decreased significantly in Group-IV and V ($p < 0.001$). In Group-II, and Group-III does not significantly difference occurred as compared to control. NAC and KP treatments restored POD activity.

3.5 Activity of CAT

3.5.1 Kidney

The activity of CAT in kidney decreased significantly in Group-IV and V ($p < 0.001$) While in Group-VI and VII Increased ($p < 0.05$) as compared to control. NAC and KP treatment restored CAT actions in treatment groups particularly in Group-VI and Group-VII.

3.5.2 Liver

The activity of CAT decreased significantly in liver of Group-IV and V ($p < 0.001$), While in group Group-II, and Group-III Non-significantly changed occurred as compared to control. In NAC and KP treated groups, i.e., Group-VIII, Group-IX, VI, VII and Group-XI, the activity of CAT was restored.

3.6 Activity of Reduced Glutathione- GSH

3.6.1 Kidney

In kidney, the level of GSH decreased significantly in Group-IV and V ($p < 0.001$) and non-significantly in Group-II, and Group-III as compared to control. NAC and KP treatment however, restored GSH activity in the treatment Group.

3.6.2 Liver

In liver, the level of GSH decreased significantly in Group-IV and V ($p < 0.001$), While in Group-II, and Group-III non- significantly difference as compared to control. NAC and KP treatment restored GSH level.

3.7 Analysis of Protein

3.7.1 Kidney

In kidney, the level of protein increased significantly in Group-IV and V ($p < 0.001$) and no significant difference was observed in other groups as compared to control. NAC and KP treatment restored the level of protein both in 4ppm and 10ppm dose.

3.7.2 Liver

In liver, the level of protein increased significantly as in Group-IV and group V ($p < 0.001$) While in other non-significantly changed occurred as compared to control. In NAC and KP treatment however restored the level of protein both in 4ppm and 10ppm dose.

3.8 Biochemical parameters

3.8.1 Liver function test

AST/GOT

The level of AST in serum increased significantly in Group-IV(4ppm) and V(10ppm) ($p < 0.001$) and no significant difference in Group-II, and Group-III as compared to control. However, the level of AST was restored in a treatment groups both in alone and combined treatments.

ALT-GTP

The level of ALT in serum increased significantly in Group-IV(4ppm) and V(10ppm) ($p < 0.001$) and no significant difference in Group-II, and Group-III as compared to control. However, the level of ALT was restored in a treatment group both in alone and combined treatments.

ALK

The level of ALK in serum also increased significantly in Group-IV and V ($p < 0.001$) and no significant difference occurred in Group-II, and Group-III as compared to control. However, the level of ALK was restored in a Group-VI,VII, and alone treatments of kp and NAC.

3.8.2 Renal function test

Creatinine

The level of creatinine in serum also increased significantly in Group-IV and V ($p < 0.001$) and no significant difference occurred in Group-II, and Group-III as compared to control. However, the level of creatinine was restored in a Group-VI,VII, and alone treatments of kp and NAC.

3.8.3 Total lipid profile

Total cholesterol level

In serum, the level of cholesterol increased significantly in Group-IV and V and non-significantly in Group-II and Group-III as compared to control. The level of cholesterol was restored in a in Group-VI and Group-VII as compared Sodium arsenite treated group.

Triglyceride

The level of triglyceride in serum increased significantly in Group-IV and V ($p < 0.001$) and non-significantly in Group-II, and Group-III as compared to control. Triglyceride level was restored in in Group-VI and Group- VII as compared to Sodium arsenite group.

3.9 Calculation of Organ Mass Index

Organ mass index (OMI) calculation showed a non-significant decrease in OMI values of mice , kidney and liver as compared to control.

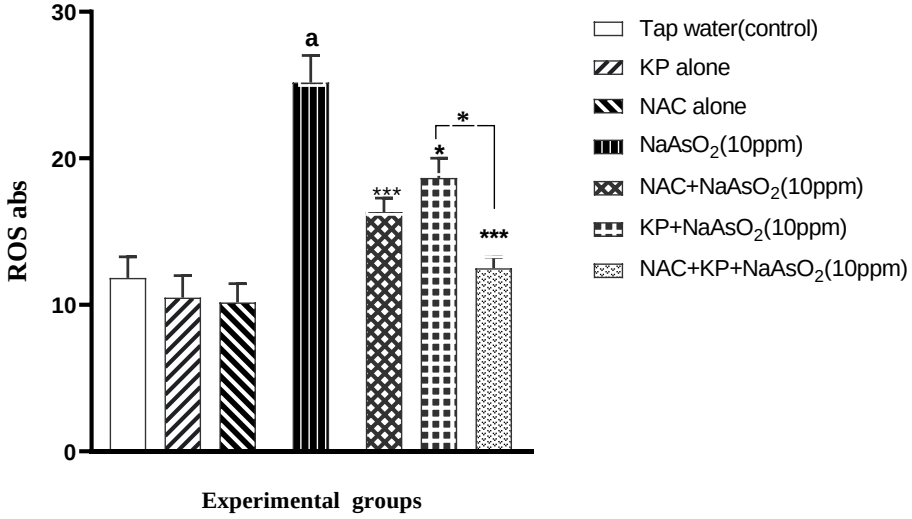

Fig.3.1. Mean ROS in adult mice liver (n=6/group) upon 35 days exposure to 10 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups. Letter "a" shows significant increase (Group-V NaAsO2 alone, $p < 0.001$) from control. In group-IX(NAC+NaAsO2), group-XI(KP+NaAsO2) and group VII (KP+NAC+NaAsO2) decreased the ROS level as compared to NaAsO2 treated group.

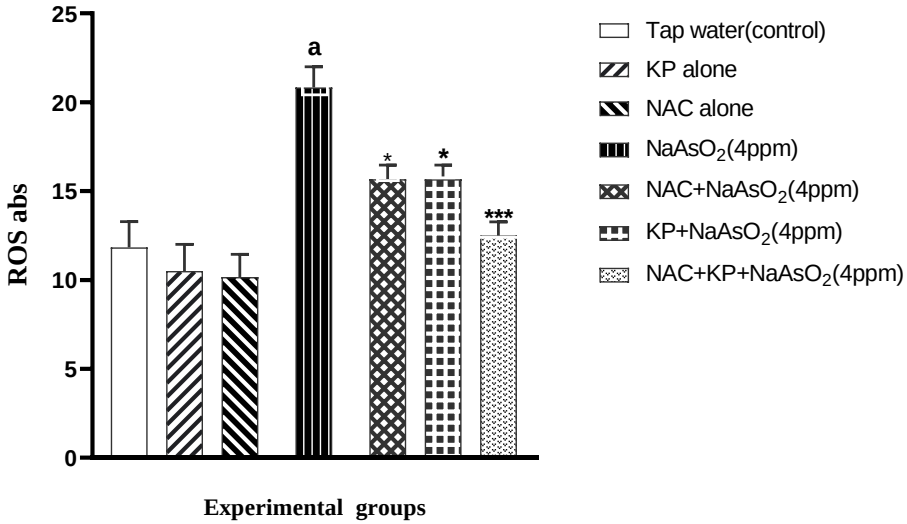

Fig.3.2. Mean ROS in adult mice liver (n=6/group) upon 35 days exposure to 4 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups . Letter "a" shows significant increase (Group-IV NaAsO2 alone, $p < 0.001$) from control. In group-VIII(NAC+NaAsO2), group-X(KP+NaAsO2) and group VI (KP+NAC+NaAsO2) significantly decreased the ROS level as compared to NaAsO2 treated group.

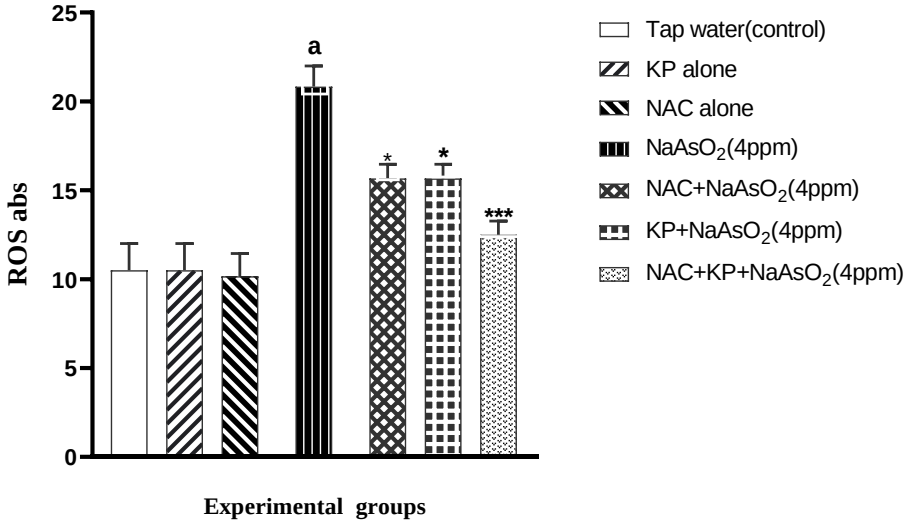

Fig.3.3. Mean ROS in adult mice kidney (n=6/group) upon 35 days exposure to 4 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups . Letter "a" shows significant increase (Group-IV NaAsO2 alone, $p < 0.001$) from control. In group-VIII(NAC+NaAsO2), group-X(KP+NaAsO2) and group VI (KP+NAC+NaAsO2) Shown significantly difference as compared to Sodium arsenite only group.

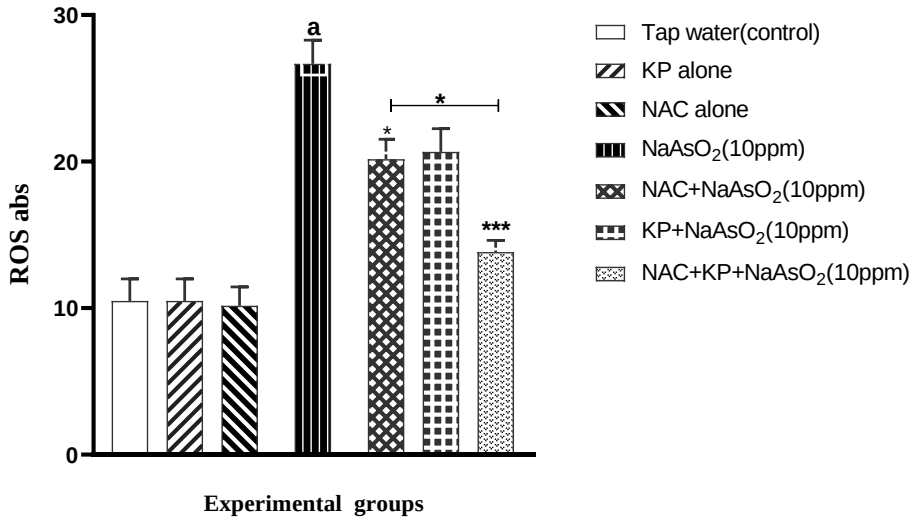

Fig.3.4. Mean ROS in adult mice kidney (n=6/group) upon 35 days exposure to 10 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups .

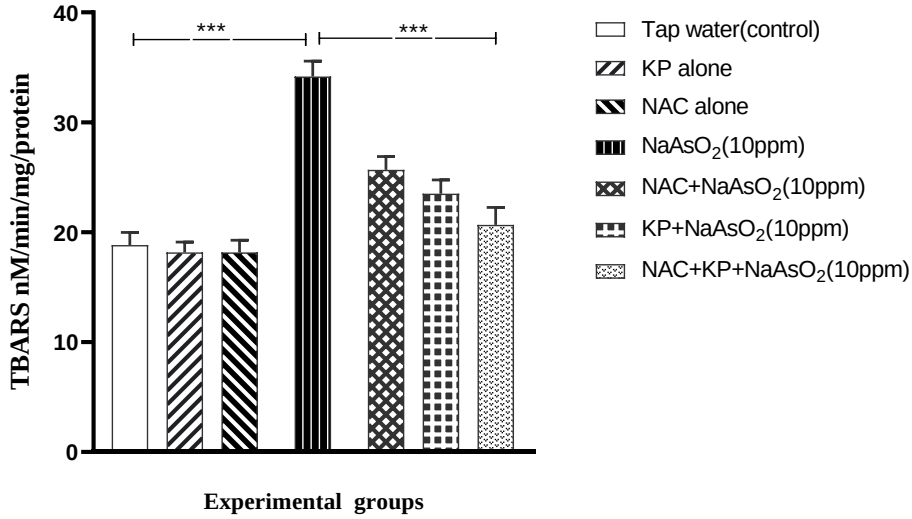

Fig.3.5. Mean of TBARS in adult mice liver (n=6/group) upon 35 days exposure to 10 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups.

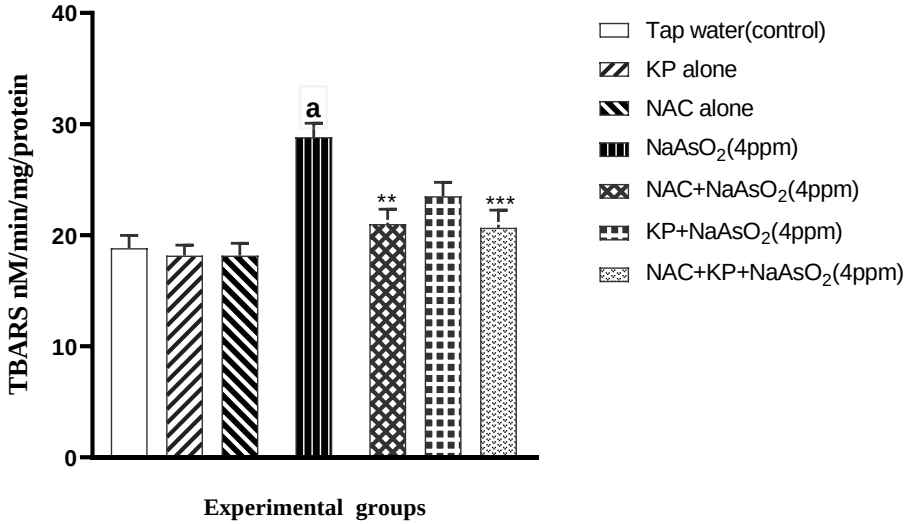

Fig.3.6. In adult mice liver, Mean of TBARS increase significantly in Sodium arsenite treated (Group-IV $NaAsO_2$ alone, $p < 0.001$) as compared to control shown by letter "a". In group VIII (NAC+ $NaAsO_2$)($p < 0.01$) and group-VI(NAC+KP+NAC+KP+ $NaAsO_2$) ($p < 0.001$) Shown significantly difference as compared to Sodium arsenite treated group.

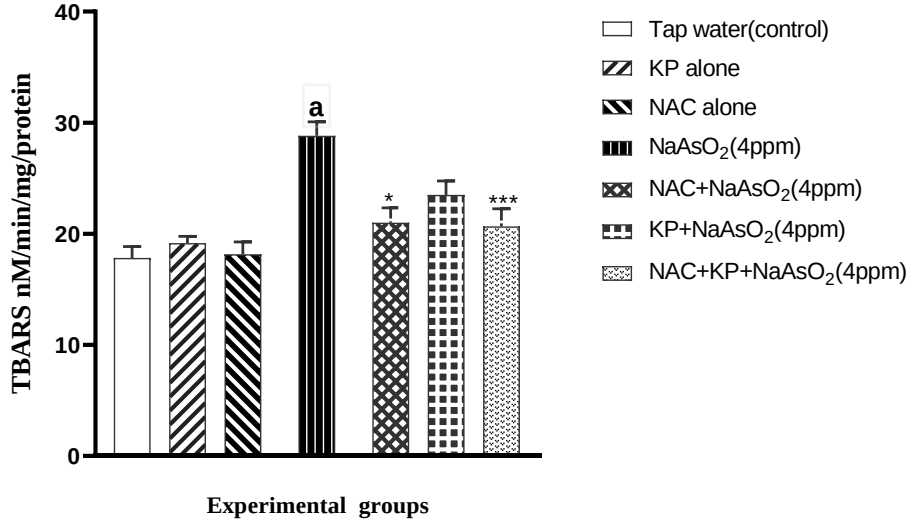

Fig.3.7. Mean TBARS in adult mice kidney (n=6/group) upon 35 days exposure to 4 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups. Letter "a" shown significant increase (Group-IV$NaAsO_2$ alone, $p < 0.001$) from control while in group VI (NAC+KP+ $NaAsO_2$) significantly decrease ($p < 0.001$) the TBARS levels as compared to group X (KP+ $NaAsO_2$).

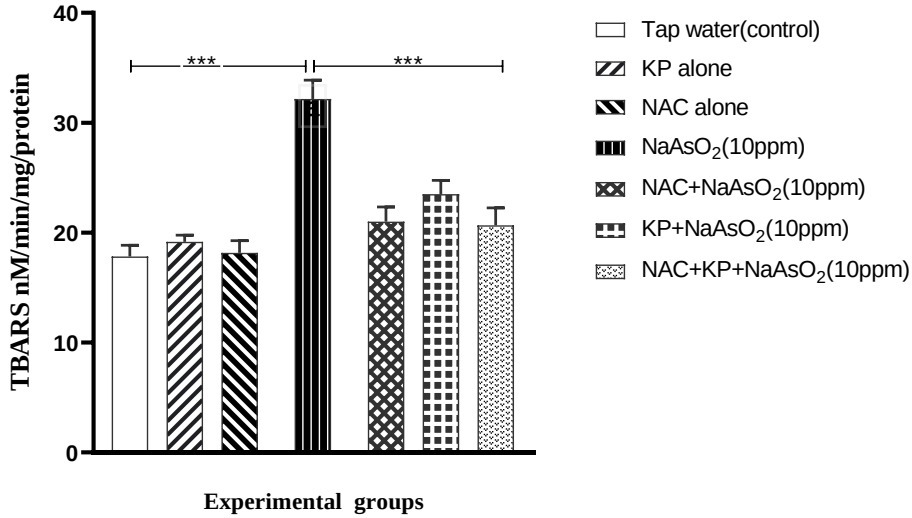

Fig.3.8. Mean TBARS in adult mice kidney (n=6/group) upon 35 days exposure to 10 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups.

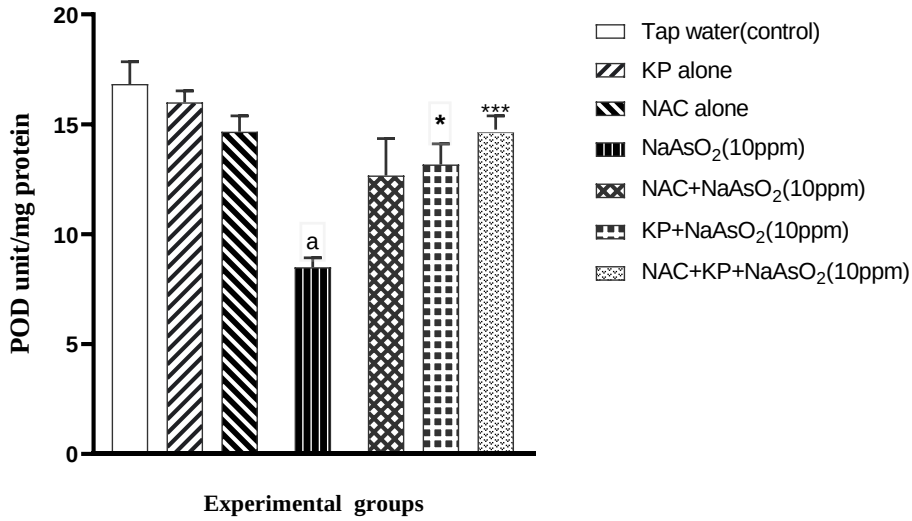

Fig.3.9. Mean POD in adult mice liver (n=6/group) upon 35 days exposure to 10 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups. Letter "a" shown significant decrease (Group-V $NaAsO_2$ alone, $p < 0.001$) from control. In group-XI (KP+$NaAsO_2$, $p < 0.05$) and group- VII (NAC+KP+ $NaAsO_2$, $p < 0.001$) significantly increase the POD level as compared to $NaAsO_2$ treated group.

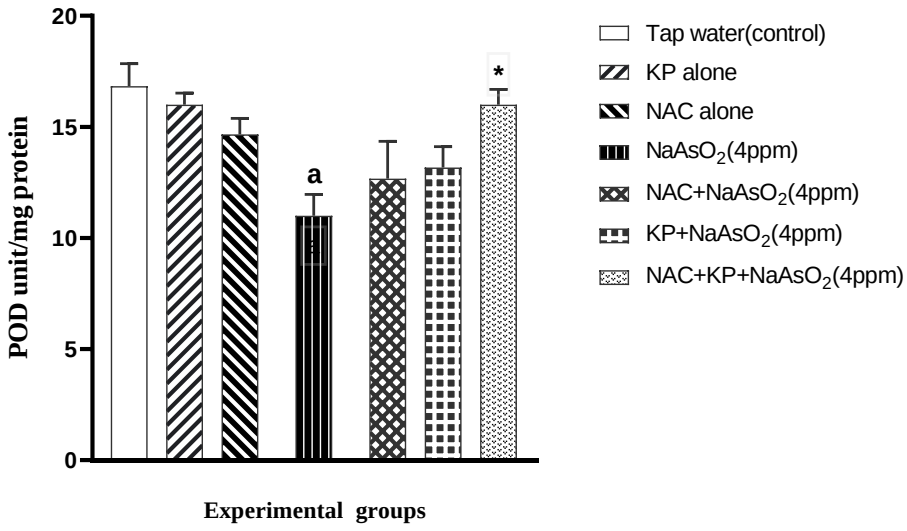

Fig.3.10. Mean POD in adult mice liver (n=6/group) upon 35 days exposure to 4 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups. Letter "a" shown significant decrease (Group-IV $NaAsO_2$ alone, $p < 0.05$) from control.

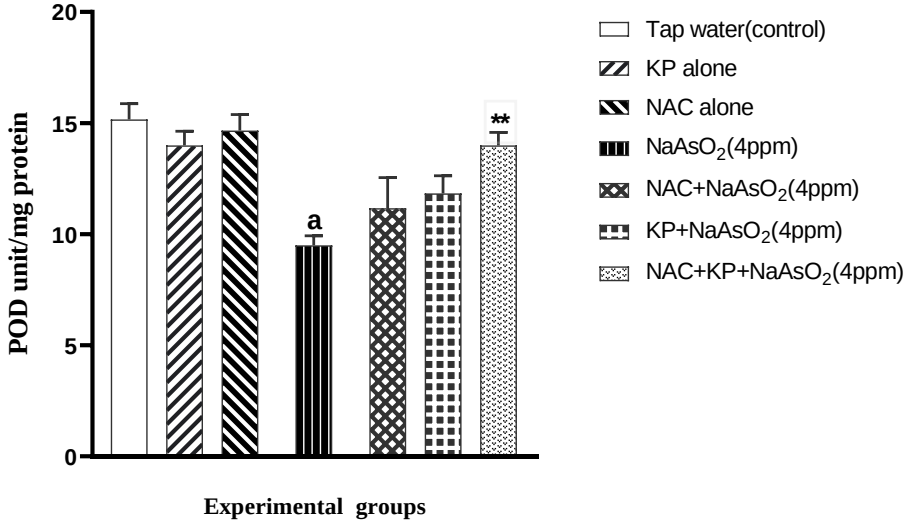

Fig.3.11. Mean POD in adult mice kidney (n=6/group) upon 35 days exposure to 4 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups. Letter "a" shown significant decrease (Group-IV $NaAsO_2$ alone, $p < 0.001$) from control. In group VI (NAC+KP+ $NaAsO_2$, $p < 0.01$) significantly increase the POD level as compared to $NaAsO_2$ treated group.

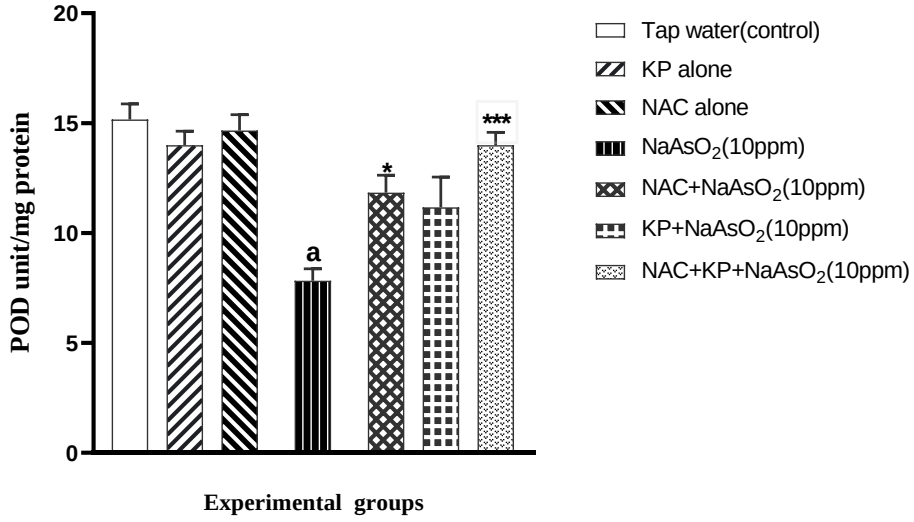

Fig.3.12. Mean POD in adult mice Kidney(n=6/group) upon 35 days exposure to 10 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups. Letter "a" shown significant decrease (Group-V $NaAsO_2$ alone, $p < 0.001$) from control. In group-IX (NAC+$NaAsO_2$, $p < 0.05$) and group- VII (NAC+KP+ $NaAsO_2$, $p < 0.001$) significantly increase the POD level as compared to $NaAsO_2$ treated group.

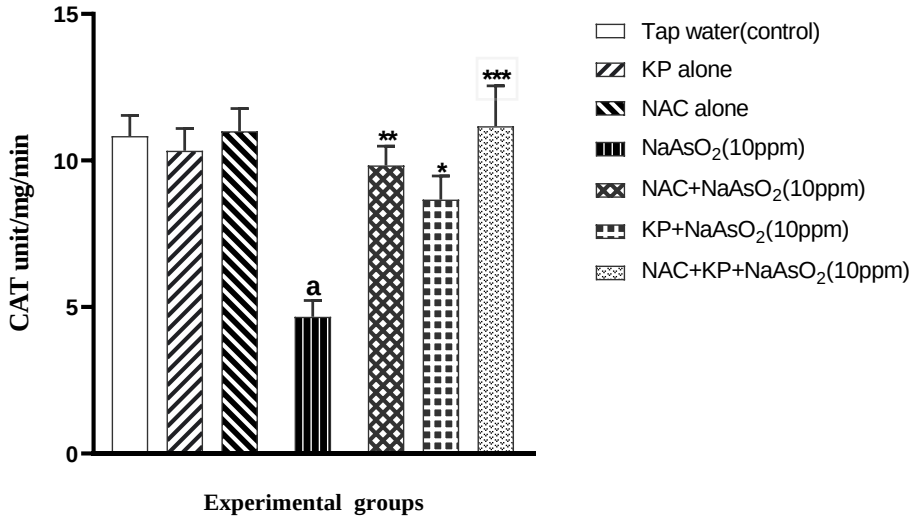

Fig.3.13. Mean CAT in adult mice liver (n=6/group) upon 35 days exposure to 10 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups. Letter "a" shown significant decrease (Group-V $NaAsO_2$ alone, $p < 0.001$) from control. In group-IX (NAC-75+$NaAsO_2$, $p < 0.01$),group XI(KP+ $NaAsO_{2,P<0.05}$) and group-VII (NAC+KP+ $NaAsO_2$, $p < 0.001$) significantly increase the CAT level as compared to $NaAsO_2$ treated group.

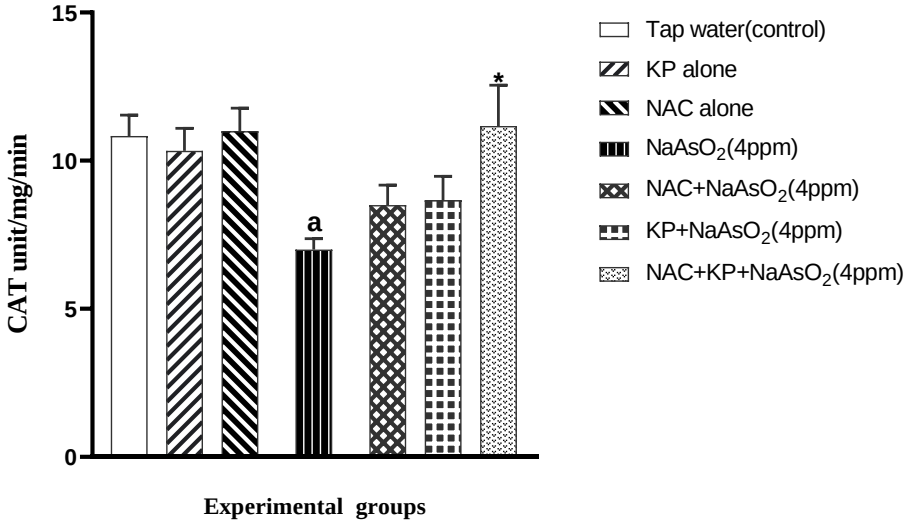

Fig.3.14. Mean CAT in adult mice liver (n=6/group) upon 35 days exposure to 4 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups. Letter "a" shown significant decrease (Group-IV $NaAsO_2$ alone, $p < 0.05$) from control. In group-VI (NAC+KP+ $NaAsO_2$, $p < 0.05$) significantly increase the CAT level as compared to $NaAsO_2$ treated group.

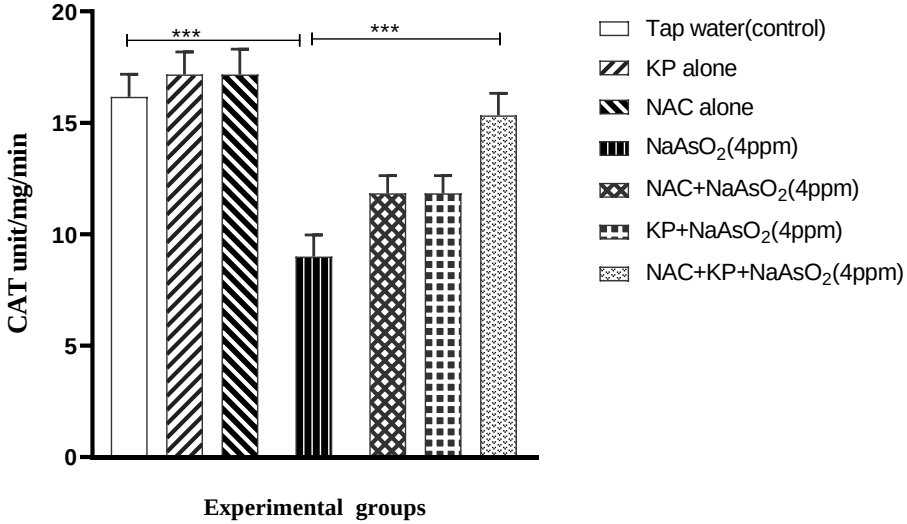

Fig.3.15. Mean CAT in adult mice Kidney (n=6/group) upon 35 days exposure to 4 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups.

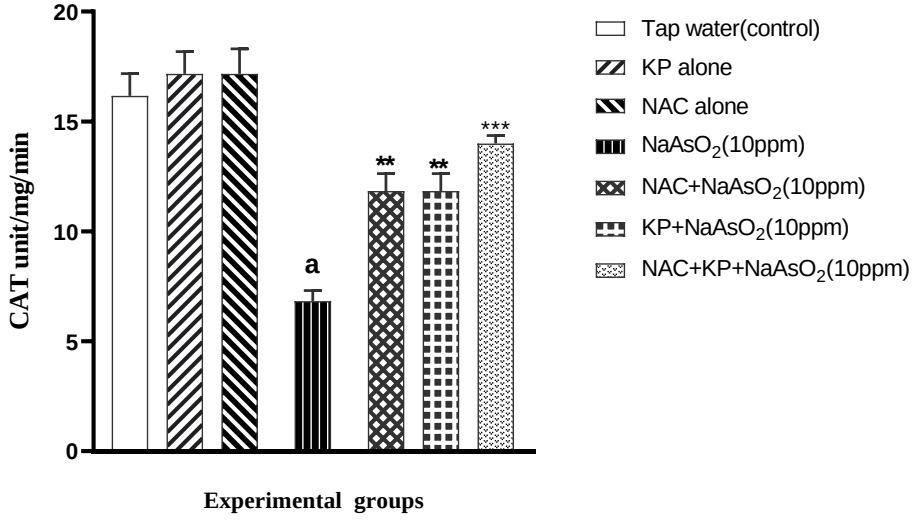

Fig.3.16. Mean CAT in adult mice kidney (n=6/group) upon 35 days exposure to 10 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups. Letter "a" shown significant decrease (Group-V $NaAsO_2$ alone, $p < 0.001$) from control. In group-IX (NAC-75+$NaAsO_2$, $p < 0.01$),group XI(KP+ $NaAsO_{2,P<0.01}$) and group-VII (NAC+KP+ $NaAsO_2$, $p < 0.001$) significantly increase the CAT level as compared to $NaAsO_2$ treated group.

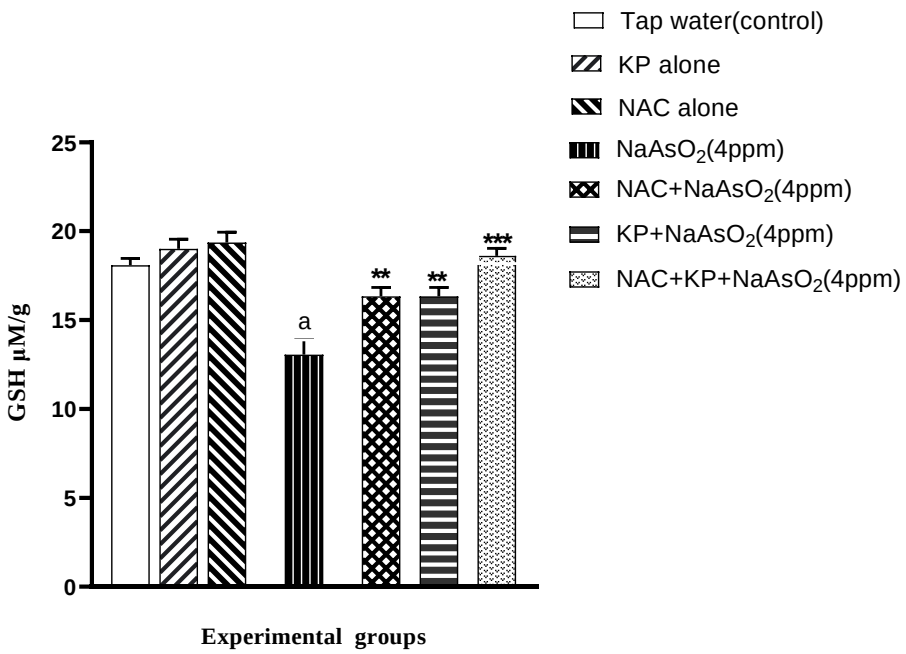

Fig.3.17. Mean GSH in adult mice liver (n=6/group) upon 35 days exposure to 4 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups. Letter "a" shown significant decrease (Group-IV $NaAsO_2$ alone, $p < 0.001$) from control In group-VIII (NAC-75+$NaAsO_2$, $p < 0.01$),group X(KP+ $NaAsO_{2,P<0.01}$) and group-VI (NAC+KP+ $NaAsO_2$, $p < 0.001$) significantly increase the GSH level as compared to $NaAsO_2$ treated group.

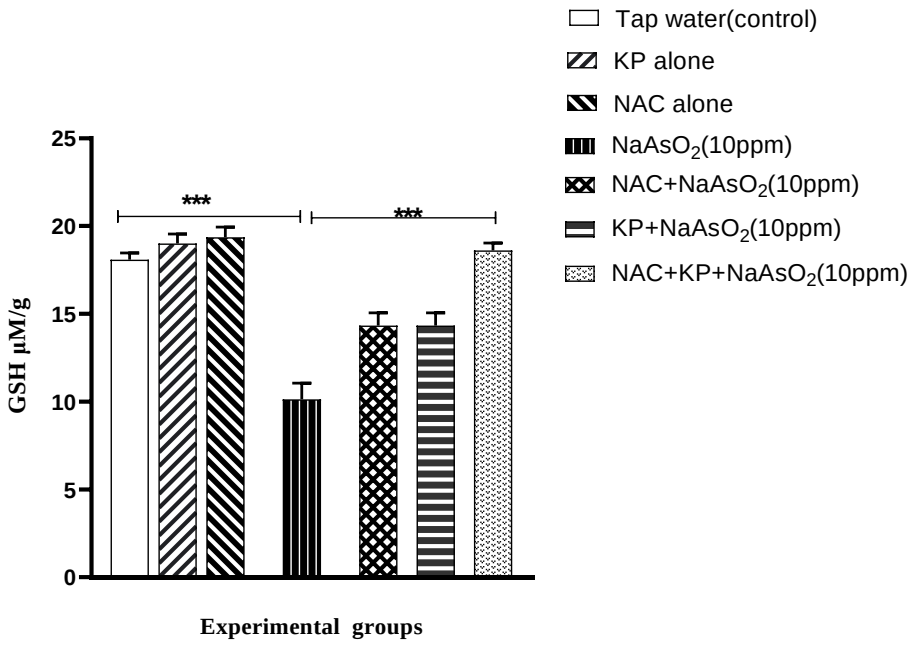

Fig.3.18. Mean GSH in adult mice liver (n=6/group) upon 35 days exposure to 10 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups.

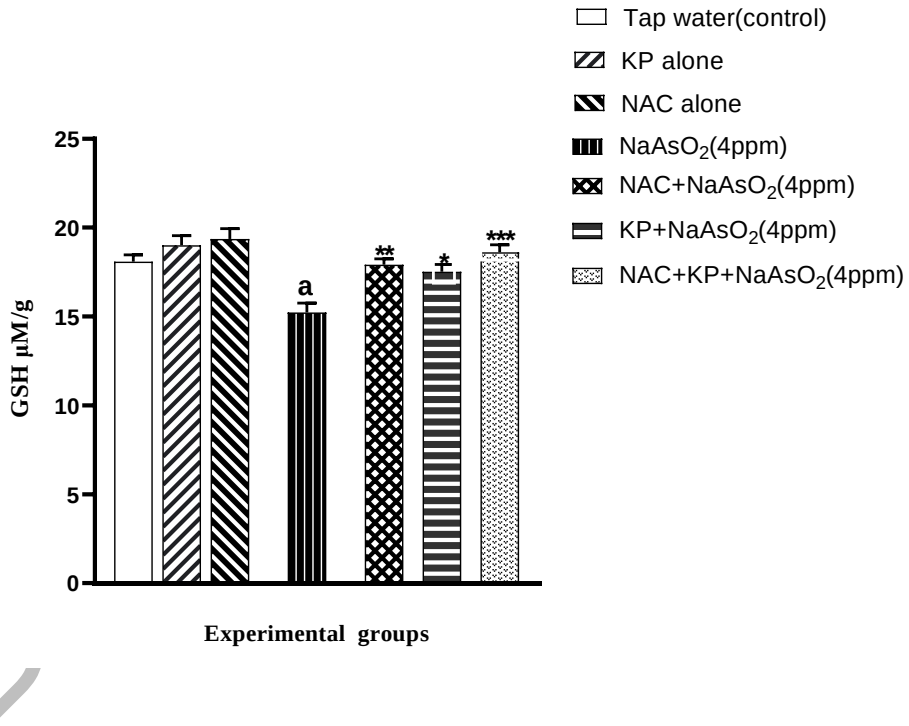

Fig.3.19. Mean GSH in adult mice Kidney (n=6/group) upon 35 days exposure to 4 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups. Letter "a" shown significant decrease (Group-IV $NaAsO_2$ alone, $p < 0.01$) from control In group-VIII (NAC-75+$NaAsO_2$, $p < 0.01$),group X(KP+ $NaAsO_{2,P<0.05}$) and group-VI (NAC+KP+ $NaAsO_2$, $p < 0.001$) significantly increase the GSH level as compared to $NaAsO_2$ treated group.

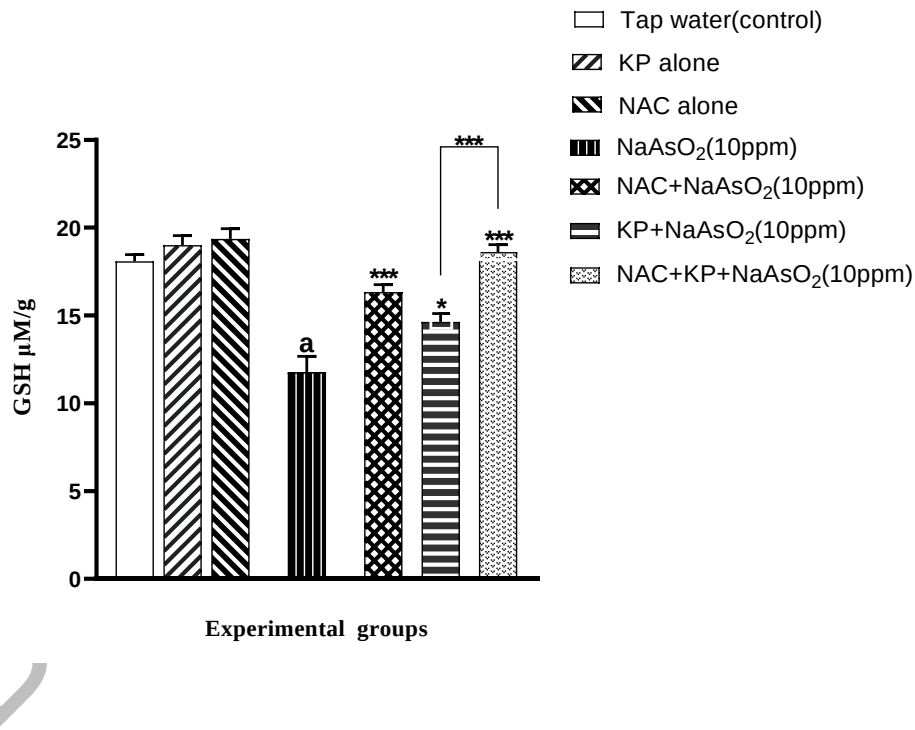

Fig.3.20. Mean GSH in adult mice Kidney (n=6/group) upon 35 days exposure to 10ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). Letter "a" shown significant decrease (Group-V $NaAsO_2$ alone, $p < 0.001$) from control. In group-IX (NAC-75+$NaAsO_2$, $p < 0.001$), group XI (KP+ $NaAsO_2$, $P<0.05$) and group-VII (NAC+KP+ $NaAsO_2$, $p < 0.001$) significantly increase the CAT level as compared to $NaAsO_2$ treated group. In group VII (KP+NAC+ $NaAsO_2$, $P<0.001$ significantly increase the GSH level as compared to group XI (KP+ $NaAsO_2$).

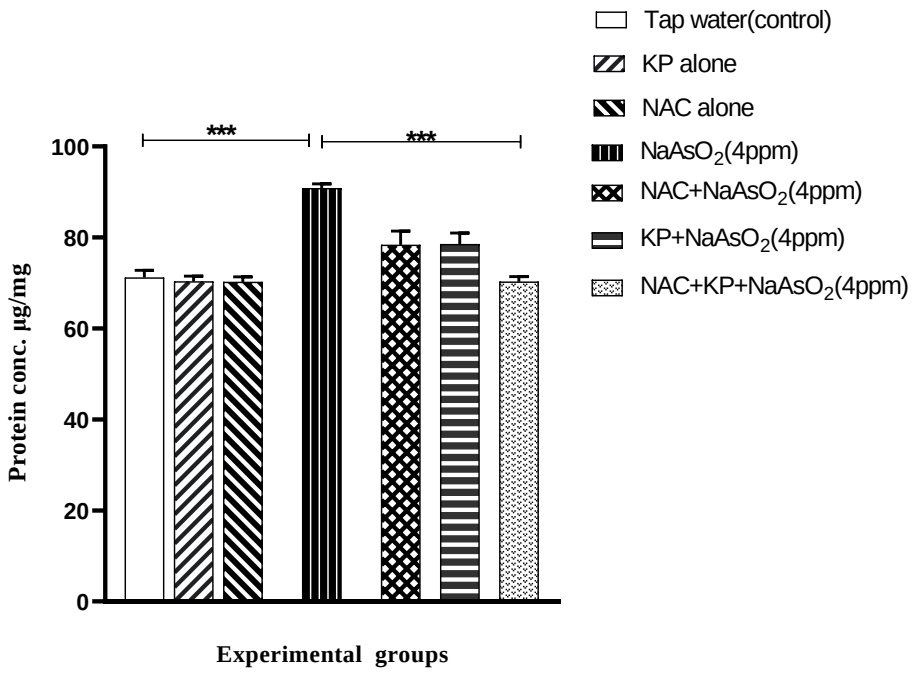

Fig.3.21. Mean Protein concentration in adult mice liver (n=6/group) upon 35 days exposure to 4 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups.

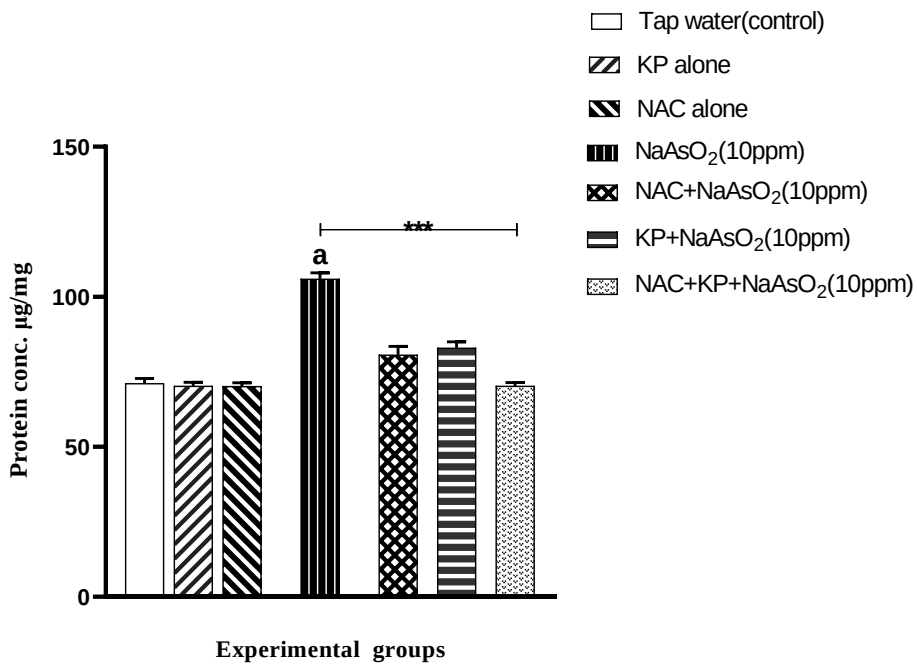

Fig.3.22. Mean Protein concentration in adult mice liver (n=6/group) upon 35 days exposure to 10ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups. Letter "a" shown significant increase (Group-V $NaAsO_2$ alone, $p < 0.001$) from control In group-IX (NAC-75+$NaAsO_2$, $p < 0.001$),group XI(KP+ $NaAsO_2$,$P<0.001$) and group-VII (NAC+KP+ $NaAsO_2$, $p < 0.001$) significantly decrease the Protein level as compared to $NaAsO_2$ treated group.

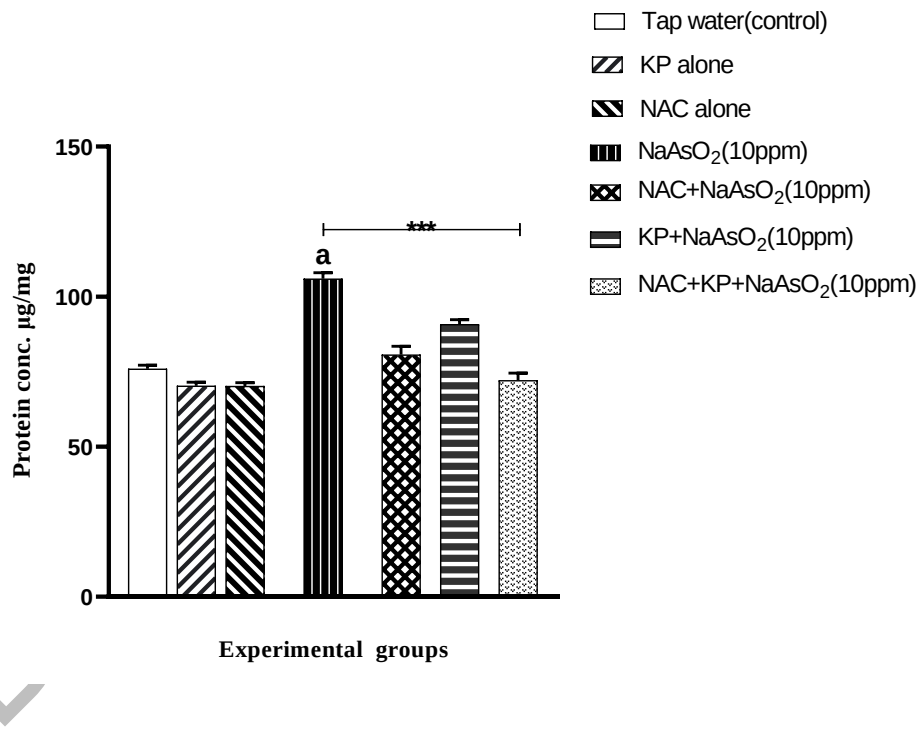

Fig.3.23. Mean Protein concentration in adult mice kidney (n=6/group) upon 35 days exposure to 10ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). Letter "a" shown significant increase (Group-V $NaAsO_2$ alone, $p < 0.001$) from control In group-IX (NAC-75+$NaAsO_2$, $p < 0.001$),group XI(KP+ $NaAsO_2$,$P<0.001$) and group-VII (NAC+KP+ $NaAsO_2$, $p < 0.001$) significantly decrease the Protein level as compared to $NaAsO_2$ treated group.

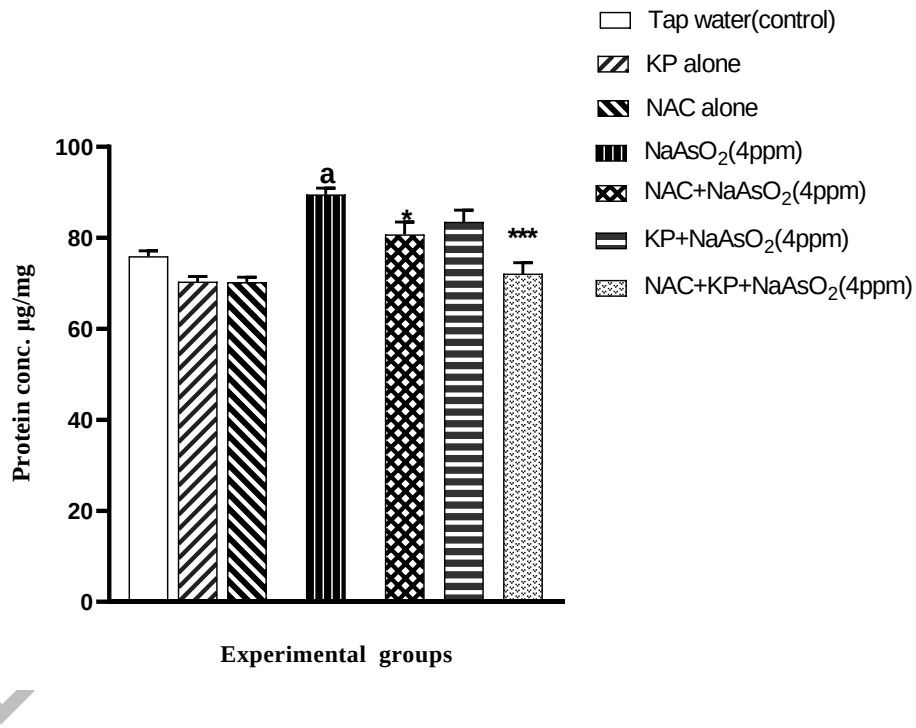

Fig.3.24. Mean Protein concentration in adult mice kidney (n=6/group) upon 35 days exposure to 4ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). Letter "a" shown significant increase (Group-IV $NaAsO_2$ alone, $p < 0.001$) from control. In group-VIII (NAC-75+$NaAsO_2$, $p < 0.05$), and group-VI (NAC+KP+ $NaAsO_2$, $p < 0.001$) significantly decrease the Protein level as compared to $NaAsO_2$ treated group.

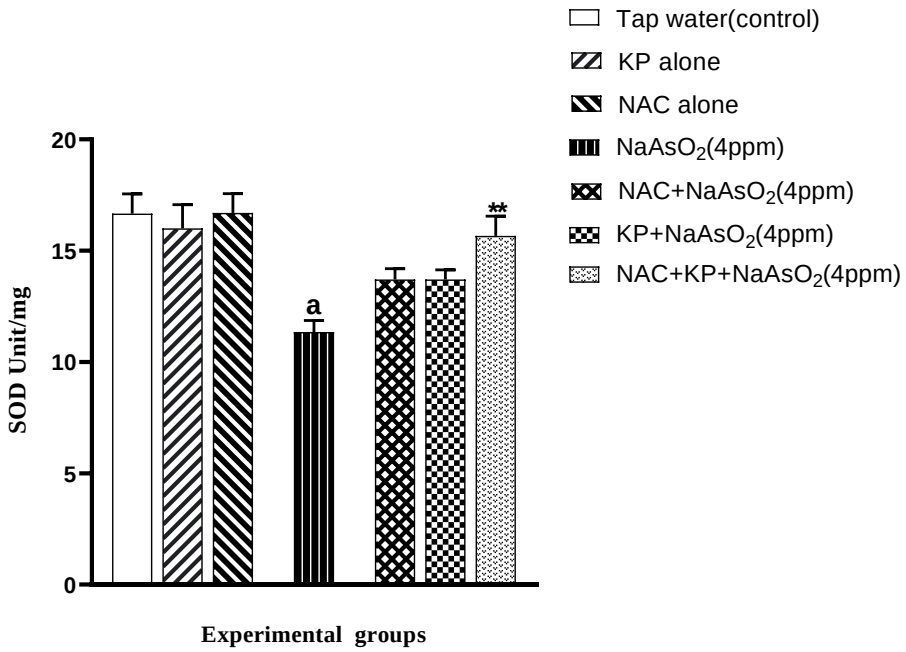

Fig.3.25. Mean SOD in adult mice liver (n=6/group) upon 35 days exposure to 4ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups. Letter "a" shown significant decrease (Group-IV $NaAsO_2$ alone, $p < 0.001$) from control. In group- VI (NAC+KP+ $NaAsO_2$, $p < 0.01$) significantly increase the SOD level as compared to $NaAsO_2$ treated group.

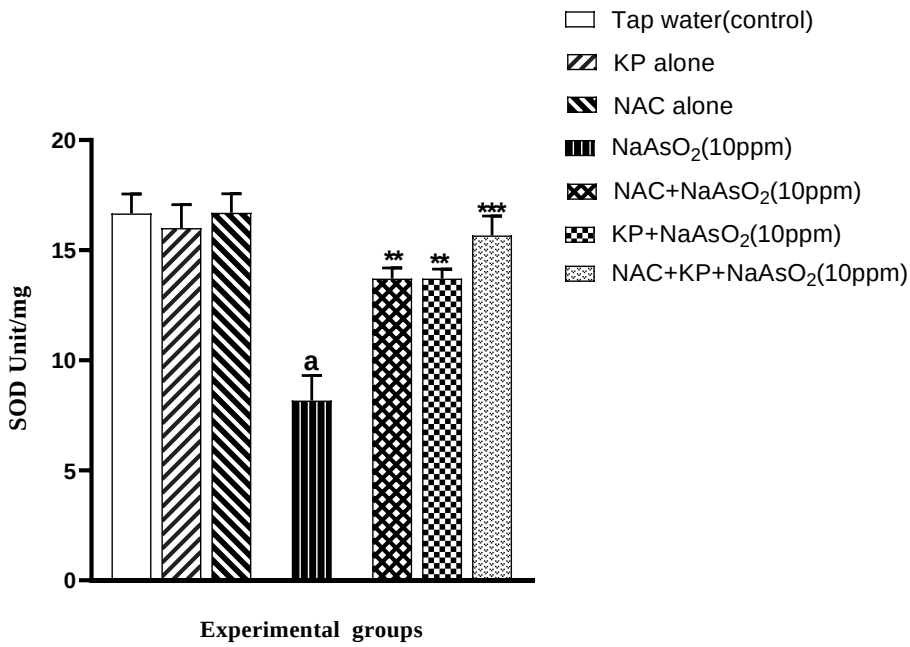

Fig.3.26. Mean SOD in adult mice liver (n=6/group) upon 35 days exposure to 10 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups. Letter "a" shown significant decrease (Group-V $NaAsO_2$ alone, $p < 0.001$) from control. In group-XI ($KP+NaAsO_2$, $p < 0.01$),group-IX($NAC+ NaAsO_{2,P<0.01}$) and group- VII ($NAC+KP+ NaAsO_2$, $p < 0.001$) significantly increase the SOD level as compared to $NaAsO_2$ treated group.

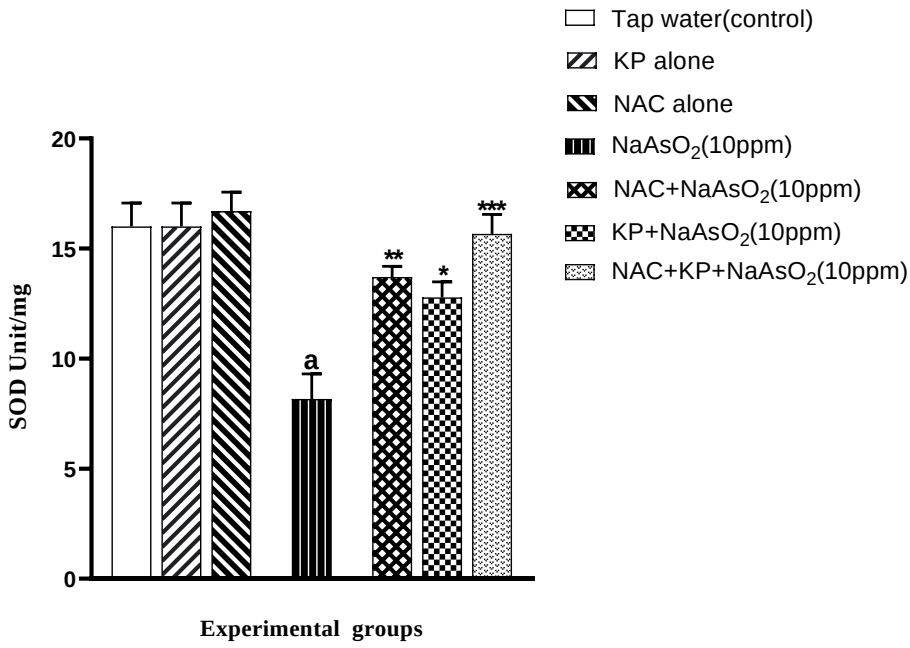

Fig.3.27. Mean SOD in adult mice Kidney (n=6/group) upon 35 days exposure to 10 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups. Letter "a" shown significant decrease (Group-V $NaAsO_2$ alone, $p < 0.001$) from control. In group-XI ($KP+NaAsO_2$, $p < 0.05$),group-IX($NAC+ NaAsO_{2,P<0.01}$) and group- VII ($NAC+KP+ NaAsO_2$, $p < 0.001$) significantly increase the SOD level as compared to $NaAsO_2$ treated group.

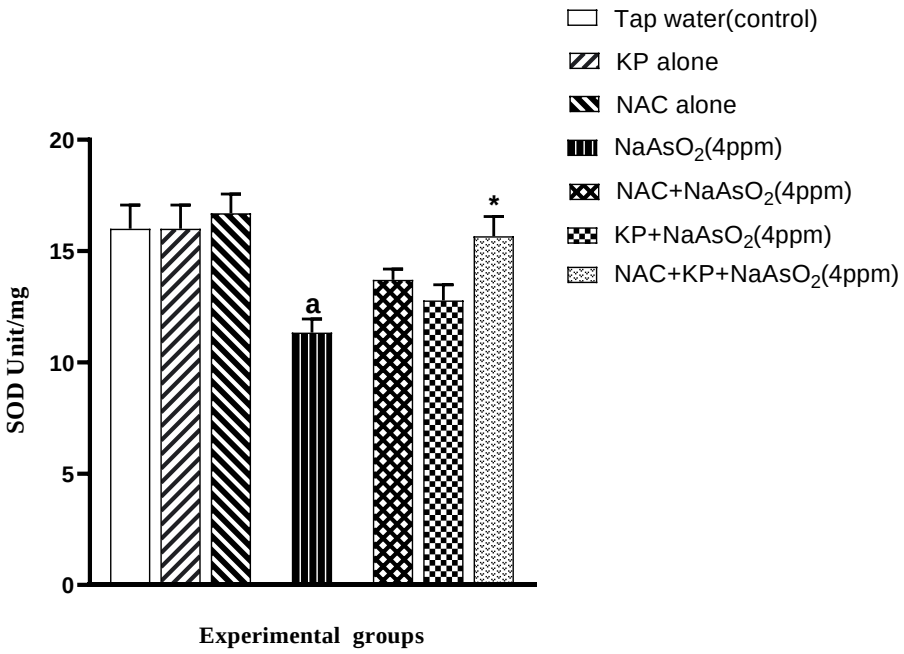

Fig.3.28. Mean SOD in adult mice Kidney (n=6/group) upon 35 days exposure to 4ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups. Letter "a" shown significant decrease (Group-IV $NaAsO_2$ alone, $p < 0.05$) from control. In group- VI (NAC+KP+ $NaAsO_2$, $p < 0.01$) significantly increase the SOD level as compared to $NaAsO_2$ treated group.

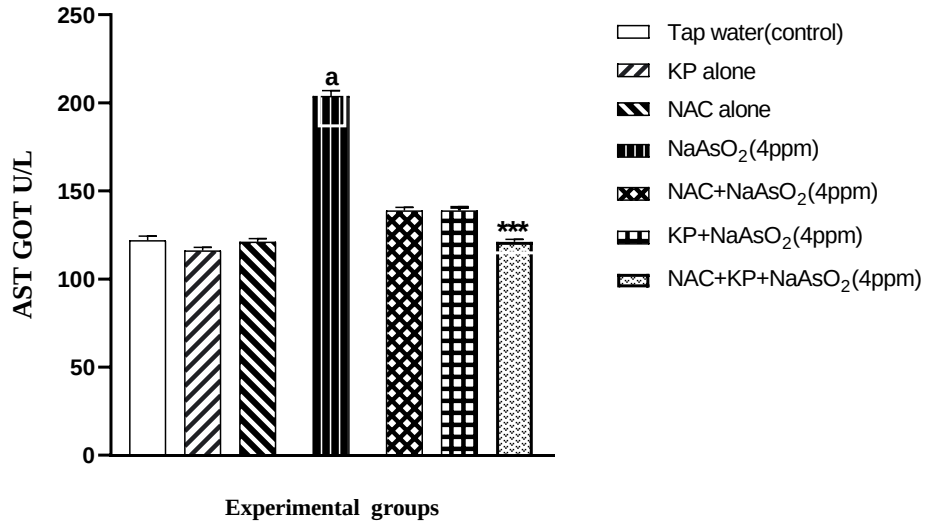

Fig.3.29. Mean AST in adult mice serum (n=6/group) upon 35 days exposure to 4 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). Letter "a" shown significant increase (Group-IV $NaAsO_2$ alone, $p < 0.001$) from control. In group-VI (NAC+KP+$NaAsO_2$, $p < 0.001$) significantly decrease the AST level as compared to $NaAsO_2$ treated group.

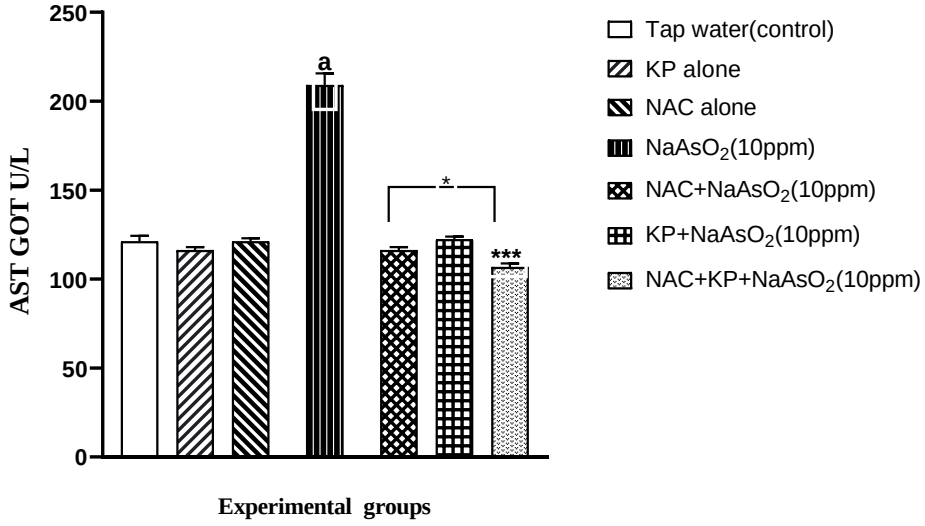

Fig.3.30. Mean AST in adult mice serum (n=6/group) upon 35 days exposure to 10 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). Letter "a" shown significant increase (Group-V $NaAsO_2$ alone, $p < 0.001$) from control. In group-VII (NAC+KP+$NaAsO_2$, $p < 0.001$) significantly decrease the AST level as compared to $NaAsO_2$ treated group. * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups

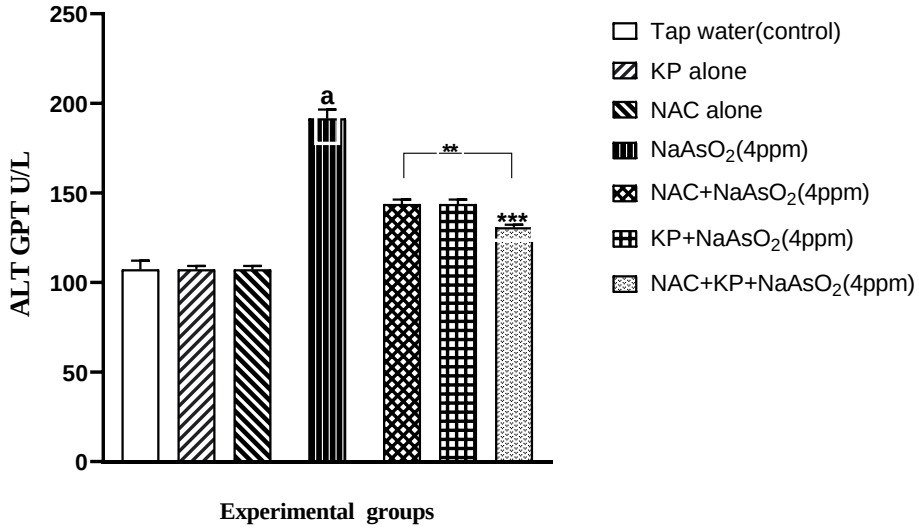

Fig.3.31. Mean ALT in adult mice serum (n=6/group) upon 35 days exposure to 4 ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). Letter "a" shown significant increase (Group-IV NaAsO2 alone, $p < 0.001$) from control.. In group-VI (NAC+KP+$NaAsO_2$, $p < 0.001$) significantly decrease the ALT level as compared to $NaAsO_2$ treated group. ** $p<0.01$ & *** $p<0.001$ show significant difference between respective treatment groups.

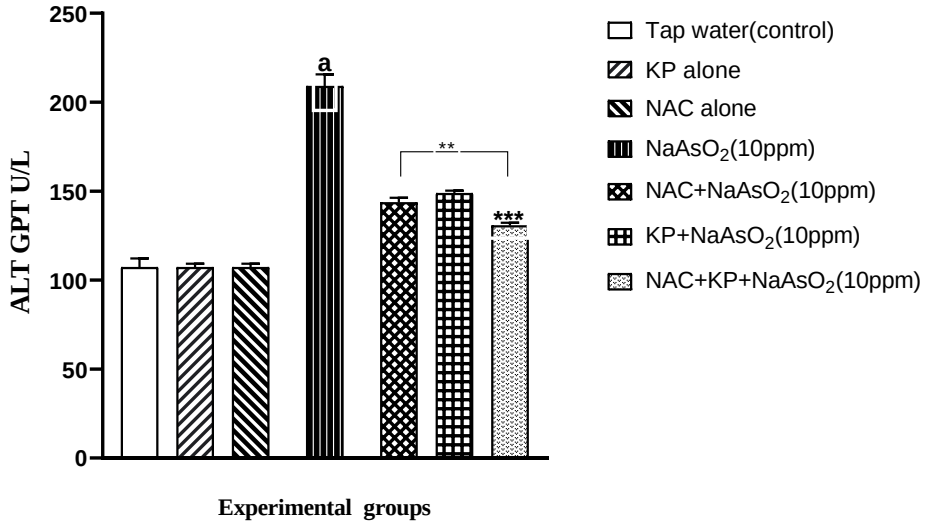

Fig.3.32. Mean ALT in adult mice serum (n=6/group) upon 35 days exposure to 10ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). Letter "a" shown significant increase (Group-V NaAsO2 alone, $p < 0.001$) from control.. In group-VII (NAC+KP+$NaAsO_2$, $p < 0.001$) significantly decrease the ALT level as compared to $NaAsO_2$ treated group. ** $p<0.01$ & *** $p<0.001$ show significant difference between respective treatment groups

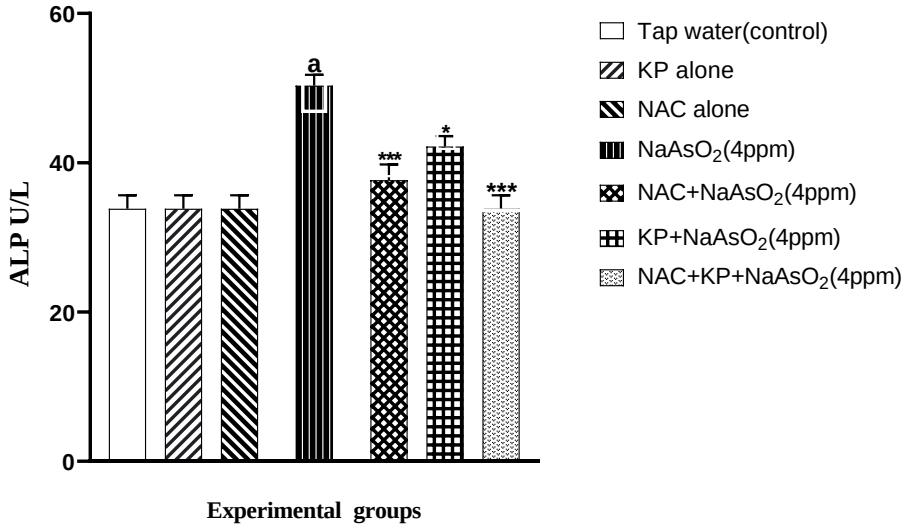

Fig.3.33. Mean ALP in adult mice serum (n=6/group) upon 35 days exposure to 4ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). Letter "a" shown significant increase (Group-IV NaAsO2 alone, $p < 0.001$) from control.. In group-VI (NAC+KP+$NaAsO_2$, $p < 0.001$) significantly decrease the ALP level as compared to $NaAsO_2$ treated group. * $p<0.05$ & *** $p<0.001$ show significant difference between respective treatment groups

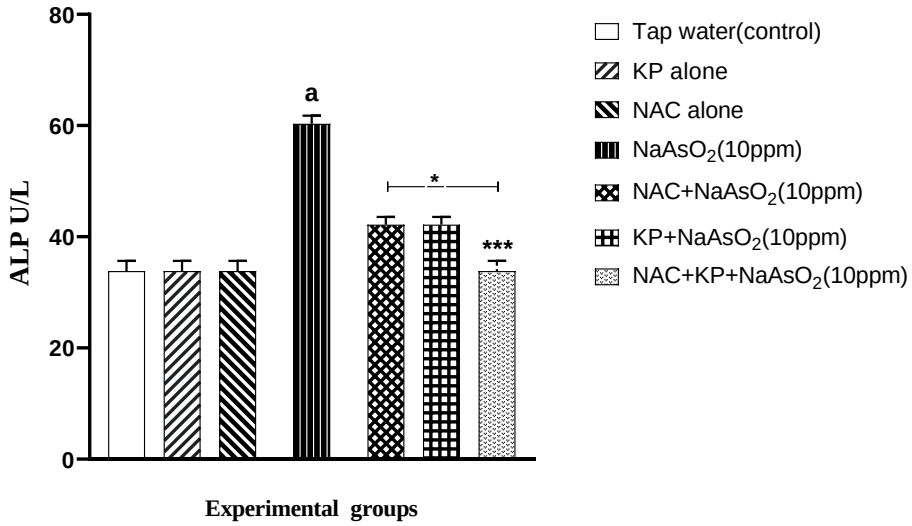

Fig.3.34. Mean ALP in adult mice serum (n=6/group) upon 35 days exposure to 10ppm dose of $NaAsO_2$, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). Letter "a" shown significant increase (Group-V NaAsO2 alone, $p < 0.001$) from control. In group-VII (NAC+KP+$NaAsO_2$, $p < 0.001$) significantly decrease the ALP level as compared to $NaAsO_2$ treated group. * $p<0.05$ show significant difference between respective alone and combined treatment groups.

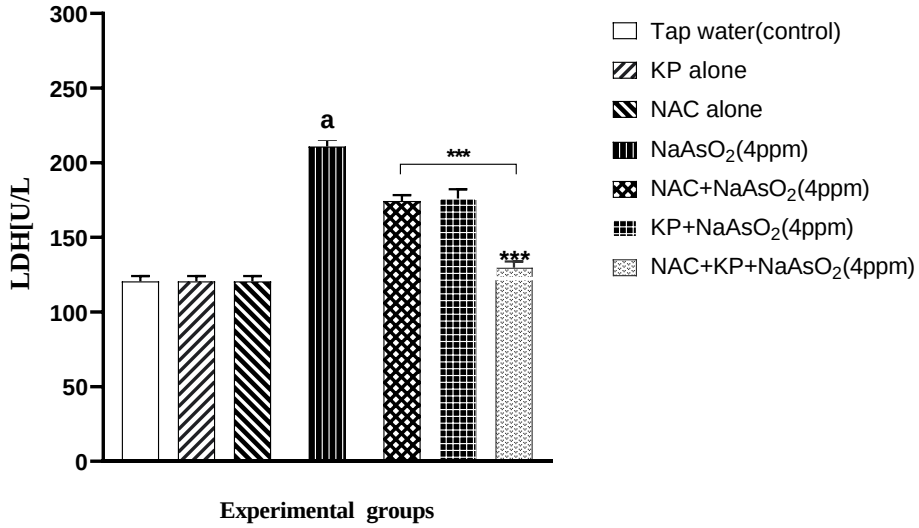

Fig. 3.35. Mean LDH level [U/L] in adult male mice serum following 35-days exposure to 4 ppm dose of NaAsO2, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). Letter "a" shown significant increase (Group-IV NaAsO2 alone, $p < 0.001$) from control . *** $p<0.001$ show significant difference between respective treatment groups.

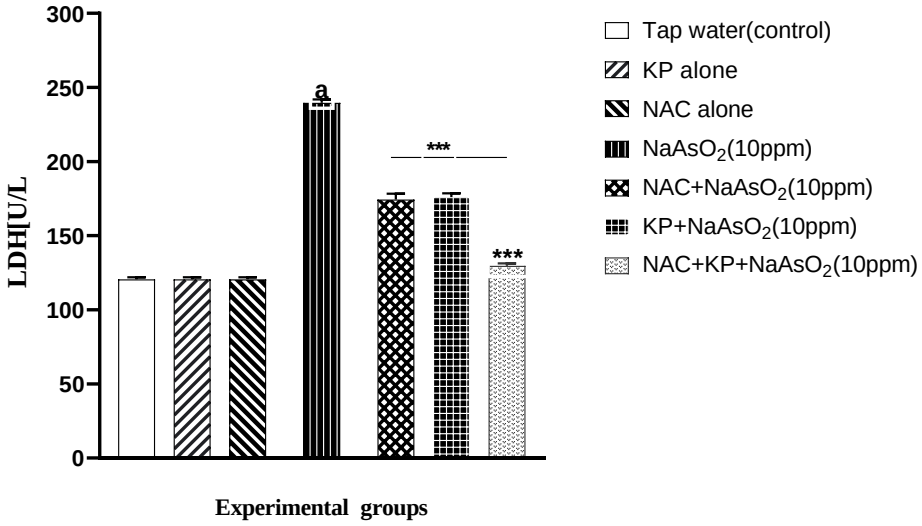

Fig. 3.36. Mean LDH level [U/L] in adult male mice serum following 35-days exposure to 10ppm dose of NaAsO2, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). Letter "a" shown significant increase (Group-V NaAsO2 alone, $p < 0.001$) from control . In group-VII (NAC+KP+$NaAsO_2$, $p < 0.001$) significantly decrease the LDH level as compared to $NaAsO_2$ treated group. *** $p<0.001$ show significant difference between respective treatment groups.

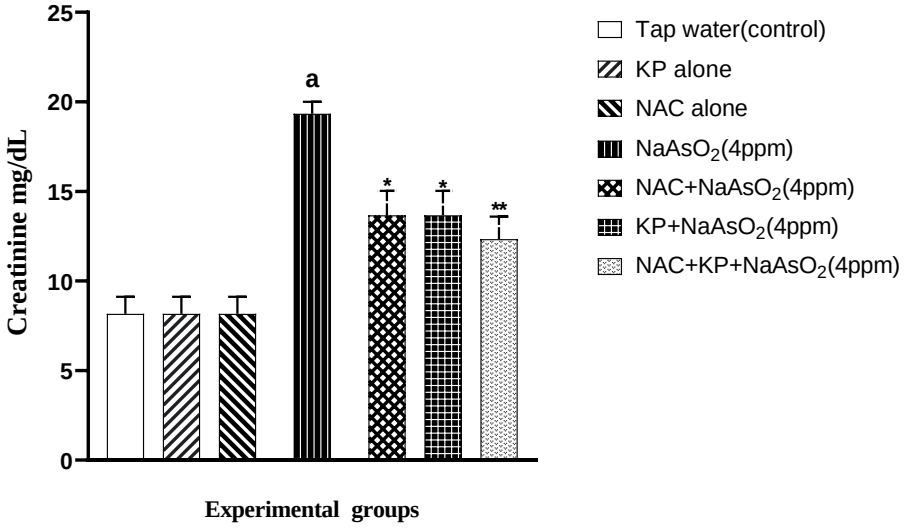

Fig.3.37. Mean Creatinine in adult male mice serum (n=6/group) upon 35 days exposure to 4ppm dose of NaAsO2, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). Letter "a" shown significant increase (Group-IV NaAsO2 alone, $p < 0.001$) from control In group-VIII (NAC-75+NaAsO2, $p < 0.05$) and group-X (KP+ NaAsO2, $p < 0.05$) and group VI(NAC+KP+ NaAsO2,$p<0.01$) significantly decrease the Creatinine level as compared to NaAsO2 treated group

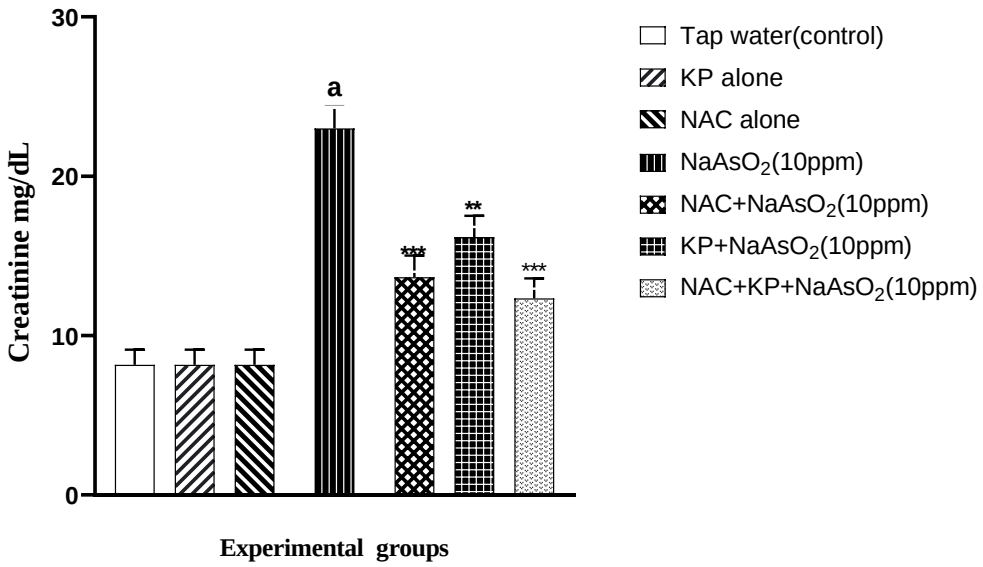

Fig.3.38. Mean Creatinine in adult male mice serum (n=6/group) upon 35 days exposure to 10 ppm dose of NaAsO2, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). Letter "a" shown significant increase (Group-V NaAsO2 alone, $p < 0.001$) from control In group-IX (NAC-75+NaAsO2, $p < 0.001$) and group-XI (KP+ NaAsO2, $p < 0.01$) and group VII(NAC+KP+ NaAsO2,$p<0.001$) significantly decrease the Creatinine level as compared to NaAsO2 treated group

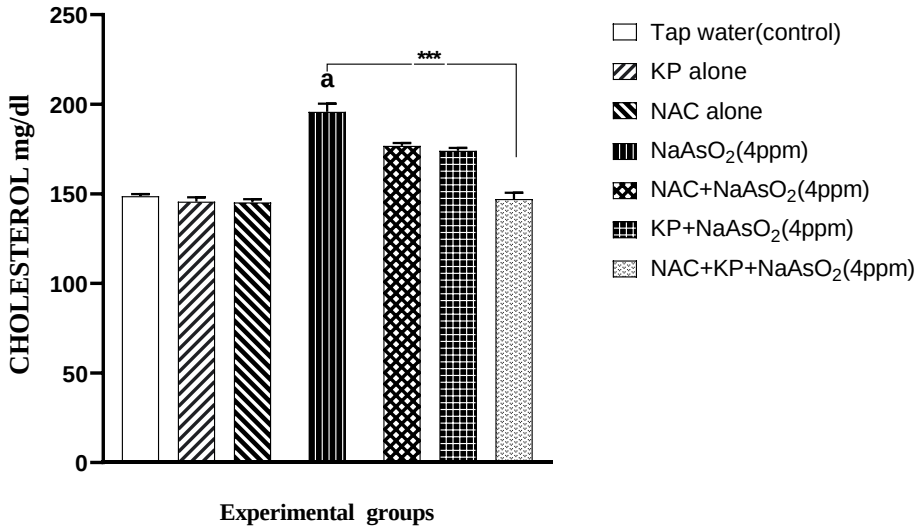

Fig.3.39. Mean Cholesterol in adult male mice serum (n=6/group) upon 35 days exposure to 4 ppm dose of NaAsO2, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). Letter "a" shown significant increase (Group-IV NaAsO2 alone, $p < 0.001$) from control. *** $p<0.001$ show significant difference between respective treatment groups

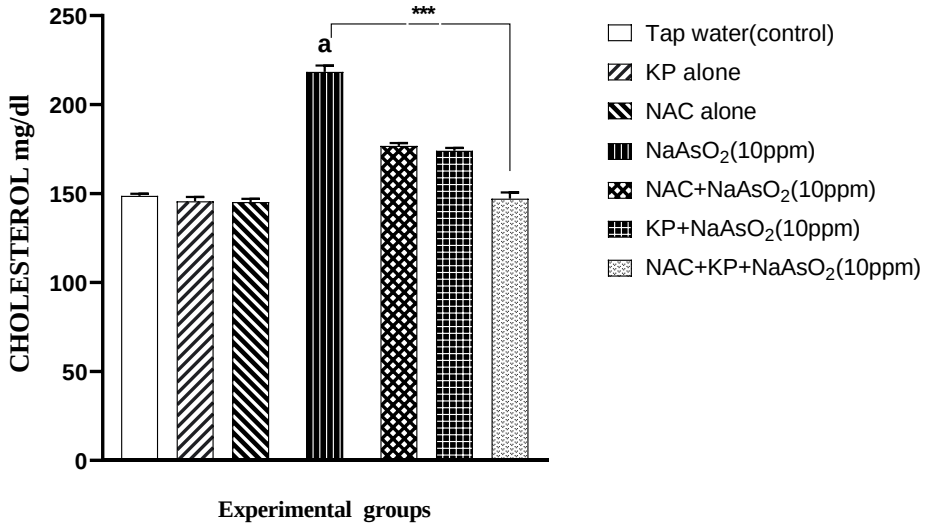

Fig.3.40. Mean Cholesterol in adult male mice serum (n=6/group) upon 35 days exposure to 10 ppm dose of NaAsO2, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). Letter "a" shown significant increase (Group-V NaAsO2 alone, $p < 0.001$) from control. *** $p<0.001$ show significant difference between Group-V NaAsO2 and group VII(NAC+KP+ NaAsO2) treatment group.

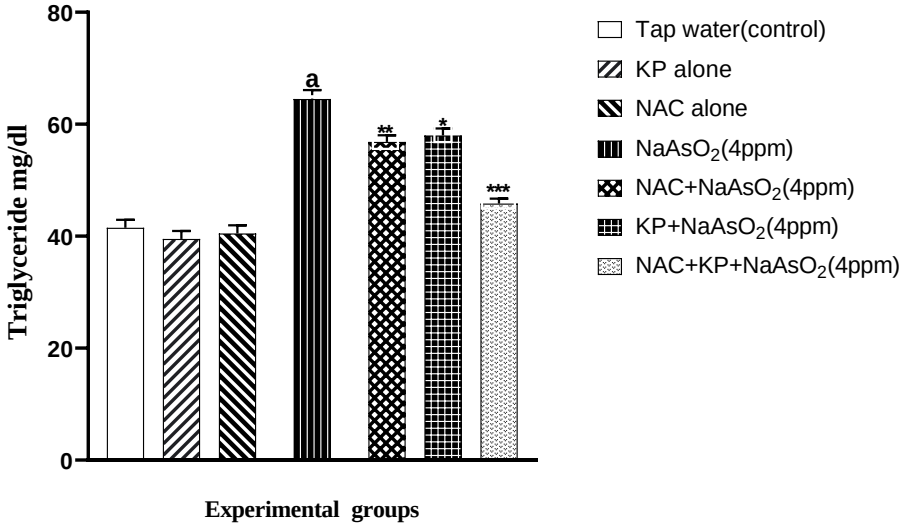

Fig.3.41. Mean Triglyceride in adult mice serum (n=6/group) upon 35 days exposure to 4ppm dose of NaAsO2, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). Letter "a" shown significant increase (Group-IV NaAsO2 alone, $p < 0.001$) from control. In group-VIII (NAC-75+NaAsO2, $p < 0.01$) ,group-X (KP + NaAsO2, $p < 0.05$) and group VI(NAC+KP+NaAsO2,$P<0.001$) ,significantly decrease the Triglyceride level as compared to NaAsO2 treated group.

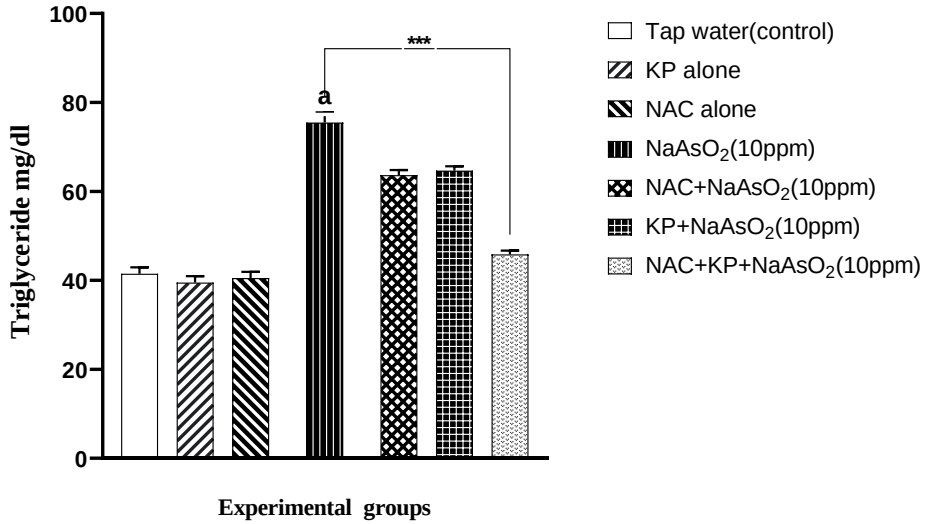

Fig.3.42. Mean Triglyceride in adult mice serum (n=6/group) upon 35 days exposure to 10 ppm dose of NaAsO2, KP-10 (50 nmol/day once a week) and NAC (75 mg/kg b.w. for 5 alternative days). Letter "a" shown significant increase (Group-V $NaAsO_2$ alone, $p < 0.001$) from control. *** $p<0.001$ show significant difference between Group-V NaAsO2 and group VII(NAC+KP+ NaAsO2) treatment group.

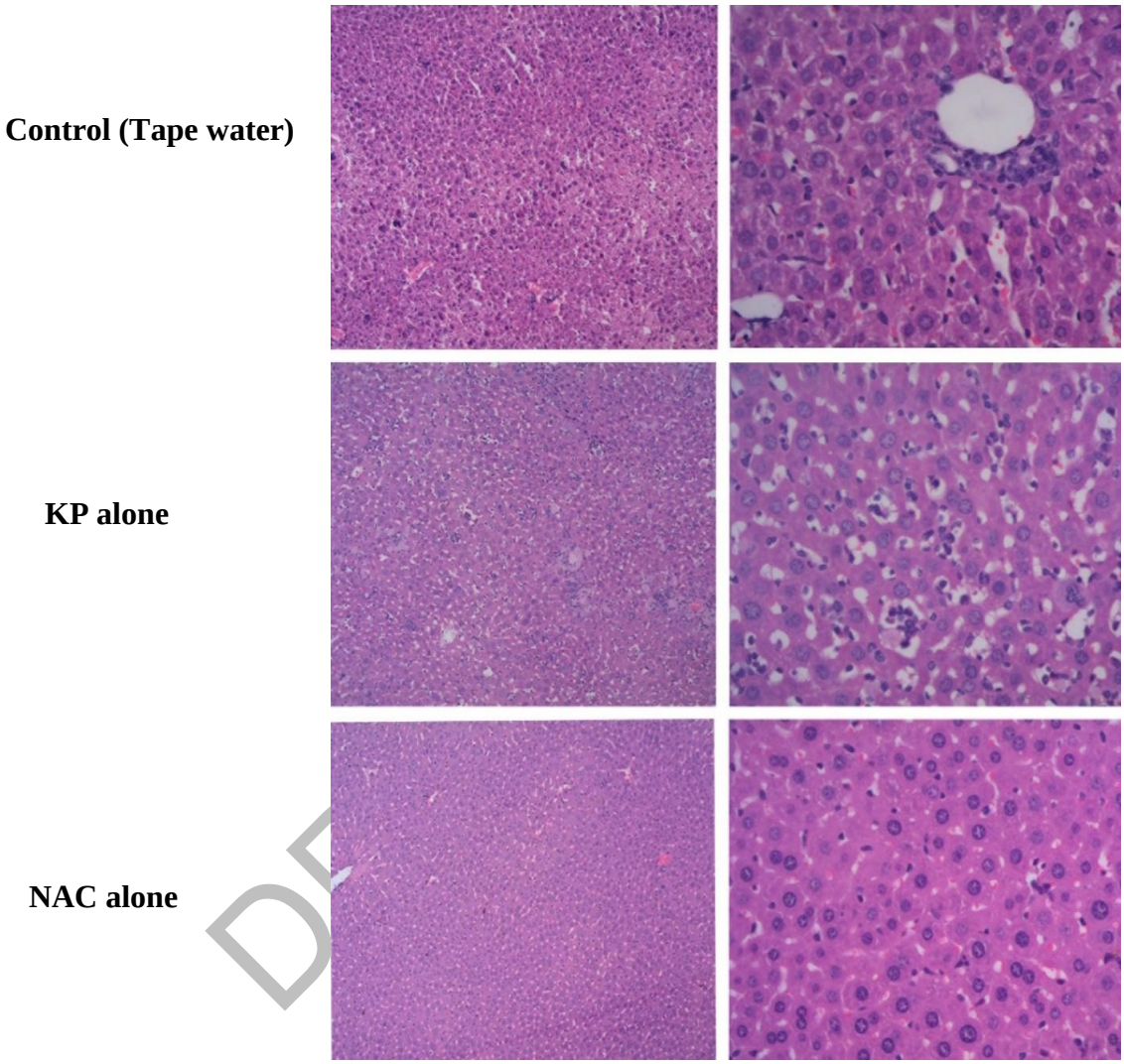

Fig. 3.43. Photomicrographs of mice liver treated with tape water(control) show normal morphology. N-acetyl cysteine alone and KP alone treated groups shows (10X and 40X) normal liver parenchyma; liver cells show fair distribution of hepatocytes, presence of sinusoid with intact capsule.

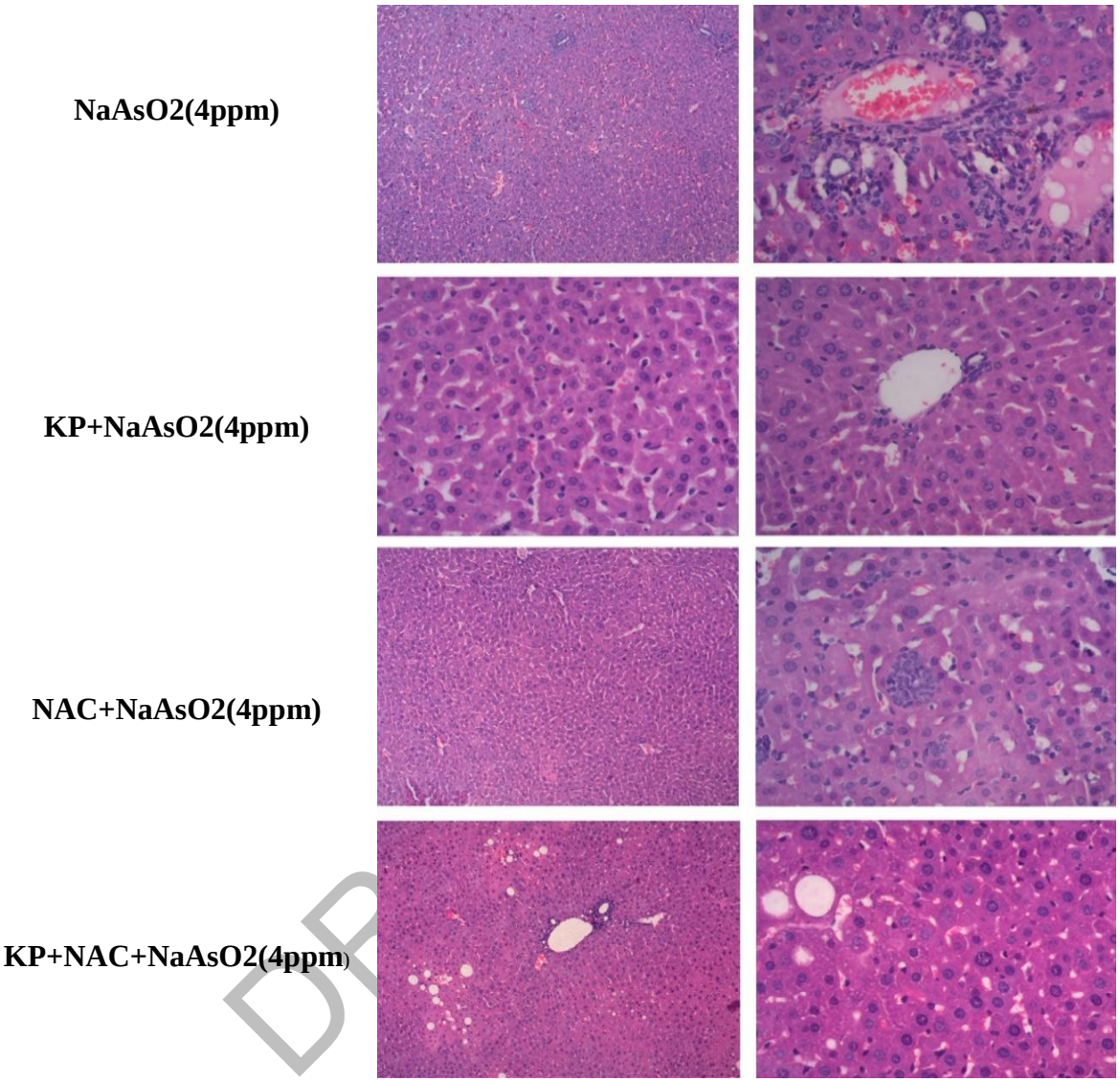

Fig. 3.44. Photomicrographs of mice liver, treated with sodium arsenite(4ppm) showing altered morphology having compromised sinusoidal space and altered hepatocyte distribution as compared to control (tap water). NAC-75+ NaAsO2 alone and KP+ NaAsO2 alone treatments shows mild restored morphology of the sinusoidal space but still shows widen space between the hepatocyte cells while NAC+KP+ NaAsO2 shows recovery of the sinusoidal space and recovered the space between the hepatocyte cells as compared to sodium arsenite(4ppm).

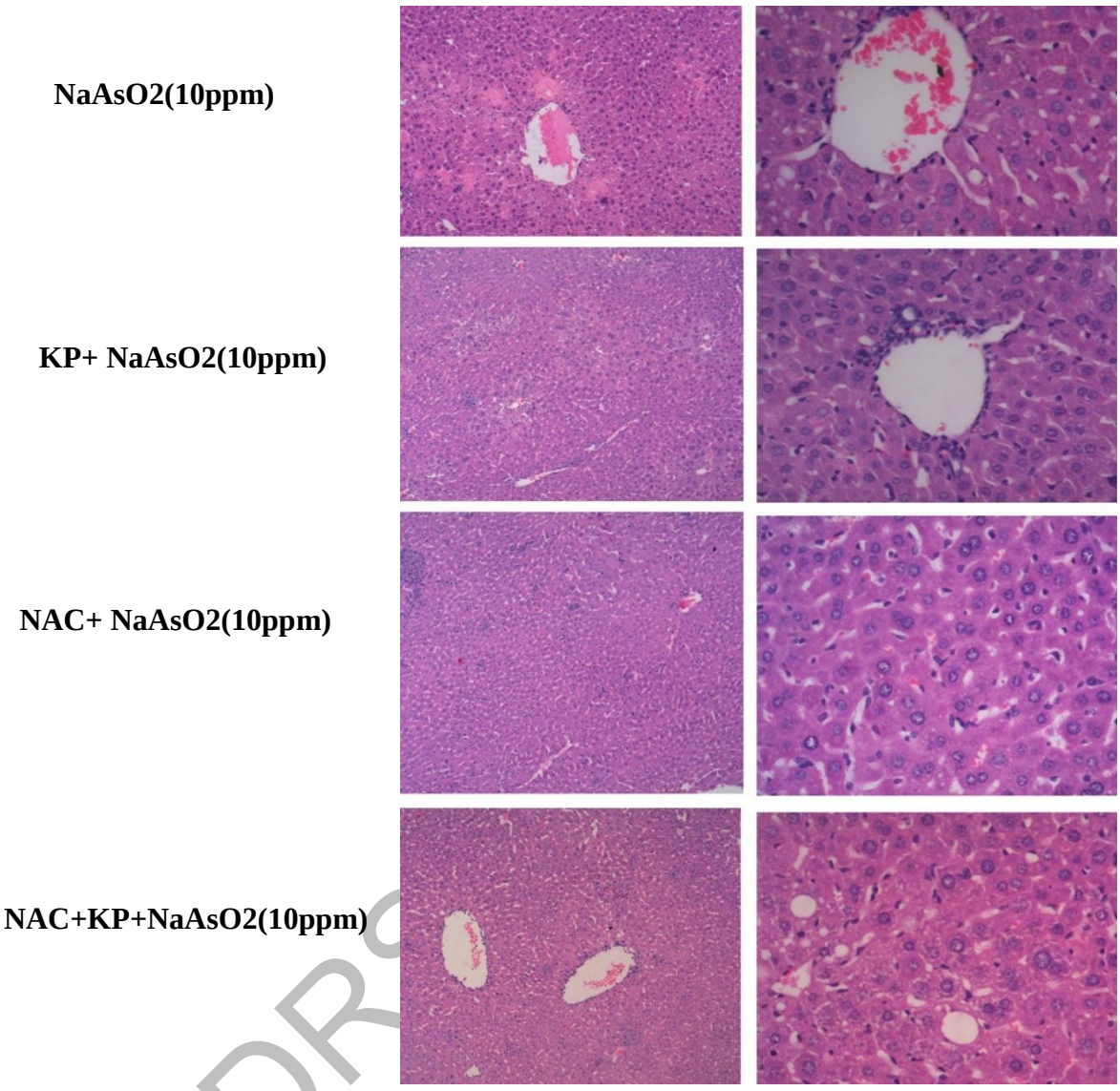

Fig. 3.45. Photomicrographs of mice liver, treated with sodium arsenite(10ppm) showing altered morphology having compromised sinusoidal space and altered hepatocyte distribution as compared to control. NAC-75+ NaAsO2 alone and KP+ NaAsO2 alone treatments shows mild restored morphology of the sinusoidal space but still shows widen space between the hepatocyte cells while NAC+KP+ NaAsO2 shows recovery of the sinusoidal space and recovered the space between the hepatocyte cells as compared to sodium arsenite(10ppm).

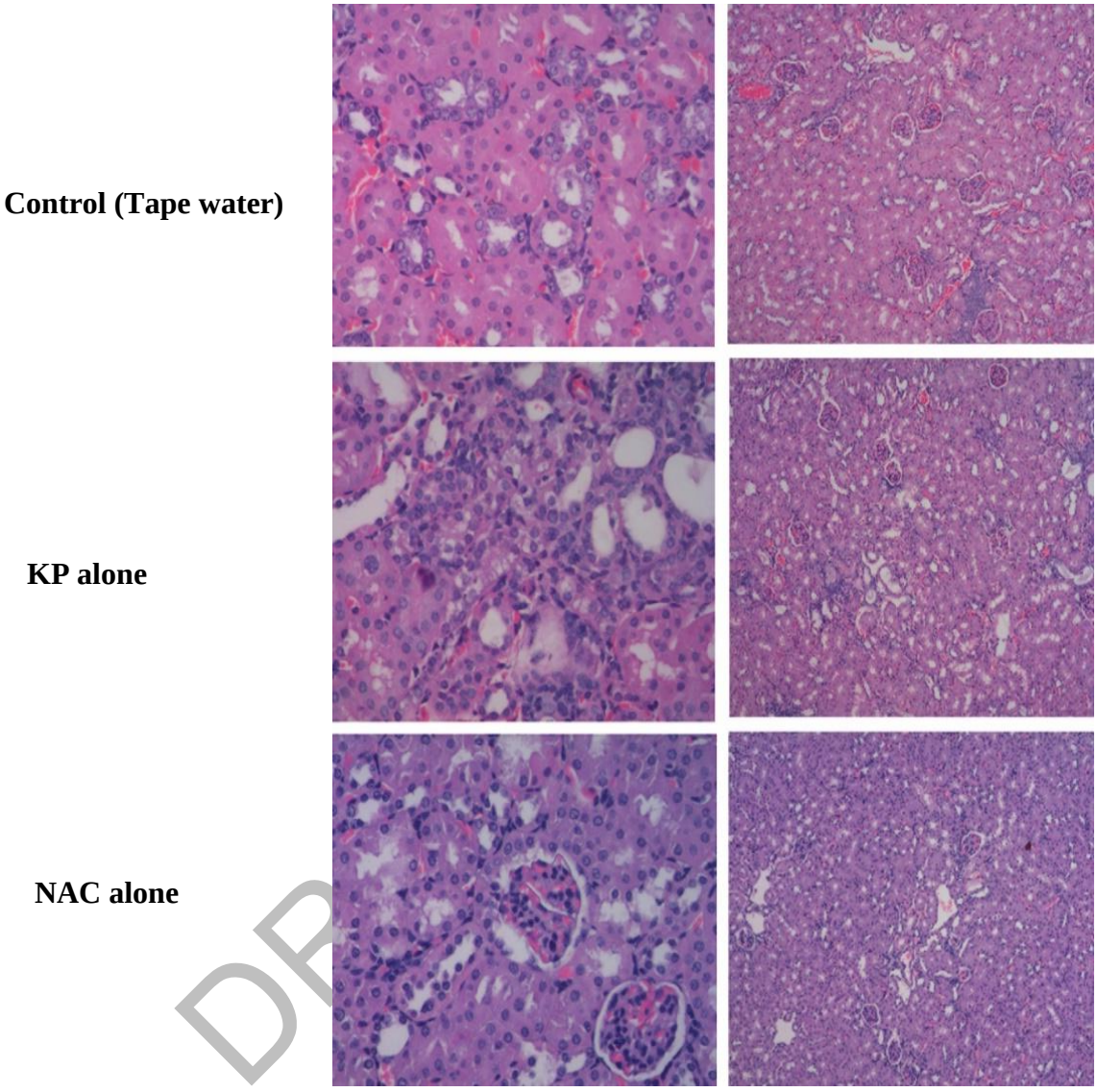

Fig. 3.46. Photomicrographs of mice kidney treated with tap water (control) show normal morphology. N-acetyl cysteine alone and KP alone treated groups shows (10X and 40X) no distinctive morphological alterations normal glomeruli and intact capsule of kidney as compared to control.

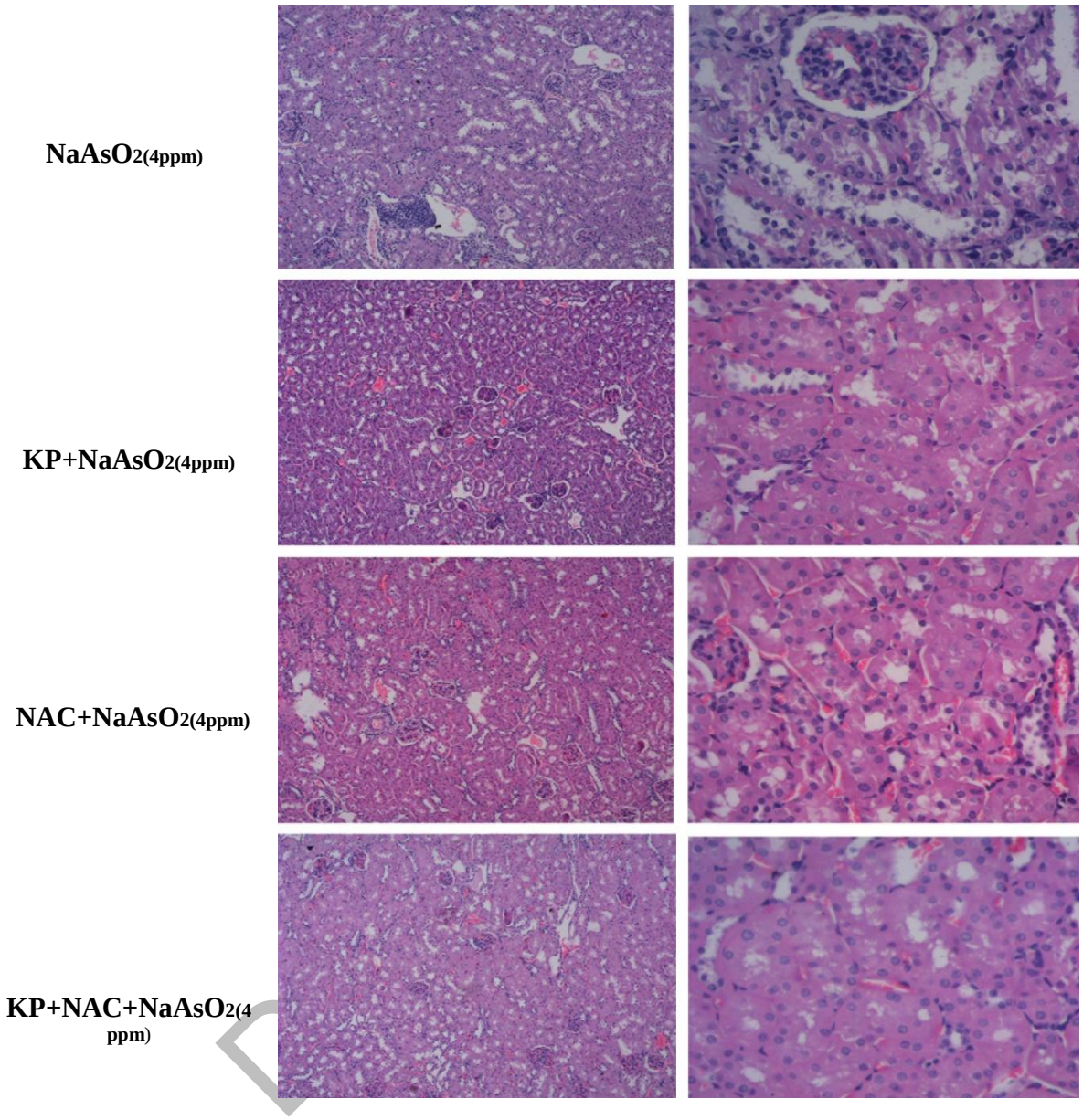

Fig.3.47. Photomicrographs of mice kidney treated with sodium arsenite(4ppm) show shrunken glomerulus, degenerative collecting ducts, vacuole formation and increased bowman space as compared to control (10X and 40X). NAC-75+ NaAsO2 alone and KP+ NaAsO2 alone treatments show mild restored morphology of tubules but still show widen space at bowman capsule while NAC+KP +NaAsO2 shows recovery of the glomerulus and tubules morphology as compared to sodium arsenite(4ppm).

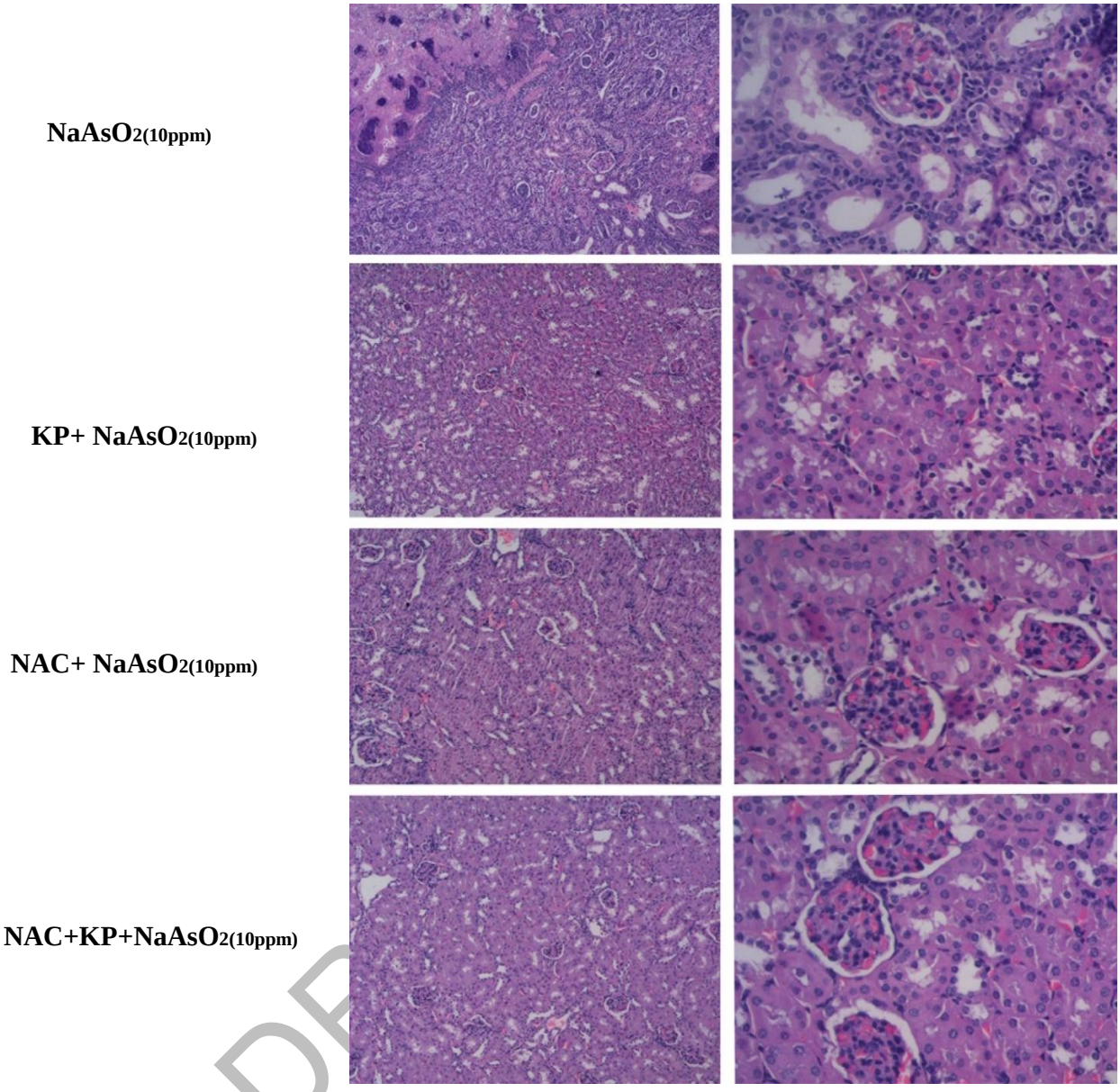

Fig.3.48. Photomicrographs of mice kidney treated with sodium arsenite(10ppm) shows shrunken glomerulus, degenerative collecting ducts, vacuole formation and increased bowman space as compared to control (10X and 40X). NAC-75+ NaAsO2 alone and KP+ NaAsO2 alone treatments shows mild restored morphology of tubules but still shows widen space at bowman capsule while NAC+KP +NaAsO2 shows recovery of the glomerulus and tubules morphology as compared to sodium arsenite(10ppm).

DISCUSSION

Natural and man-made events have polluted the environment by toxic components. (Kuzu *et al*., 2021). Arsenic, the heavy metals (metalloid element), even at low concentrations causes toxicity. Inorganic forms of arsenic are more toxic than organic forms (Shin *et al*., 2013; Parker *et al*., 2019; Habib *et al*., 2020). Arsenic is frequently found in water sources as a pollutant and used in the production of rodenticides, pesticides, herbicides, dyes, soaps, and skin protector (Shanahan and Lorimier, 2016; Oyagbemi *et al*., 2017). Cardiovascular diseases, pulmonary diseases, endocrine system, developmental toxicity, reproductive disorders, cancer, diabetes, behavior change, physiological, skin lesions, neurological and mental health problems have all been linked to its use (Li *et al*., 2013; Calderón-Segura *et al*., 2018; Mouro *et al*., 2018; Chandravanshi *et al*., 2019; Durappanavar *et al*., 2019; Jana *et al*., 2020). Sodium arsenite induced carcinogenicity and mutagenicity have been linked to oxidative stress, with increased ROS causing DNA damage, chromosomal breaks, and cell transformations (Jiang *et al*., 2013). Regarding toxicity, it has been suggested that due to its similar biochemical properties to phosphate, it may inhibit ATP synthesis by replacing phosphate into phosphorylation reactions and may bind to enzymes and sulfhydryl groups (Fattorini and Regoli, 2004; Nozohour and Jalilzadeh-Amin, 2019).

The current study investigated the toxic effects of sodium arsenite, on Balb/c male albino mice following exposure for 35 days. All biochemical and morphological changes were observed. Significant increases occur in oxidative stress markers; ROS and TBARS were elevated, while noticeable decrease occurred in the antioxidant enzymes, SOD, POD, CAT and GSH. In the liver enzyme significant increases occurred, in ALT, ALP, and AST. Increases occurred in creatinine, triglyceride, cholesterol, and in tissue total proteins. Tissue histology demonstrated alteration in cellular morphology, which indicated necrosis.

Monitoring biochemical indices in the kidney and liver can be used to assess metal-associated tissue damage. As a result, we investigated serum biochemical parameters related to kidney and liver disease to assess organ condition in mice given sodium arsenite. The present study showed that the sodium arsenite treated group have increased the levels of AST, ALT and ALP that's leads to the structural damage and liver dysfunction, which is supported by a previous study, like increased of liver transaminase enzymes, particularly AST and ALT indicate liver damage (Murugavel and Pari, 2010). Liver enzyme ALT, AST and ALP elevated when mice F0 and F1 generation treated with

sodium arsenite 10 mg/kg for 60 days (Biswas *et al*., 2020). Another study showed that the elevated levels of ALT, AST, and ALP levels when mice treated with sodium arsenite 10 mg/kg for 90 days. Exposure of As via drinking water alters the biochemical parameters of kidney and liver (Biswas *et al*., 2019). A high AST level specifically destroys liver cells. Aspartate aminotransferase, PLP-dependent enzyme, initiates the conversion of α-ketoglutarate and aspartate to glutamate and oxaloacetate.

The present study also indicated increase levels of creatinine, cholesterol, and triglycerides. These findings agree with results and findings reported in literature Mice treated with 5 mg/kg NaAsO2 for 28 days, induced oxidative damage, inflammation, renal dysfunction, and apoptosis, as shown by marked increases in creatinine and urea levels supplemented by a decrease in the kidney index (AL- Megrin *et al*., 2020). Rats treated with sodium arsenite for 4 weeks also showed nephrotoxicity. Picrosirius red staining and Hematoxylin-eosin revealed pathological alterations in rat kidneys (Sharma *et al*., 2021). These arsenic effects were accompanied by increased Ca2+ATPase inhibition, mitochondrial swelling, mitochondrial calcium depletion and cytochrome c oxidase inhibition. The hepatocytes' viability is primarily determined by membrane systems. Mitochondrial oxidative metabolism in hepatocytes is the primary source for energy (Muthumani and Prabu, 2012).

The primary defence against arsenic oxidative ability is the SOD, which catalyses the conversion of O_2 to H_2O_2, while the CAT enzyme hastens the decomposition of H_2O_2 into H_2O. The superoxide anion (O^{2-}) are vital free radicals produced by arsenic exposure. Arsenic exposure reduces SOD activity and results in an excess of O^{2-} that not dismutated to H2O2. As a result, a decrease in antioxidants such as catalase (CAT) and SOD causes changes in mitochondrial functions such as a significant decrease in mitochondrial outer membrane potential, the generation of caspase-3 and caspase -9, elevated mitochondrial inflammation, ROS levels, the release of cytochrome c which leads to apoptosis. The overexpression of inflammatory moderators such as IL-1, IL-6 and NF- κB, confirmed inflammation. Arsenic has the greatest potential redox potential and blocking the complex I respiratory chain which stimulates hepatocyte gene expression (Bodaghi-Namileh *et al*., 2018).

Liver is the main target for arsenic that leads to generate ROS, lipid, protein, and DNA damage occurred within the cell due to the direct reaction of biomolecules with ROS, therefore leading to apoptosis (Dua *et al*., 2016; Shafik and El Batsh, 2016). In the organism arsenic metabolism causes ROS production and oxidative stress, resulting in

hepatic injury. Arsenic toxicity causes oxidative stress in the liver, which increases lipid peroxides, protein carbonyls, decreases glutathione and antioxidant enzymes. Arsenic activates caspase cascade signaling (III). When changes occur in mitochondrial functional proteins, such as BcL2 associated X apoptosis regulator (Bax), mitochondrial permeability transition (MPT), cytochrome c and B-cell lymphoma 2 (BcL2) cause cell apoptosis. When caspase activity increases it activates the endoplasmic reticulum (ER) stress pathway, as a result decreases glutathione levels with cellular antioxidant mechanisms, it is causing disruption in the DNA repair system and an increase in cellular oxidative damage (Mir-Jamal *et al*., 2013).

The current study found that As exposure increases oxidative pressure in the kidney tissue, as shown by increase in ROS and TBARS, as well as a depletion of nonenzymatic and enzymatic antioxidant molecules. Previous research has suggested that As causes oxidative stress by cycling between metal oxidation–reduction (redox) reactions (e.g., Fe and As) or by inhibiting antioxidant enzymes, resulting in cellular free radical accumulation and inflammation (Flora, 2011).

Experimental mice were supplemented with KP-10 and NAC through intraperitoneal injections along with exposure to NaAsO2 via drinking water. In addition to alone administration of both potential therapeutics, their combined treatment was also provided for evaluation of their synergistic effect against arsenic-induced kidney and liver toxicity. The obtained results revealed the decrease in ROS and TBARS, increase in the activities of antioxidant enzymes, including SOD, POD, CAT, and that of non-enzymatic GSH, and other biochemical parameters including LDH within normal range upon supplementation with KP-10 and NAC in treatment groups exposed to both 4ppm and 10ppm doses of NaAsO2.

Previous data shown that NAC restores the serum markers of liver damage (Bilirubin, ALT, ALP, and AST) that are induced by thioacetamide (TAA) and carbon tetrachloride (CCl4) (Nissar *et al*., 2013). Arsenic-induced testicular damage is restored by NAC, as evidenced by increased of CAT and SOD activities, increased testicular steroidogenesis, decrease testicular lipid peroxidation products, decrease arsenic level in testis and restore testicular weight (Reddy *et al*., 2011). NAC action to restore of cellular antioxidant potential, glutathione level and scavenging the ROS, as well as the inhibition of TNF production and neutrophil activity (Dhouib *et al*., 2016). NAC inhibits apoptosis and neural inflammation through inhibiting oxidative stress (Fan *et al*., 2020). Kisspeptin increased the activities of SOD and catalase. When compared to the control group, the

levels of malondialdehyde, TOS and AST were lower, but levels of BUN, cholesterol, HDL, and AD were higher in the other three groups (Aydin *et al.*, 2010).

Apparently, both KP-10 and NAC prevented the toxic effects of NaAsO2 separately but synergistic effect of combined administration of KP-10 and NAC against NaAsO2 exposure at both 4ppm and 10ppm doses was significantly greater than KP alone supplementation along with NaAsO2 exposure.

No significant difference was observed in the body weights of control and treatment groups but, it was discovered that As-intoxicated mice (Group IV and V) had significantly lower body weight and kidney weight than control mice (Group I). However, relative weights of liver and kidney demonstrated significant decline upon exposure to 4ppm and 10 ppm doses of NaAsO2. However, both alone and combined supplementation with KP-10 and NAC restored the relative organ weights near to control thus indicating their protective effects against arsenic toxicity. This study was supported by Thangapandiyan *et al*., (2019), Arsenic exposure reduced water and food intake and damaged the renal tubular epithelium, resulting in reductions in body and kidney weights and disrupted kidney function, as indicated by creatinine and urea levels. Similar effects of KP-10 have been previously reported against 4ppm and 10ppm doses of $NaAsO_2$ in adult male mice (Fatima and Qureshi, 2022).

Histopathological results of present study indicated widen eradicated sinusoidal space, tissue degenerations, tissue necrosis, central vein congestion and hemorrhages. In the liver tissue section of arsenic treated mice showed less compactness of the cells while control mice did show any abrasions of pathological significance. The kidney tissue also showed less compactness and necrosis. The results obtained in the current comparative study reveal the histoprotective effects of NAC supplementation along with NaASO2 exposure. Similar observations were noticed upon KP-10 supplementation. However, no difference was observed between combined or alone supplementation of NAC and KP-10 along with NaAsO2 exposure.

KP-10 and NAC alone treatments revealed no significant difference in biochemical and histological parameters not only from tap water control but also from each other. These included unaltered levels of oxidative stress parameters, LDH, SOD, POD, CAT levels. In addition, serum parameters and tissue histology also revealed no significant change upon either KP alone or NAC alone administration in mice provided with drinking tap water. These findings agree with our previous results and findings reported by Reddy

et al. (2011) who found no significant change in respective parameters upon NAC alone treatment when compared to control.

Current findings also revealed significantly elevated kidney and liver LDH levels in mice exposed to 4 and 10 ppm doses of NaAsO2 alone. KP-10 and NAC supplementation however equally prevented this increase and maintained the LDH levels near control. Furthermore, concomitant supplementation of KP-10 and NAC was found to be more efficient in lowering the LDH level as compared to KP-10 alone supplementation with NaAsO2 exposure. These results are supported by Kumar et al. (2013) who reported the significant decline in testicular LDH level upon NAC supplementation in rats treated with respective heavy metals in his research. Although underlying mechanism mediating these effects may vary for KP-10 and NAC which need further investigation.

In conclusion, the current study found that sodium arsenite has a negative effect on overall body physiology in mice, causing biochemical and histomorphological changes in kidney, and liver tissue. It has the potential to cause oxidative stress, as evidenced by a significant decrease in antioxidant enzymes and significant increase in ROS and TBARS. This oxidative damage caused an increase in serum toxic biomarkers, which are indicators of kidney and liver tissue damage. In contrast, NAC and Kisspeptin-10, the most common antioxidant, protected cells from oxidative damage and increased antioxidant activity, as evidenced by the renewal of tissue histological architecture.

A molecular study at all signaling levels is required to understand the intracellular pathways of sodium arsenite toxicity and high dose NAC toxicity.

References

Ahmad, S., Kitchin, K. T., & Cullen, W. R. (2000). Arsenic species that cause release of iron from ferritin and generation of activated oxygen. *Archives of Biochemistry and Biophysics*, *382*(2), 195-202.

Akinboro, A., Adedosu, O. T., Badmus, J. A., Adegbola, P. I., Abubakar, M. Y., James, R. O., & Aborisade, A. B. (2022). Aspalathus Linearis extract ameliorate Haematological disorder, dyslipidaemia and tissue toxicity associated with arsenic exposure in Rats. *Phytomedicine Plus*, *2*(1), 100171.

Akkaya, H., Kilic, E., Eyuboglu Dinc, S., & Yilmaz, B. (2014). Postacute effects of kisspeptin- 10 on neuronal injury induced by L- methionine in rats. *Journal of Biochemical and Molecular Toxicology*, *28*(8), 373-377.

Al Osman, M., Yang, F., & Massey, I. Y. (2019). Exposure routes and health effects of heavy metals on children. *Biometals*, *32*, 563-573.

Alloway, B. J. (2013). Sources of heavy metals and metalloids in soils. *Heavy Metals in Soils: Trace Metals and Metalloids in Soils and Their Bioavailability*, 11-50.

Amin, A. F., Shaaban, O. M., & Bediawy, M. A. (2008). N-acetyl cysteine for treatment of recurrent unexplained pregnancy loss. *Reproductive Biomedicine Online*, *17*(5), 722-726.

Angajala, A., Lim, S., Phillips, J. B., Kim, J. H., Yates, C., You, Z., & Tan, M. (2018). Diverse roles of mitochondria in immune responses: novel insights into immuno-metabolism. *Frontiers in Immunology*, *9*, 1605.

Aono, J., Yanagawa, T., Itoh, K., Li, B., Yoshida, H., Kumagai, Y., ... & Ishii, T. (2003). Activation of Nrf2 and accumulation of ubiquitinated A170 by arsenic in osteoblasts. *Biochemical and Biophysical Research Communications*, *305*(2), 271-277.

Arnold, H. L., Odam, R. B., & James, W. D. (1990). Disease of the skin. *Clinical Dermatology. Philadelphia: WB Saunders*, 121-122.

Arsenic, W. H. O. (1981). Environmental health criteria 18. *Geneva: World Health Organization*, *82*.

Arsenic-eaters, Boston Med. Surg. J. 51 (1854) 189

Aschengrau, A., Zierler, S., & Cohen, A. (1989). Quality of community drinking water and the occurrence of spontaneous abortion. *Archives of Environmental Health: An International Journal*, *44*(5), 283-290.

Aslan, M., Senturk, G. E., Akkaya, H., Sahin, S., & Yılmaz, B. (2017). The effect of oxytocin and Kisspeptin-10 in ovary and uterus of ischemia-reperfusion injured rats. *Taiwanese Journal of Obstetrics and Gynecology*, *56*(4), 456-462.

Atkuri, K. R., Mantovani, J. J., Herzenberg, L. A., & Herzenberg, L. A. (2007). N-Acetylcysteine—a safe antidote for cysteine/glutathione deficiency. *Current opinion in Pharmacology*, *7*(4), 355-359.

ATSDR. Priority list of hazardous substances. 2019 [cited 2021 May 20]; Available from: https://www.atsdr.cdc.gov/spl/index.html#2019spl.

Avenant-Oldewage, A. & Marx, H. M. (2000). Bioaccumulation of chromium, copper and iron in the organs and tissues of Clarias gariepinus in the Olifants River, Kruger National Park. *Water SA*, *26*(4), 569-582.

Aydilek, N., Varisli, O., Kocyigit, A. B. D. Ü. R. R. A. H. İ. M., Taskin, A., & Kaya, M. S. (2015). Effect of dietary restriction on sperm characteristic and oxidative status on testicular tissue in young rats exposed to long- term heat stress. *Andrologia*, *47*(9), 1055-1061.

Aydin, M., Oktar, S., Yonden, Z., Ozturk, O. H., & Yilmaz, B. (2010). Direct and indirect effects of kisspeptin on liver oxidant and antioxidant systems in young male rats. *Cell Biochemistry and Function: Cellular Biochemistry and its Modulation by Active Agents or Disease*, *28*(4), 293-299.

Azadmanesh, J., Lutz, W. E., Weiss, K. L., Coates, L., & Borgstahl, G. E. (2018). Redox manipulation of the manganese metal in human manganese superoxide dismutase for neutron diffraction. *Acta Crystallographica Section F: Structural Biology Communications*, *74*(10), 677-687.

Backholer, K., Smith, J. T., Rao, A., Pereira, A., Iqbal, J., Ogawa, S., ... & Clarke, I. J. (2010). Kisspeptin cells in the ewe brain respond to leptin and communicate with neuropeptide Y and proopiomelanocortin cells. *Endocrinology*, *151*(5), 2233-2243.

Baker, D. E., & Chesnin, L. (1975). Chemical monitoring of soils for environmental quality and animal and human health. *Advances in agronomy*, *27*, 305-374.

Bánfalvi, G. (2011). Heavy metals, trace elements and their cellular effects. *Cellular effects of heavy metals*, 3-28.

Bashir, S., Sharma, Y., Irshad, M., Nag, T. C., Tiwari, M., Kabra, M., & Dogra, T. D. (2006). Arsenic induced apoptosis in rat liver following repeated 60 days exposure. *Toxicology*, *217*(1), 63-70.

Becker, J. A., Mirjolet, J. F., Bernard, J., Burgeon, E., Simons, M. J., Vassart, G., ... & Libert, F. (2005). Activation of GPR54 promotes cell cycle arrest and apoptosis of human tumor cells through a specific transcriptional program not shared by other Gq-coupled receptors. *Biochemical and biophysical research communications*, *326*(3), 677-686.

Belhadj Slimen, I., Najar, T., Ghram, A., Dabbebi, H., Ben Mrad, M., & Abdrabbah, M. (2014). Reactive oxygen species, heat stress and oxidative-induced mitochondrial damage. A review. *International journal of hyperthermia*, *30*(7), 513-523.

Bennett, G. F. (1985). Handbook of toxic and hazardous chemicals and carcinogens, 2nd edn., Noyes Publications, Park Ridge, NJ, 1985, 950 pages, $96.

Bhattacharya P, Frisbie SH, Smith E, Naidu R, Jacks G, Sarkar B (2002). Arsenic in the environment, from a global perspective. In: Sarkar B, editor. Handbook of heavy metals in the environment. New York: Marcell Dekker;pp. 147–215.

Bodaghi-Namileh, V., Sepand, M. R., Omidi, A., Aghsami, M., Seyednejad, S. A., Kasirzadeh, S., & Sabzevari, O. (2018). Acetyl-l-carnitine attenuates arsenic-induced liver injury by abrogation of mitochondrial dysfunction, inflammation, and apoptosis in rats. *Environmental toxicology and pharmacology*, *58*, 11-20.

Bode, A. M., & Dong, Z. (2002). The paradox of arsenic: molecular mechanisms of cell transformation and chemotherapeutic effects. *Critical reviews in oncology/hematology*, *42*(1), 5-24.

Bowe, J. E., Hill, T. G., Hunt, K. F., Smith, L. I., Simpson, S. J., Amiel, S. A., & Jones, P. M. (2019). A role for placental kisspeptin in β cell adaptation to pregnancy. *JCI insight*, *4*(20).

Bowen, H. J. M. (1966). Trace elements in biochemistry. *Trace elements in biochemistry.*

Bowen, H. J. M. (1979). *Environmental chemistry of the elements*. Academic Press.

Brinkel, J., Khan, M. H., & Kraemer, A. (2009). A systematic 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.

Buchet, J. P., Lauwerys, R., & Roels, H. (1981). Comparison of the urinary excretion of arsenic metabolites after a single oral dose of sodium arsenite, monomethylarsonate, or dimethylarsinate in man. *International archives of occupational and environmental health*, *48*, 71-79.

Buchet, J. P., Lauwerys, R., & Roels, H. (1981). Urinary excretion of inorganic arsenic and its metabolites after repeated ingestion of sodium metaarsenite by

volunteers. *International archives of occupational and environmental health*, *48*, 111-118.

Bundschuh, J., & Garcia, M. E. (2008). Rural Latin America—A forgotten part of the global groundwater arsenic problem?. In *Groundwater for Sustainable Development* (pp. 335-346). CRC Press.

Cárdenas-González, M., Osorio-Yáñez, C., Gaspar-Ramírez, O., Pavković, M., Ochoa-Martinez, A., López-Ventura, D., ... & Vaidya, V. S. (2016). Environmental exposure to arsenic and chromium in children is associated with kidney injury molecule-1. *Environmental research*, *150*, 653-662.

Castellano, J. M., & Tena-Sempere, M. (2016). Metabolic control of female puberty: potential therapeutic targets. *Expert opinion on therapeutic targets*, *20*(10), 1181-1193.

Castellano, J. M., Navarro, V. M., Fernandez-Fernandez, R., Castano, J. P., Malagon, M. M., Aguilar, E., ... & Tena-Sempere, M. (2006). Ontogeny and mechanisms of action for the stimulatory effect of kisspeptin on gonadotropin-releasing hormone system of the rat. *Molecular and cellular endocrinology*, *257*, 75-83.

Castellano, J. M., Navarro, V. M., Fernandez-Fernandez, R., Roa, J., Vigo, E., Pineda, R., ... & Tena-Sempere, M. (2006). Effects of galanin-like peptide on luteinizing hormone secretion in the rat: sexually dimorphic responses and enhanced sensitivity at male puberty. *American Journal of Physiology-Endocrinology and Metabolism*, *291*(6), E1281-E1289.

Cerrato, F., Shagoury, J., Kralickova, M., Dwyer, A., Falardeau, J., Ozata, M., ... & Seminara, S. B. (2006). Coding sequence analysis of GNRHR and GPR54 in patients with congenital and adult-onset forms of hypogonadotropic hypogonadism. *European Journal of Endocrinology*, *155*(Supplement_1), S3-S10.

Chen, A. T., Chibnall, J. T., & Nasrallah, H. A. (2016). Placebo-controlled augmentation trials of the antioxidant NAC in schizophrenia: A review. *Annals of clinical psychiatry: official journal of the American Academy of Clinical Psychiatrists*, *28*(3), 190-196.

Chin, N. P. (2010). Environmental toxins: Physical, social, and emotional. *Breastfeeding Medicine*, *5*(5), 223.

Chiou, H. Y., Huang, W. I., Su, C. L., Chang, S. F., Hsu, Y. H., & Chen, C. J. (1997). Dose-response relationship between prevalence of cerebrovascular disease and ingested inorganic arsenic. *Stroke*, *28*(9), 1717-1723.

Chouchane, S., & Snow, E. T. (2001). In vitro effect of arsenical compounds on glutathione-related enzymes. *Chemical research in toxicology*, *14*(5), 517-522.

Clarke, I. J. (2002). Two decades of measuring GnRH secretion. *Reproduction (Cambridge, England). Supplement*, *59*, 1-13.

Clarkson, J., & Herbison, A. E. (2006). Postnatal development of kisspeptin neurons in mouse hypothalamus; sexual dimorphism and projections to gonadotropin-releasing hormone neurons. *Endocrinology*, *147*(12), 5817-5825.

ClinicalTrials.gov. Available online: https://clinicaltrials.gov (accessed on 2 April 2019).

Compounds, W. A. (2001). Environmental Health Criteria 224. *World Health Organisation, Geneva*.

Concha, G., Vogler, G., Nermell, B., & Vahter, M. (1998). Low-level arsenic excretion in breast milk of native Andean women exposed to high levels of arsenic in the drinking water. *International archives of occupational and environmental health*, *71*, 42-46.

Cordier, W., Yousaf, M., Nell, M. J., & Steenkamp, V. (2021). Underlying mechanisms of cytotoxicity in HepG2 hepatocarcinoma cells exposed to arsenic, cadmium and mercury individually and in combination. *Toxicology in Vitro*, *72*, 105101.

Corsini, E., Asti, L., Viviani, B., Marinovich, M., & Galli, C. L. (1999). Sodium arsenate induces overproduction of interleukin-1α in murine keratinocytes: role of mitochondria. *Journal of investigative dermatology*, *113*(5), 760-765.

Cotter, M. A., Thomas, J., Cassidy, P., Robinette, K., Jenkins, N., Florell, S. R., ... & Grossman, D. (2007). N-acetylcysteine protects melanocytes against oxidative stress/damage and delays onset of ultraviolet-induced melanoma in mice. *Clinical Cancer Research*, *13*(19), 5952-5958.

Cox, D. P., & Alexander, M. (1973). Effect of phosphate and other anions on trimethylarsine

Crecelius, E. A., Johnson, C. J., & Hofer, G. C. (1974). Contamination of soils near a copper smelter by arsenic, antimony and lead. *Water, air, and soil pollution*, *3*, 337-342.

Crespy, V., & Williamson, G. (2004). A review of the health effects of green tea catechins in in vivo animal models. *The Journal of nutrition*, *134*(12), 3431S-3440S.

Crook, M. (2006). *Clinical chemistry & metabolic medicine* (Vol. 426). London: Hodder Arnold.

Cuadrado, A., Martín-Moldes, Z., Ye, J., & Lastres-Becker, I. (2014). Transcription factors NRF2 and NF-κB are coordinated effectors of the Rho family, GTP-binding protein RAC1 during inflammation. *Journal of Biological Chemistry*, *289*(22), 15244-15258.

d'Anglemont de Tassigny, X., & Colledge, W. H. (2010). The role of kisspeptin signaling in reproduction. *Physiology*, *25*(4), 207-217.

d'Anglemont de Tassigny, X., Fagg, L. A., Dixon, J. P., Day, K., Leitch, H. G., Hendrick, A. G., ... & Colledge, W. H. (2007). Hypogonadotropic hypogonadism in mice lacking a functional Kiss1 gene. *Proceedings of the National Academy of Sciences*, *104*(25), 10714-10719.

d'Anglemont de Tassigny, X., Jayasena, C., Murphy, K. G., Dhillo, W. S., & Colledge, W. H. (2017). Mechanistic insights into the more potent effect of KP-54 compared to KP-10 in vivo. *PLoS One*, *12*(5), e0176821.

De Oliveira, C. P. M. S., Stefano, J. T., De Siqueira, E. R. F., Silva, L. S., de Campos Mazo, D. F., Lima, V. M. R., ... & Carrilho, F. J. (2008). Combination of N- acetylcysteine and metformin improves histological steatosis and fibrosis in patients with non-alcoholic steatohepatitis. *Hepatology Research*, *38*(2), 159-165.

De Roux, N., Genin, E., Carel, J. C., Matsuda, F., Chaussain, J. L., & Milgrom, E. (2003). Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proceedings of the National Academy of Sciences*, *100*(19), 10972-10976.

Del Razo, L. M., Quintanilla-Vega, B., Brambila-Colombres, E., Calderón-Aranda, E. S., Manno, M., & Albores, A. (2001). Stress proteins induced by arsenic. *Toxicology and applied pharmacology*, *177*(2), 132-148.

Demers-Lamarche, J., Guillebaud, G., Tlili, M., Todkar, K., Bélanger, N., Grondin, M., ... & Germain, M. (2016). Loss of mitochondrial function impairs lysosomes. *Journal of biological chemistry*, *291*(19), 10263-10276.

DG Environment, Ambient air pollution by As, Cd and Ni compounds, Position Paper, Working Group on Arsenic,Cadmium and Nickel Compounds, DG Environment European Commission, 2000.

Dhar, D. K., Naora, H., Kubota, H., Maruyama, R., Yoshimura, H., Tonomoto, Y., ... & Nagasue, N. (2004). Downregulation of KiSS- 1 expression is responsible for tumor invasion and worse prognosis in gastric carcinoma. *International Journal of Cancer*, *111*(6), 868-872.

Dhillo, W. S., Chaudhri, O. B., Patterson, M., Thompson, E. L., Murphy, K. G., Badman, M. K., ... & Bloom, S. R. (2005). Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males. *The Journal of Clinical Endocrinology & Metabolism*, *90*(12), 6609-6615.

Diniz, Y. S., Rocha, K. K., Souza, G. A., Galhardi, C. M., Ebaid, G. M., Rodrigues, H. G., ... & Novelli, E. L. (2006). Effects of N-acetylcysteine on sucrose-rich diet-induced hyperglycaemia, dyslipidemia and oxidative stress in rats. *European journal of pharmacology*, *543*(1-3), 151-157.

Dinkova-Kostova, A. T., Holtzclaw, W. D., Cole, R. N., Itoh, K., Wakabayashi, N., Katoh, Y., ... & Talalay, P. (2002). Direct evidence that sulfhydryl groups of Keap1 are the sensors soxidants. *Proceedings of the National Academy of Sciences*, *99*(18), 11908-11913.

Dopp, E., Kligerman, A. D., & Diaz-Bone, R. A. (2010). Organoarsenicals. Uptake, metabolism, and toxicity. *Organometallics in Environment and Toxicology*, *7*.

Drobná, Z., Jaspers, I., Thomas, D. J., & Stýblo, M. (2003). Differential activation of AP- 1 in human bladder epithelial cells by inorganic and methylated arsenicals. *The FASEB Journal*, *17*(1), 67-69

Du, X., Tian, M., Wang, X., Zhang, J., Huang, Q., Liu, L., & Shen, H. (2018). Cortex and hippocampus DNA epigenetic response to a long-term arsenic exposure via drinking water. *Environmental pollution*, *234*, 590-600.

Duan, X., Gao, S., Li, J., Wu, L., Zhang, Y., Li, W., ... & Li, B. (2017). Acute arsenic exposure induces inflammatory responses and CD4+ T cell subpopulations differentiation in spleen and thymus with the involvement of MAPK, NF-kB, and Nrf2. *Molecular immunology*, *81*, 160-172.

Dudek, M., Ziarniak, K., & Sliwowska, J. H. (2018). Kisspeptin and metabolism: the brain and beyond. *Frontiers in endocrinology*, *9*, 145.

Dutré, V. A. N. D. E. C. A. S. T. E. E. L. E., & Vandecasteele, C. (1995). Solidification/stabilisation of arsenic-containing waste: leach tests and behaviour of arsenic in the leachate. *Waste Management*, *15*(1), 55-62.

Echevarria, M. A. N., Reis, T. A., Capatti, G. R., Soares, V. S., da Silveira, D. X., & Fidalgo, T. M. (2017). N-acetylcysteine for treating cocaine addiction–A systematic review. *Psychiatry research*, *251*, 197-203.

El-Demerdash, F. M., Yousef, M. I., & Radwan, F. M. (2009). Ameliorating effect of curcumin on sodium arsenite-induced oxidative damage and lipid peroxidation in different rat organs. *Food and Chemical Toxicology*, *47*(1), 249-254.

Ellinsworth, D. C. (2015). Arsenic, reactive oxygen, and endothelial dysfunction. *Journal of Pharmacology and Experimental Therapeutics*, *353*(3), 458-464.

El-Orabi, N. F., Rogers, C. B., Edwards, H. G., & Schwartz, D. D. (2011). Heat-induced inhibition of superoxide dismutase and accumulation of reactive oxygen species leads to HT-22 neuronal cell death. *Journal of Thermal Biology*, *36*(1), 49-56.

EPA (1984). Health Assessment Document for inorganic arsenic. Final report, EPA, 600/8-83-021F. USEPA. Environmental criteria and assessment office. Research Triangle Park, N.C.

Ercal, N., Gurer-Orhan, H., & Aykin-Burns, N. (2001). Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. *Current topics in medicinal chemistry*, *1*(6), 529-539.

F.M. Fordyce, T.M. Williams, A. Paijitpapapon, P.Charoenchaisei, British Geol. Survey, Keyworth, 1995.

Farkhondeh, T., Naseri, K., Esform, A., Aramjoo, H., & Naghizadeh, A. (2021). Drinking water heavy metal toxicity and chronic kidney diseases: a systematic review. *Reviews on Environmental Health*, *36*(3), 359-366.

Fatoki, J. O., Adeleke, G. E., Badmus, J. A., Afolabi, O. K., Adedosu, O. T., Kehinde, S. A., & Adekunle, A. S. (2019). Time–Course of Sodium Arsenate Induced Hepatotoxicity and Nephrotoxicity in Male Wistar Rats. *microcirculation*, *9*(4)..

Feed Additive Compendium, vol. 13, Munneapolis, The Miller Publishing Company, 1975, p. 330.

Fernandois, D., Cruz, G., Na, E. K., Lara Peñaloza, H., & Paredes, A. (2017). Kisspeptin level in the aging ovary is regulated by the sympathetic nervous system.

Finch, L. E., Hillyer, M. M., & Leopold, M. C. (2015). Quantitative analysis of heavy metals in children's toys and jewelry: a multi-instrument, multitechnique exercise in analytical chemistry and public health. *Journal of chemical education*, *92*(5), 849-854.

Flora, S. J. (2011). Arsenic-induced oxidative stress and its reversibility. *Free Radical Biology and Medicine*, *51*(2), 257-281.

formation by Candida humicola. *Applied Microbiology*, *25*(3), 408-413.

Fu, L. Y., & van den Pol, A. N. (2010). Kisspeptin directly excites anorexigenic proopiomelanocortin neurons but inhibits orexigenic neuropeptide Y cells by an indirect synaptic mechanism. *Journal of Neuroscience*, *30*(30), 10205-10219.

Fulghesu, A. M., Ciampelli, M., Muzj, G., Belosi, C., Selvaggi, L., Ayala, G. F., & Lanzone, A. (2002). N-acetyl-cysteine treatment improves insulin sensitivity in women with polycystic ovary syndrome. *Fertility and sterility*, *77*(6), 1128-1135.

Funes, S., Hedrick, J. A., Vassileva, G., Markowitz, L., Abbondanzo, S., Golovko, A., ... & Gustafson, E. L. (2003). The KiSS-1 receptor GPR54 is essential for the development of the murine reproductive system. *Biochemical and biophysical research communications*, *312*(4), 1357-1363.

Ghafarizadeh, A., Malmir, M., Naderi Noreini, S., & Faraji, T. (2021). Antioxidant effects of N- acetylcysteine on the male reproductive system: A systematic review. *Andrologia*, *53*(1), e13898.

Goddard, M. J., Tanhehco, J. L., & Dau, P. C. (1992). Chronic arsenic poisoning masquerading as Landry-Guillain-Barré syndrome. *Electromyography and clinical neurophysiology*, *32*(9), 419-423.

Goebel, H. H., Schmidt, P. F., Bohl, J., Tettenborn, B., Krämer, G., & Gutmann, L. (1990). Polyneuropathy due to acute arsenic intoxication: biopsy studies. *Journal of neuropathology and experimental neurology*, *49*(2), 137-149.

Gottsch, M. L., Clifton, D. K., & Steiner, R. A. (2009). From KISS1 to kisspeptins: an historical perspective and suggested nomenclature. *Peptides*, *30*(1), 4-9.

Gottsch, M. L., Cunningham, M. J., Smith, J. T., Popa, S. M., Acohido, B. V., Crowley, W. F., ... & Steiner, R. A. (2004). A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. *Endocrinology*, *145*(9), 4073-4077.

Grandjean, P., Weihe, P., Needham, L. L., Burse, V. W., Patterson Jr, D. G., Sampson, E. J., ... & Vahter, M. (1995). Effect of a seafood diet on mercury, selenium, arsenic, and PCBs and other organochlorines in human milk. *Environ Res*, *71*, 29-38.

Green, J. L., Heard, K. J., Reynolds, K. M., & Albert, D. (2013). Oral and intravenous acetylcysteine for treatment of acetaminophen toxicity: A systematic review and meta-analysis. *Western Journal of Emergency Medicine*, *14*(3), 218.

Grimsrud, T. K., & Andersen, A. (2012). Unrecognized risks of nickel-related respiratory cancer among Canadian electrolysis workers. *Scandinavian journal of work, environment & health*, 503-515.

Grimsrud, T. K., Berge, S. R., Martinsen, J. I., & Andersen, A. (2003). Lung cancer incidence among Norwegian nickel-refinery workers 1953–2000. *Journal of environmental monitoring*, *5*(2), 190-197.

Guha Mazumder, D., Purkayastha, I., Ghose, A., Mistry, G., Saha, C., Nandy, A. K., ... & Majumdar, K. K. (2012). Hypertension in chronic arsenic exposure: A case control study in West Bengal. *Journal of Environmental Science and Health, Part A*, *47*(11), 1514-1520.

Guidarelli, A., Fiorani, M., Cerioni, L., Scotti, M., & Cantoni, O. (2017). Arsenite induces DNA damage via mitochondrial ROS and induction of mitochondrial permeability transition. *Biofactors*, *43*(5), 673-684.

Guidarelli,A.; Fiorani, M.; Cerioni, L.; Scotti, M.; Cantoni, O. Arsenite induces DNA damage via mitochondrial ROS and induction of mitochondrial permeability transition. Biofactors 2017, 43, 673–684. [CrossRef]

Guidelines for Drinking Water Quality, Recommendations, 2nd ed., vol. 2, World Health Organisation,Geneva, 1996.

Gul, N., Shah, M. T., Khan, S., Khattak, N. U., & Muhammad, S. (2015). Arsenic and heavy metals contamination, risk assessment and their source in drinking water of the Mardan District, Khyber Pakhtunkhwa, Pakistan. *Journal of water and health*, *13*(4), 1073-1084.

Gulledge, J. H., & O'Connor, J. T. (1973). Removal of arsenic (V) from water by adsorption on aluminum and ferric hydroxides. *Journal- American Water Works Association*, *65*(8), 548-552.

Guo, F., Li, Y., Wang, J., Li, Y., Li, Y., & Li, G. (2013). Stanniocalcin1 (STC1) inhibits cell proliferation and invasion of cervical cancer cells. *PloS one*, *8*(1), e53989.

Hambidge, K. M., & Krebs, N. F. (2007). Zinc deficiency: a special challenge. *The Journal of nutrition*, *137*(4), 1101-1105.

Han, S. K., Gottsch, M. L., Lee, K. J., Popa, S. M., Smith, J. T., Jakawich, S. K., ... & Herbison, A. E. (2005). Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. *Journal of Neuroscience*, *25*(49), 11349-11356.

Harter, C. J., Kavanagh, G. S., & Smith, J. T. (2018). The role of kisspeptin neurons in reproduction and metabolism. *Journal of Endocrinology*, *238*(3), R173-R183.

Harvey, P. J., Handley, H. K., & Taylor, M. P. (2015). Identification of the sources of metal (lead) contamination in drinking waters in north-eastern Tasmania using lead isotopic compositions. *Environmental Science and Pollution Research*, *22*, 12276-1228

Hayyan, M., Hashim, M. A., & AlNashef, I. M. (2016). Superoxide ion: generation and chemical implications. *Chemical reviews*, *116*(5), 3029-30.

He, J., Wang, M., Jiang, Y., Chen, Q., Xu, S., Xu, Q., ... & Liu, L. Z. (2014). Chronic arsenic exposure and angiogenesis in human bronchial epithelial cells via the ROS/miR-199a-5p/HIF-1 α/COX-2 pathway. *Environmental health perspectives*, *122*(3), 255-261.

He, X., & Ma, Q. (2009). NRF2 cysteine residues are critical for oxidant/electrophile-sensing, Kelch-like ECH-associated protein-1-dependent ubiquitination-proteasomal degradation, and transcription activation. *Molecular pharmacology*, *76*(6), 1265-1278.

Healy, S. M., Wildfang, E., Zakharyan, R. A., & Aposhian, H. V. (1999). Diversity of inorganic arsenite biotransformation. *Biological trace element research*, *68*, 249-266.

Hopenhayn-Rich, C., Browning, S. R., Hertz-Picciotto, I., Ferreccio, C., Peralta, C., & Gibb, H. (2000). Chronic arsenic exposure and risk of infant mortality in two areas of Chile. *Environmental health perspectives*, *108*(7), 667-673

Hopf, F. W. (2017). Do specific NMDA receptor subunits act as gateways for addictive behaviors?. *Genes, Brain and Behavior*, *16*(1), 118-138.

Hou, Y., Wang, X., Ping, J., Lei, Z., Gao, Y., Ma, Z., ... & Su, J. (2017). Metabonomics approach to assessing the modulatory effects of kisspeptin-10 on liver injury induced by heat stress in rats. *Scientific Reports*, *7*(1), 7020.

Hou, Y., Wang, Y., Wang, H., & Xu, Y. (2014). Induction of glutathione synthesis in human hepatocytes by acute and chronic arsenic exposure: differential roles of mitogen-activated protein kinases. *Toxicology*, *325*, 96-106.

Hrabovszky, E., Ciofi, P., Vida, B., Horvath, M. C., Keller, E., Caraty, A., ... & Kallo, I. (2010). The kisspeptin system of the human hypothalamus: sexual dimorphism and relationship with gonadotropin- releasing hormone and neurokinin B neurons. *European Journal of Neuroscience*, *31*(11), 1984-1998.

Hrabovszky, E., Molnár, C. S., Sipos, M. T., Vida, B., Ciofi, P., Borsay, B. A., ... & Liposits, Z. (2011). Sexual dimorphism of kisspeptin and neurokinin B immunoreactive neurons in the infundibular nucleus of aged men and women. *Frontiers in endocrinology*, *2*, 80.

Hrycay, E. G., & Bandiera, S. M. (2015). Involvement of cytochrome P450 in reactive oxygen species formation and cancer. *Advances in Pharmacology*, *74*, 35-84.

Hu, Y., Jin, X., & Snow, E. T. (2002). Effect of arsenic on transcription factor AP-1 and NF-κB DNA binding activity and related gene expression. *Toxicology letters*, *133*(1), 33-45.

Huang, Q., Zhan, L., Cao, H., Li, J., Lyu, Y., Guo, X., ... & Xing, J. (2016). Increased mitochondrial fission promotes autophagy and hepatocellular carcinoma cell survival through the ROS-modulated coordinated regulation of the NFKB and TP53 pathways. *Autophagy*, *12*(6), 999-1014.

Hunter, T. (2014). The genesis of tyrosine phosphorylation. *Cold Spring Harbor perspectives in biology*, *6*(5), a020644.

Hurtado de Catalfo, G. E., Ranieri- Casilla, A., Marra, F. A., De Alaniz, M. J., & Marra, C. A. (2007). Oxidative stress biomarkers and hormonal profile in human patients undergoing varicocelectomy. *International journal of andrology*, *30*(6), 519-530.

IARC (1980). Monograph arsenic and its compounds. Lyons International Agency for Research on Cancer. 23:39-41.

IARC monographs on the evaluation of carcinogenic risk to human. Volume 100C. Lyon, International Agency for Research on Cancer, 2012.

Ikeguchi, M., Hirooka, Y., & Kaibara, N. (2003). Quantitative reverse transcriptase polymerase chain reaction analysis for KiSS-1 and orphan G-protein-coupled receptor (hOT7T175) gene expression in hepatocellular carcinoma. *Journal of cancer research and clinical oncology*, *129*, 531-535.

Ikeguchi, M., Yamaguchi, K. I., & Kaibara, N. (2004). Clinical Significance of the Loss of KiSS-1 and orphan G-Protein–Coupled receptor (hOT7T175) gene expression in esophageal squamous cell carcinoma. *Clinical Cancer Research*, *10*(4), 1379-1383.

Irwig, M. S., Fraley, G. S., Smith, J. T., Acohido, B. V., Popa, S. M., Cunningham, M. J., ... & Steiner, R. A. (2004). Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat. *Neuroendocrinology*, *80*(4), 264-272.

Islam, L. N., Nurun Nabi, A. H. M., Rahman, M. M., & Zahid, M. S. H. (2007). Association of respiratory complications and elevated serum immunoglobulins with drinking water arsenic toxicity in human. *Journal of Environmental Science and Health, Part A*, *42*(12), 1807-1814.

Israni, A. K., & Kasiske, B. L. (2008). *Brenner & Rector's The Kidney*.

Itoh, K., Chiba, T., Takahashi, S., Ishii, T., Igarashi, K., Katoh, Y., ... & Nabeshima, Y. I. (1997). An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochemical and biophysical research communications*, *236*(2), 313-322.

Jain, A., Lamark, T., Sjøttem, E., Larsen, K. B., Awuh, J. A., Øvervatn, A., ... & Johansen, T. (2010). p62/SQSTM1 is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription. *Journal of biological chemistry*, *285*(29), 22576-22591.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., & Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary toxicology*, *7*(2), 60.

Janani, R., Gurunathan, B., Sivakumar, K., Varjani, S., Ngo, H. H., & Gnansounou, E. (2022). Advancements in heavy metals removal from effluents employing nano-adsorbents: way towards cleaner production. *Environmental Research*, *203*, 111815.

Janasik, B., Reszka, E., Stanislawska, M., Jablonska, E., Kuras, R., Wieczorek, E., ... & Wasowicz, W. (2018). Effect of arsenic exposure on NRF2-KEAP1 pathway and epigenetic modification. *Biological trace element research*, *185*, 11-19.

Jayasena, C. N., Abbara, A., Narayanaswamy, S., Comninos, A. N., Ratnasabapathy, R., Bassett, P., ... & Dhillo, W. S. (2015). Direct comparison of the effects of intravenous kisspeptin-10, kisspeptin-54 and GnRH on gonadotrophin secretion in healthy men. *Human Reproduction*, *30*(8), 1934-1941.

Jayasumana, C., Paranagama, P. A., Amarasinghe, M. D., Wijewardane, K. M. R. C., Dahanayake, K. S., Fonseka, S. I., ... & Senanayake, V. K. (2013). Possible link of chronic arsenic toxicity with chronic kidney disease of unknown etiology in Sri Lanka.

Jiang, J., Tam, L. M., Wang, P., & Wang, Y. (2018). Arsenite targets the RING finger domain of Rbx1 E3 ubiquitin ligase to inhibit proteasome-mediated degradation of Nrf2. *Chemical research in toxicology*, *31*(5), 380-387.

Jiang, X., Chen, C., Zhao, W., & Zhang, Z. (2013). Sodium arsenite and arsenic trioxide differently affect the oxidative stress, genotoxicity and apoptosis in A549 cells: an implication for the paradoxical mechanism. *Environmental toxicology and pharmacology*, *36*(3), 891-902.

Jiang, X., Zou, B., Feng, H., Tang, J., Tu, Y., & Zhao, X. (2019). Spatial distribution mapping of Hg contamination in subclass agricultural soils using GIS enhanced multiple linear regression. *Journal of Geochemical Exploration*, *196*, 1-7.

Jing, J., & Teschke, R. (2018). Traditional Chinese medicine and herb-induced liver injury: comparison with drug-induced liver injury. *Journal of clinical and translational hepatology*, *6*(1), 57

Johnson, D. L. (1972). Bacterial reduction of arsenate in sea water. *Nature*, *240*(5375), 44-45.

Jomova, K., Jenisova, Z., Feszterova, M., Baros, S., Liska, J., Hudecova, D., ... & Valko, M. (2011). Arsenic: toxicity, oxidative stress and human disease. *Journal of Applied Toxicology*, *31*(2), 95-107.

Kabata-Pendias, H. Pendias, CRC Press, Boca Raton, FL, 1984, p. 31

Kang, S., Chung, J. H., Lee, J. H., Fisher, G. J., Wan, Y. S., Duell, E. A., & Voorhees, J. J. (2003). Topical N-acetyl cysteine and genistein prevent ultraviolet-light-induced signaling that leads to photoaging in human skin in vivo. *Journal of Investigative Dermatology*, *120*(5), 835-841.

Kapaj, S., Peterson, H., Liber, K., & Bhattacharya, P. (2006). Human health effects from chronic arsenic poisoning–a review. *Journal of Environmental Science and Health, Part A*, *41*(10), 2399-2428.

Kato, K., Hayashi, H., Hasegawa, A., Yamanaka, K., & Okada, S. (1994). DNA damage induced in cultured human alveolar (L-132) cells by exposure to dimethylarsinic acid. *Environmental health perspectives*, *102*(suppl 3), 285-288.

Kauffman, A. S., Gottsch, M. L., Roa, J., Byquist, A. C., Crown, A., Clifton, D. K., ... & Tena-Sempere, M. (2007). Sexual differentiation of Kiss1 gene expression in the brain of the rat. *Endocrinology*, *148*(4), 1774-1783.

Kermani, T., Hossein, S. F., Talaei-Khozani, T., & Aliabadi, E. (2020). Pre-Incubation with Kisspeptin Improves the Adverse Effects of Freeze-Thawed Human Ejaculated Sperms.

Khan, F., Momtaz, S., Niaz, K., Hassan, F. I., & Abdollahi, M. (2017). Epigenetic mechanisms underlying the toxic effects associated with arsenic exposure and the development of diabetes. *Food and Chemical Toxicology*, *107*, 406-417.

Khlifi, R., & Hamza-Chaffai, A. (2010). Head and neck cancer due to heavy metal exposure via tobacco smoking and professional exposure: a review. *Toxicology and applied pharmacology*, *248*(2), 71-88.

Kokilavani, V., Devi, M. A., Sivarajan, K., & Panneerselvam, C. (2005). Combined efficacies of dl-α-lipoic acid and meso 2, 3 dimercaptosuccinic acid against arsenic induced toxicity in antioxidant systems of rats. *Toxicology letters*, *160*(1), 1-7..

Komatsu, M., Kurokawa, H., Waguri, S., Taguchi, K., Kobayashi, A., Ichimura, Y., ... & Yamamoto, M. (2010). The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nature cell biology*, *12*(3), 213-223.

Kotani, M., Detheux, M., Vandenbogaerde, A., Communi, D., Vanderwinden, J. M., Le Poul, E., ... & Parmentier, M. (2001). The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. *Journal of Biological Chemistry*, *276*(37), 34631-34636.

Lamas, G. A., Navas-Acien, A., Mark, D. B., & Lee, K. L. (2016). Heavy metals, cardiovascular disease, and the unexpected benefits of chelation therapy. *Journal of the American College of Cardiology*, *67*(20), 2411-2418.

Lau, A., Zheng, Y., Tao, S., Wang, H., Whitman, S. A., White, E., & Zhang, D. D. (2013). Arsenic inhibits autophagic flux, activating the Nrf2-Keap1 pathway in a p62-dependent manner. *Molecular and cellular biology*, *33*(12), 2436-2446.

Le, X. C., Lu, X., Ma, M., Cullen, W. R., Aposhian, H. V., & Zheng, B. (2000). Speciation of key arsenic metabolic intermediates in human urine. *Analytical Chemistry*, *72*(21), 5172-5177.

Lee, D. K., Nguyen, T., O'Neill, G. P., Cheng, R., Liu, Y., Howard, A. D., ... & O'Dowd, B. F. (1999). Discovery of a receptor related to the galanin receptors. *FEBS letters*, *446*(1), 103-107.

Lee, J. H., Miele, M. E., Hicks, D. J., Phillips, K. K., Trent, J. M., Weissman, B. E., & Welch, D. R. (1996). KiSS-1, a novel human malignant melanoma metastasis-suppressor gene. *JNCI: Journal of the National Cancer Institute*, *88*(23), 1731-1737.

Lewis, D. R., Southwick, J. W., Ouellet-Hellstrom, R., Rench, J., & Calderon, R. L. (1999). Drinking water arsenic in Utah: A cohort mortality study. *Environmental health perspectives*, *107*(5), 359-365.

Li, Z., Tian, J., Cui, G., Wang, M., & Yu, D. (2016). Effects of local testicular heat treatment on Leydig cell hyperplasia and testosterone biosynthesis in rat testes. *Reproduction, Fertility and Development*, *28*(9), 1424-1432.

Lin, Q., Li, S., Jiang, N., Shao, X., Zhang, M., Jin, H., ... & Ni, Z. (2019). PINK1-parkin pathway of mitophagy protects against contrast-induced acute kidney injury via decreasing mitochondrial ROS and NLRP3 inflammasome activation. *Redox biology*, *26*, 101254.

Liu, J., Zhao, H., Wang, Y., Shao, Y., Zong, H., Zeng, X., & Xing, M. (2019). Arsenic trioxide and/or copper sulfate induced apoptosis and autophagy associated with oxidative stress and perturbation of mitochondrial dynamics in the thymus of Gallus gallus. *Chemosphere*, *219*, 227-235.

Liu, Q., Hu, Y., Zhang, M., Yan, Y., Yu, H., & Ge, L. (2018). microRNA-451 protects neurons against ischemia/reperfusion injury-induced cell death by targeting CELF2. *Neuropsychiatric Disease and Treatment*, 2773-2782.

Liu, W., Beck, B. H., Vaidya, K. S., Nash, K. T., Diers, A. R., Feeley, K. P., ... & Welch, D. R. (2013). The KISS1 metastasis suppressor appears to reverse the Warburg effect by enhancing mitochondria biogenesis. *Cancer Research*, *73*(8_Supplement), 3866-3866.291..

Liu, X., Ling, M., Chen, C., Luo, F., Yang, P., Wang, D., ... & Liu, Q. (2017). Impaired autophagic flux and p62-mediated EMT are involved in arsenite-induced transformation of L-02 cells. *Toxicology and Applied Pharmacology*, *334*, 75-87.

Lokuge, K. M., Smith, W., Caldwell, B., Dear, K., & Milton, A. H. (2004). The effect of arsenic mitigation interventions on disease burden in Bangladesh. *Environmental Health Perspectives*, *112*(11), 1172-1177.

M.N. Bates, A.M. Smith, C.H. Rick, Am. J. Epidemiol.135 (1992) 462

Ma, Y., Egodawatta, P., McGree, J., Liu, A., & Goonetilleke, A. (2016). Human health risk assessment of heavy metals in urban stormwater. *Science of the Total Environment*, *557*, 764-772.

Macho, A. P., Lozano-Durán, R., & Zipfel, C. (2015). Importance of tyrosine phosphorylation in receptor kinase complexes. *Trends in plant science*, *20*(5), 269-272.

Madden, E. F., & Fowler, B. A. (2000). Mechanisms of nephrotoxicity from metal combinations: a review. *Drug and chemical toxicology*, *23*(1), 1-12.

Malmir, M., Faraji, T., Noreini, N. S., & Mehranjani, S. M. (2018). Protective antioxidant effects of N-acetylcysteine on testicular tissue and serum testosterone in paranonylphenol-treated mice (A stereological analysis). *Reprod Syst Sex Disord*, *7*(2), 1000225.

Maloney, M. E. (1996). Arsenic in dermatology. *Dermatologic surgery*, *22*(3), 301-3

Mandal, B. K., & Suzuki, K. T. (2002). Arsenic round the world: a review. *Talanta*, *58*(1), 201-235.

Mandal, B. K., Ogra, Y., & Suzuki, K. T. (2001). Identification of dimethylarsinous and monomethylarsonous acids in human urine of the arsenic-affected areas in West Bengal, India. *Chemical research in toxicology*, *14*(4), 371-378.

Marafante, E., Bertolero, F., Edel, J., Pietra, R., & Sabbioni, E. (1982). Intracellular interaction and biotransformation of arsenite in rats and rabbits. *Science of the Total Environment*, *24*(1), 27-39.

Marcelet, H. (1913). L'arsenic etle manganèse dans quelques végétaux marins. *Bull. Soc. Sci. pharm.*, *20*, 271-275.

Marmolino, D., & Manto, M. (2010). Past, present and future therapeutics for cerebellar ataxias. *Current neuropharmacology*, *8*(1), 41-61.

Martina, V., Masha, A., Gigliardi, V. R., Brocato, L., Manzato, E., Berchio, A., ... & Iannone, A. (2008). Long-term N-acetylcysteine and L-arginine administration reduces endothelial activation and systolic blood pressure in hypertensive patients with type 2 diabetes. *Diabetes care*, *31*(5), 940-944.

Mazumder, D. G., Chakraborty, A. K., Ghose, A., Gupta, J. D., Chakraborty, D. P., Dey, S. B., & Chattopadhyay, N. (1988). Chronic arsenic toxicity from drinking tubewell water in rural West Bengal. *Bulletin of the World Health Organization*, *66*(4), 499.

Mazumder, D. N. G., Haque, R., Ghosh, N., De, B. K., Santra, A., Chakraborti, D., & Smith, A. H. (2000). Arsenic in drinking water and the prevalence of respiratory effects in West Bengal, India. *International journal of epidemiology*, *29*(6), 1047-1052.

Mazumder, D. N., Santra, A., Pal, A., Ghose, A., & Sarkar, S. (1998). Chronic arsenic toxicity in west Bengal--the worst calamity in the world. *Journal of the Indian Medical Association*, *96*(1), 4-7.

McBride, B. C., & Wolfe, R. S. (1971). Biosynthesis of dimethylarsine by Methanobacterium. *Biochemistry*, *10*(23), 4312-4317.

McClure, E. A., Gipson, C. D., Malcolm, R. J., Kalivas, P. W., & Gray, K. M. (2014). Potential role of N-acetylcysteine in the management of substance use disorders. *CNS drugs*, *28*, 95-106.

Meliker, J. R., Wahl, R. L., Cameron, L. L., & Nriagu, J. O. (2007). Arsenic in drinking water and cerebrovascular disease, diabetes mellitus, and kidney disease in Michigan: a standardized mortality ratio analysis. *Environmental Health*, *6*(1), 1-11.

Menendez, D., Mora, G., Salazar, A. M., & Ostrosky-Wegman, P. (2001). ATM status confers sensitivity to arsenic cytotoxic effects. *Mutagenesis*, *16*(5), 443-448.

Messager, S., Chatzidaki, E. E., Ma, D., Hendrick, A. G., Zahn, D., Dixon, J., ... & Aparicio, S. A. (2005). Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. *Proceedings of the National Academy of Sciences*, *102*(5), 1761-1766.

Millea, P. J. (2009). N-acetylcysteine: multiple clinical applications. *American family physician*, *80*(3), 265-269.

Milton, A. H., Hasan, Z., Rahman, A., & Rahman, M. (2001). Chronic arsenic poisoning and respiratory effects in Bangladesh. *Journal of occupational health*, *43*(3), 136-14.

Milton, A. H., Hasan, Z., Rahman, A., & Rahman, M. (2003). Non-cancer effects of chronic arsenicosis in Bangladesh: preliminary results. *Journal of Environmental Science and Health, Part A*, *38*(1), 301-305.

Milton, N. G., Chilumuri, A., Rocha-Ferreira, E., Nercessian, A. N., & Ashioti, M. (2012). Kisspeptin prevention of amyloid-β peptide neurotoxicity in vitro. *ACS chemical neuroscience*, *3*(9), 706-719.

Milton, N. G., Chilumuri, A., Rocha-Ferreira, E., Nercessian, A. N., & Ashioti, M. (2012). Kisspeptin prevention of amyloid-β peptide neurotoxicity in vitro. *ACS chemical neuroscience*, *3*(9), 706-719.

Mitsuishi, Y., Taguchi, K., Kawatani, Y., Shibata, T., Nukiwa, T., Aburatani, H., ... & Motohashi, H. (2012). Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. *Cancer cell*, *22*(1), 66-79.

Mizuta, N., Mizuta, M., Ito, F., Ito, T., Uchida, H., Watanabe, Y., ... & Matsumura, H. (1956). AN OUTBLEAK OF ACUTE ARSENIC POISONING CAUSED BY ARSENIC CONTAMINATED SOY-SAUCE (SH? YU): A CLINICAL REPORT OF 220 CACES. *山口医科大学紀要*, *2*, 131-149.

Mohammadi, R. M., Sobhan, A. S., Karimi, H., & Sorooshnia, R. (2015). Natural and anthropogenic source of heavy metals pollution in the soil samples of an industrial complex; a case study.

Morris, B. J. (2013). Seven sirtuins for seven deadly diseases ofaging. *Free Radical Biology and Medicine*, *56*, 133-171.

Motohashi, H., Katsuoka, F., Engel, J. D., & Yamamoto, M. (2004). Small Maf proteins serve as transcriptional cofactors for keratinocyte differentiation in the Keap1–Nrf2 regulatory pathway. *Proceedings of the National Academy of Sciences*, *101*(17), 6379-6384.

Muir, A. I., Chamberlain, L., Elshourbagy, N. A., Michalovich, D., Moore, D. J., Calamari, A., ... & Harrison, D. C. (2001). AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1. *Journal of Biological Chemistry*, *276*(31), 28969-28975.

Murugesan, P., Balaganesh, M., Balasubramanian, K., & Arunakaran, J. (2007). Effects of polychlorinated biphenyl (Aroclor 1254) on steroidogenesis and antioxidant system in cultured adult rat Leydig cells. *Journal of endocrinology*, *192*(2), 325-338.

Muthuvel, R., Venkataraman, P., Krishnamoorthy, G., Gunadharini, D. N., Kanagaraj, P., Stanley, A. J., ... & Arunakaran, J. (2006). Antioxidant effect of ascorbic acid on PCB (Aroclor 1254) induced oxidative stress in hypothalamus of albino rats. *Clinica Chimica Acta*, *365*(1-2), 297-303.

Nagajyoti, P. C., Lee, K. D., & Sreekanth, T. V. M. (2010). Heavy metals, occurrence and toxicity for plants: a review. *Environmental chemistry letters*, *8*, 199-216.

Naranmandura, H., Xu, S., Koike, S., Pan, L. Q., Chen, B., Wang, Y. W., ... & Suzuki, N. (2012). The endoplasmic reticulum is a target organelle for trivalent dimethylarsinic acid (DMAIII)-induced cytotoxicity. *Toxicology and applied pharmacology*, *260*(3), 241-249.

Navarro, V. M. (2020). Metabolic regulation of kisspeptin—the link between energy balance and reproduction. *Nature reviews endocrinology*, *16*(8), 407-420.

Navarro, V. M., & Tena-Sempere, M. (2012). Neuroendocrine control by kisspeptins: role in metabolic regulation of fertility. *Nature Reviews Endocrinology*, *8*(1), 40-53.

Nelson, K. W. (1977). Industrial contributions of arsenic to the environment. *Environmental Health Perspectives*, *19*, 31-34.

Nico, P. S., Fendorf, S. E., Lowney, Y. W., Holm, S. E., & Ruby, M. V. (2004). Chemical structure of arsenic and chromium in CCA-treated wood: implications of environmental weathering. *Environmental science & technology*, *38*(19), 5253-5260.

Niki, E. (2008). Lipid peroxidation products as oxidative stress biomarkers. *Biofactors*, *34*(2), 171-180.

Nordenson, I., & Beckman, L. (1991). Is the genotoxic effect of arsenic mediated by oxygen free radicals?. *Human heredity*, *41*(1), 71-73.

Nriagu, J. O. (1994). Arsenic in the environment(Part II, Human health and ecosystem effects). *Advances in environmental science and technology*.

Nriagu, J. O. (2002). Arsenic poisoning through the ages. *Environmental chemistry of arsenic*, *1*, 1-26.

Nriagu, J. O., & Azcue, J. M. (1990). Food contamination with arsenic in the environment. *Food contamination with arsenic in the environment.*, 121-143.

Nutt, L. K., Gogvadze, V., Uthaisang, W., Mirnikjoo, B., McConkey, D. J., & Orrenius, S. (2005). Research paper indirect effects of Bax and Bak initiate the mitochondrial

alterations that lead to cytochrome c release during arsenic trioxide-induced apoptosis. *Cancer Biol Ther*, *4*, 459-467.

Ogasawara, T. D. L. W. (2008). Nilsa RD. Huang P. Redox regulation of cell survival. *Antioxid Redox Signal*, *10*, 1343-1374.

Ohtaki, T., Shintani, Y., Honda, S., Matsumoto, H., Hori, A., Kanehashi, K., ... & Fujino, M. (2001). Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature*, *411*(6837), 613-617.

Olaniyan, O. T., Meraiyebu, A. B., Auta, K. B., Dare, J. B., Anjorin, Y. D., & Shafe, M. O. (2013). Kisspeptin system: a multi-homeostatic system. *IOSR J Pharm Biol Sci*, *5*(4), 87-101.

Olbrich, T., Ziegler, E., Türk, G., Schubert, A., Emons, G., & Gründker, C. (2010). Kisspeptin-10 inhibits bone-directed migration of GPR54-positive breast cancer cells: Evidence for a dose–window effect. *Gynecologic oncology*, *119*(3), 571-578

Paintlia, M. K., Paintlia, A. S., Contreras, M. A., Singh, I., & Singh, A. K. (2008). Lipopolysaccharide-induced peroxisomal dysfunction exacerbates cerebral white matter injury: attenuation by N-acetyl cysteine. *Experimental neurology*, *210*(2), 560-576.

Pannu, N., Manns, B., Lee, H., & Tonelli, M. (2004). Systematic review of the impact of N-acetylcysteine on contrast nephropathy. *Kidney international*, *65*(4), 1366-1374.

Parhar, I. S., Ogawa, S., & Sakuma, Y. (2004). Laser-captured single digoxigenin-labeled neurons of gonadotropin-releasing hormone types reveal a novel G protein-coupled receptor (Gpr54) during maturation in cichlid fish. *Endocrinology*, *145*(8), 3613-3618.

Parodi-Rullán, R. M., Soto-Prado, J., Vega-Lugo, J., Chapa-Dubocq, X., Díaz-Cordero, S. I., & Javadov, S. (2018). Divergent effects of cyclophilin-D inhibition on the female rat heart: Acute versus chronic post-myocardial infarction. *Cellular Physiology and Biochemistry*, *50*(1), 288-303.

Parrish, A. R., Zheng, X. H., Turney, K. D., Younis, H. S., & Gandolfi, A. J. (1999). Enhanced transcription factor DNA binding and gene expression induced by arsenite or arsenate in renal slices. *Toxicological sciences: An official journal of the Society of Toxicology*, *50*(1), 98-105.

Peterson, P. J., Girling, C. A., Benson, L. M., & Zeive, R. (1981). Effect of heavy metals pollution on plants. *Effect of Trace Metals on Plant Function. Applied Science Publishers*, 279.

Pieralisi, A., Martini, C., Soto, D., Vila, M. C., Calvo, J. C., & Guerra, L. N. (2016). N-acetylcysteine inhibits lipid accumulation in mouse embryonic adipocytes. *Redox Biology*, *9*, 39-44.

Pirabbasi, E., Shahar, S., Manaf, Z. A., Rajab, N. F., & Manap, R. A. (2016). Efficacy of ascorbic acid (vitamin C) and/N-acetylcysteine (NAC) supplementation on nutritional and antioxidant status of male chronic obstructive pulmonary disease (COPD) patients. *Journal of nutritional science and vitaminology*, *62*(1), 54-61.

Piver, W. T. (1983). Mobilization of arsenic by natural and industrial processes. *Biological and environmental effects of arsenic*, *6*(1).

Platanias, L. C. (2009). Biological responses to arsenic compounds. *Journal of Biological Chemistry*, *284*(28), 18583-18587.

Prakash, S., & Verma, A. K. (2021). Arsenic: It's Toxicity and Impact on Human health. *International Journal of Biological Innovations, IJBI*, *3*(1), 38-47.

Qureshi, I. Z., & Fatima, I. (2019). Peripherally administered KP-10 prevents development of insulin-induced hypoglycemic shock in diabetic rhesus monkeys. *Physiology International*, *106*(4), 335-346.

Radomska-Leśniewska, D. M., & Skopiński, P. (2012). Review paper N-acetylcysteine as an anti-oxidant and anti-inflammatory drug and its some clinical applications. *Central European journal of immunology*, *37*(1), 57-66.

Rahman, M. M., Ng, J. C., & Naidu, R. (2009). Chronic exposure of arsenic via drinking water and its adverse health impacts on humans. *Environmental geochemistry and health*, *31*, 189-200.

Rahman, M., Vahter, M., Wahed, M. A., Sohel, N., Yunus, M., Streatfield, P. K., ... & Persson, L. Å. (2006). Prevalence of arsenic exposure and skin lesions. A population based survey in Matlab, Bangladesh. *Journal of Epidemiology & Community Health*, *60*(3), 242-248.

Rahman, Z., & Singh, V. P. (2020). Bioremediation of toxic heavy metals (THMs) contaminated sites: concepts, applications and challenges. *Environmental Science and Pollution Research*, *27*, 27563-27581.

Rai, P. K., Lee, S. S., Zhang, M., Tsang, Y. F., & Kim, K. H. (2019). Heavy metals in food crops: Health risks, fate, mechanisms, and management. *Environment international*, *125*, 365-385.

Raie, R. M. (1996). Regional variation in As, Cu, Hg, and Se and interaction between them. *Ecotoxicology and Environmental Safety*, *35*(3), 248-252.

Rapado-Castro, M., Dodd, S., Bush, A. I., Malhi, G. S., Skvarc, D. R., On, Z. X., ... & Dean, O. M. (2017). Cognitive effects of adjunctive N-acetyl cysteine in psychosis. *Psychological medicine*, *47*(5), 866-876.

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

Ravenscroft, P., Brammer, H., & Richards, K. (2011). *Arsenic pollution: a global synthesis*. John Wiley & Sons.

Ricu, M. A., Ramirez, V. D., Paredes, A. H., & Lara, H. E. (2012). Evidence for a celiac ganglion-ovarian kisspeptin neural network in the rat: intraovarian anti-kisspeptin delays vaginal opening and alters estrous cyclicity. *Endocrinology*, *153*(10), 4966-4977.

Riley, B. E., Kaiser, S. E., Shaler, T. A., Ng, A. C., Hara, T., Hipp, M. S., ... & Kopito, R. R. (2010). Ubiquitin accumulation in autophagy-deficient mice is dependent on the Nrf2-mediated stress response pathway: a potential role for protein aggregation in autophagic substrate selection. *Journal of Cell Biology*, *191*(3), 537-552.

Ringel, M. D., Hardy, E., Bernet, V. J., Burch, H. B., Schuppert, F., Burman, K. D., & Saji, M. (2002). Metastin receptor is overexpressed in papillary thyroid cancer and activates MAP kinase in thyroid cancer cells. *The Journal of Clinical Endocrinology & Metabolism*, *87*(5), 2399-2402.

Rodríguez-Vázquez, E., Tena-Sempere, M., & Castellano, J. M. (2020). Mechanisms for the metabolic control of puberty. *Current Opinion in Endocrine and Metabolic Research*, *14*, 78-84.

Rometo, A. M., Krajewski, S. J., Lou Voytko, M., & Rance, N. E. (2007). Hypertrophy and increased kisspeptin gene expression in the hypothalamic infundibular nucleus of postmenopausal women and ovariectomized monkeys. *The Journal of Clinical Endocrinology & Metabolism*, *92*(7), 2744-2750.

Rudnai, P., & Gulyas, E. (1998, July). Adverse effects of drinking water related arsenic exposure on some pregnancy outcomes in Karcag, Hungary. In *3rd International Conference on Arsenic Exposure and Health Effects* (pp. 12-15).

Safarinejad, M. R., & Safarinejad, S. (2009). Efficacy of selenium and/or N-acetyl-cysteine for improving semen parameters in infertile men: a double-blind, placebo controlled, randomized study. *The Journal of urology*, *181*(2), 741-751.

Saha, K. C. (1995). Chronic arsenical dermatoses from tube-well water in West Bengal during 1983-87. *Ind. Dermatol.*, *40*(1), 1-12.

Šalamon, Š., Kramar, B., Marolt, T. P., Poljšak, B., & Milisav, I. (2019). Medical and dietary uses of N-acetylcysteine. *Antioxidants*, *8*(5), 111.

Salmon, A. H., Ferguson, J. K., Burford, J. L., Gevorgyan, H., Nakano, D., Harper, S. J., ... & Peti-Peterdi, J. (2012). Loss of the endothelial glycocalyx links albuminuria and vascular dysfunction. *Journal of the American Society of Nephrology*, *23*(8), 1339-1350.

Sanchez-Carbayo, M., Capodieci, P., & Cordon-Cardo, C. (2003). Tumor suppressor role of KiSS-1 in bladder cancer: loss of KiSS-1 expression is associated with bladder cancer progression and clinical outcome. *The American journal of pathology*, *162*(2), 609-617.

Sarkar, B. (2002). *Heavy metals in the environment*. CRC press.

Sasaki, A., Oshima, Y., & Fujimura, A. (2007). An approach to elucidate potential mechanism of renal toxicity of arsenic trioxide. *Experimental hematology*, *35*(2), 252-262.

Schwarzmaier, C., Sierka, M., & Scheer, M. (2013). Intact As4 tetrahedra coordinated side-on to metal cations. *Angewandte Chemie International Edition*, *52*(3), 858-861.

Schwetz, T. A., Reissaus, C. A., & Piston, D. W. (2014). Differential stimulation of insulin secretion by GLP-1 and Kisspeptin-10. *PLoS One*, *9*(11), e113020.

Seminara, S. B., Messager, S., Chatzidaki, E. E., Thresher, R. R., Acierno Jr, J. S., Shagoury, J. K., ... & Colledge, W. H. (2003). The GPR54 gene as a regulator of puberty. *New England Journal of Medicine*, *349*(17), 1614-1627.

Seminara, S. B., Messager, S., Chatzidaki, E. E., Thresher, R. R., Acierno Jr, J. S., Shagoury, J. K., ... & Colledge, W. H. (2004). The GPR54 gene as a regulator of puberty. *Obstetrical & Gynecological Survey*, *59*(5), 351-353.

Shah, P., Trinh, E., Qiang, L., Xie, L., Hu, W. Y., Prins, G. S., ... & He, Y. Y. (2017). Arsenic induces p62 expression to form a positive feedback loop with Nrf2 in human epidermal keratinocytes: implications for preventing arsenic-induced skin cancer. *Molecules*, *22*(2), 194.

Shirasaki, F., Takata, M., Hatta, N., & Takehara, K. (2001). Loss of expression of the metastasis suppressor gene KiSS1 during melanoma progression and its association with LOH of chromosome 6q16. 3-q23. *Cancer research*, *61*(20), 7422-7425.

Shoji, I., Hirose, T., Mori, N., Hiraishi, K., Kato, I., Shibasaki, A., ... & Takahashi, K. (2010). Expression of kisspeptins and kisspeptin receptor in the kidney of chronic renal failure rats. *Peptides*, *31*(10), 1920-1925.

Sies, H. (1997). Oxidative stress: oxidants and antioxidants. *Experimental Physiology: Translation and Integration*, *82*(2), 291-295.

Simeonova, P. P., Wang, S., Kashon, M. L., Kommineni, C., Crecelius, E., & Luster, M. I. (2001). Quantitative relationship between arsenic exposure and AP-1 activity in mouse urinary bladder epithelium. *Toxicological sciences*, *60*(2), 279-284.

Slattery, J., Kumar, N., Delhey, L., Berk, M., Dean, O., Spielholz, C., & Frye, R. (2015). Clinical trials of N-acetylcysteine in psychiatry and neurology: a systematic review. *Neuroscience & Biobehavioral Reviews*, *55*, 294-321.

Smith, A. H., Marshall, G., Liaw, J., Yuan, Y., Ferreccio, C., & Steinmaus, C. (2012). Mortality in young adults following in utero and childhood exposure to arsenic in drinking water. *Environmental health perspectives*, *120*(11), 1527-1531.

Smith, E. R. G., Naidu, R., & Alston, A. M. (1998). Arsenic in the soil environment.

Smith, H. (1964). The interpretation of the arsenic content of human hair. *Journal of the Forensic Science Society*, *4*(4), 192-199.

Smith, J. T. (2013). Sex steroid regulation of kisspeptin circuits. *Kisspeptin signaling in reproductive biology*, 275-295.

Smith, R. A. (1976). A method to distinguish between arsenic in and on human hair. *Environmental Research*, *12*(2), 171-173.

Song, G. Q., & Zhao, Y. (2016). Kisspeptin 10 inhibits the Warburg effect in breast cancer through the Smad signaling pathway: both in vitro and in vivo. *American Journal of Translational Research*, *8*(1), 188.

Song, W. J., Mondal, P., Wolfe, A., Alonso, L. C., Stamateris, R., Ong, B. W., ... & Hussain, M. A. (2014). Glucagon regulates hepatic kisspeptin to impair insulin secretion. *Cell metabolism*, *19*(4), 667-681.

Stern, B. R. (2010). Essentiality and toxicity in copper health risk assessment: overview, update and regulatory considerations. *Journal of Toxicology and Environmental Health, Part A*, *73*(2-3), 114-127.

Stey, C., Steurer, J., Bachmann, S., Medici, T. C., & Tramer, M. R. (2000). The effect of oral N-acetylcysteine in chronic bronchitis: a quantitative systematic review. *European Respiratory Journal*, *16*(2), 253-262.

Styblo, M., Serves, S. V., Cullen, W. R., & Thomas, D. J. (1997). Comparative inhibition of yeast glutathione reductase by arsenicals and arsenothiols. *Chemical research in toxicology*, *10*(1), 27-33.

Suess-Fink, G. (2012). Arsenic Poisoning. *Chemie in Unserer Zeit*, *46*(2), 100-109.

Sullivan, R. J. (1969). Preliminary air pollution survey of manganese and its compounds. *National Air Pollution Control Administration, Raleigh, NC Publication No. APTD*, *69*, 39-54.

T. Sollman, A Manual of Pharmacology, WB Saunders Company, Philadelphia, 1957.

Talbi, R., & Navarro, V. M. (2020). Novel insights into the metabolic action of Kiss1 neurons. *Endocrine connections*, *9*(5), R124.

Tang, S. (1987). Factors affecting the geochemical background contents of heavy metals in different soils of China. *Acta Sci Circumstantiale*, *7*(3), 245-252.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. *Molecular, clinical and environmental toxicology: volume 3: environmental toxicology*, 133-164.

Terao, Y., Kumano, S., Takatsu, Y., Hattori, M., Nishimura, A., Ohtaki, T., & Shintani, Y. (2004). Expression of KiSS-1, a metastasis suppressor gene, in trophoblast giant cells of the rat placenta. *Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression*, *1678*(2-3), 102-110.

Thompson, E. L., Murphy, K. G., Patterson, M., Bewick, G. A., Stamp, G. W., Curtis, A. E., ... & Bloom, S. R. (2006). Chronic subcutaneous administration of kisspeptin-54 causes testicular degeneration in adult male rats. *American Journal of Physiology-Endocrinology and Metabolism*, *291*(5), E1074-E1082.

Thompson, E. L., Patterson, M., Murphy, K. G., Smith, K. L., Dhillo, W. S., Todd, J. F., ... & Bloom, S. R. (2004). Central and peripheral administration of kisspeptin- 10 stimulates the hypothalamic- pituitary- gonadal axis. *Journal of neuroendocrinology*, *16*(10), 850-858.

Tolson, K. P., Garcia, C., Delgado, I., Marooki, N., & Kauffman, A. S. (2016). Metabolism and energy expenditure, but not feeding or glucose tolerance, are impaired in young Kiss1r KO female mice. *Endocrinology*, *157*(11), 4192-4199.

Tolson, K. P., Garcia, C., Yen, S., Simonds, S., Stefanidis, A., Lawrence, A., ... & Kauffman, A. S. (2014). Impaired kisspeptin signaling decreases metabolism and promotes glucose intolerance and obesity. *The Journal of clinical investigation*, *124*(7), 3075-3079.

Tomko, R. L., Jones, J. L., Gilmore, A. K., Brady, K. T., Back, S. E., & Gray, K. M. (2018). N-acetylcysteine: A potential treatment for substance use disorders: This OTC antioxidant may benefit adults who use cocaine and adolescents who use marijuana. *Current psychiatry*, *17*(6), 30-39.

Tsai, S. M., Wang, T. N., & Ko, Y. C. (1999). Mortality for certain diseases in areas with high levels of arsenic in drinking water. *Archives of Environmental Health: An International Journal*, *54*(3), 186-193.

Tseng, H. P., Wang, Y. H., Wu, M. M., The, H. W., Chiou, H. Y., & Chen, C. J. (2006). Association between chronic exposure to arsenic and slow nerve conduction velocity among adolescents in Taiwan. *Journal of Health, Population and Nutrition*, 182-189.

Turrens, J. F. (2003). Mitochondrial formation of reactive oxygen species. *The Journal of physiology*, *552*(2), 335-344.

U.S. Department of the Interior Bureau of Mines, in:Minerals Yearbook, vol. 1, 1973, p. 1360.

U.S. Environmental Protection Agency, Interim Primary Drinking Water Standards, Fed. Reg. 40 (1975) 11,990.

US Environmental Protection Agency. (1980). *Ambient water quality criteria for mercury*. US Environmental Protection Agency.

USEPA (United States Environmental Protection Agency) Fed Regist 2001;66:6975–7066. Washington, DC.

Vahter, M., Marafante, E., & Dencker, L. (1984). Tissue distribution and retention of 74 As-dimethylarsinic acid in mice and rats. *Archives of environmental contamination and toxicology*, *13*, 259-264.

Valko, M., Jomova, K., Rhodes, C. J., Kuča, K., & Musílek, K. (2016). Redox-and non-redox-metal-induced formation of free radicals and their role in human disease. *Archives of toxicology*, *90*, 1-37.

Vallee, B. L., Ulmer, D. D., & Wacker, W. E. C. (1960). Arsenic toxicology and biochemistry. Arch. *Ind. Health*, *21*(1), 132.

Van Itallie, L., & Steenhauer, A. J. (1932). Rauwolfia serpentina BENTH. *Archiv der Pharmazie*, *270*(6), 313-322.

Vasudevan, D. M., & Sreekumari, S. (2005). *Text book of Biochemistry for medical student. Jaypee Brothers.*

Vertuani, S., Angusti, A., & Manfredini, S. (2004). The antioxidants and pro-antioxidants network: an overview. *Current pharmaceutical design*, *10*(14), 1677-1694.

Vickers, N. J. (2017). Animal communication: when i'm calling you, will you answer too?. *Current biology*, *27*(14), R713-R715.

Vinogradov, A. P. (1959). Geochemistry of rare and dispersed chemical elements in soils.

Voskuil, W. H. *R012: Selected readings in mineral economics*. NV Bureau of Mines & Geology.

Wang, T., Cao, Z., Shen, Z., Yang, J., Chen, X., Yang, Z., ... & Zhou, N. (2020). Existence and functions of a kisspeptin neuropeptide signaling system in a non-chordate deuterostome species. *Elife*, *9*, e53370.

Wanibuchi, H., Salim, E. I., Kinoshita, A., Shen, J., Wei, M., Morimura, K., ... & Fukushima, S. (2004). Understanding arsenic carcinogenicity by the use of animal models. *Toxicology and applied pharmacology*, *198*(3), 366-376.

Wedepohl, K. H. (1969). Composition and abundance of common sedimentary rocks. *Handbook of geochemistry*, *1*, 250-271.

West, A., Vojta, P. J., Welch, D. R., & Weissman, B. E. (1998). Chromosome localization and genomic structure of the KiSS-1 metastasis suppressor gene (KISS1). *Genomics*, *54*(1), 145-148.

Whitacre, R. W., & Pearse, C. S. (1974). *Arsenic and the Environment*. Colorado School of Mines.

Whitmarsh, A. J., & Davis, R. J. (1996). Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways. *Journal of molecular medicine*, *74*, 589-607.

Williams, J. A., & Ding, W. X. (2015). Targeting Pink1-Parkin-mediated mitophagy for treating liver injury. *Pharmacological research*, *102*, 264-269.

Woolson, E. A. (1975). Arsenical pesticides. 168th meeting of the American Chemical Society, Atlantic City, New Jersey, September 9, 1974.

Woolson, E. A. (1977). Generation of alkylarsines from soil. *Weed Science*, *25*(5), 412-416.

WOOLSON, E. A. (1983). *Emissions, cycling and effects of arsenic in soils* (pp. 51-139). Elsevier, Amsterdam.

Woolson, E. A., & Kearney, P. C. (1973). Persistence and reactions of [14C]-cacodylic acid in soils. *Environmental science & technology*, *7*(1), 47-50.

Worsztynowicz, A., & Mill, W. (1995). Potential ecological risk due to acidification of heavy industrialized areas—the Upper Silesia case. In *Studies in Environmental Science* (Vol. 64, pp. 353-365). Elsevier.

Wu, J., Fu, W., Huang, Y., & Ni, Y. (2012). Effects of kisspeptin-10 on lipid metabolism in cultured chicken hepatocytes. *Asian-Australasian journal of animal sciences*, *25*(9), 1229-1236

Xiao, R., Guo, D., Ali, A., Mi, S., Liu, T., Ren, C., ... & Zhang, Z. (2019). Accumulation, ecological-health risks assessment, and source apportionment of heavy metals in paddy soils: A case study in Hanzhong, Shaanxi, China. *Environmental Pollution*, *248*, 349-357.

Xu, M., Niu, Q., Hu, Y., Feng, G., Wang, H., & Li, S. (2019). Proanthocyanidins antagonize arsenic-induced oxidative damage and promote arsenic methylation through activation of the Nrf2 signaling pathway. *Oxidative medicine and cellular longevity*, *2019*.

Yamanaka, K., & Okada, S. (1994). Induction of lung-specific DNA damage by metabolically methylated arsenics via the production of free radicals. *Environmental Health Perspectives*, *102*(suppl 3), 37-40.

Yamanaka, K., Hayashi, H., Tachikawa, M., Kato, K., Hasegawa, A., Oku, N., & Okada, S. (1997). Metabolic methylation is a possible genotoxicity-enhancing process of inorganic arsenics. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *394*(1-3), 95-101.

Yang, L. Y. (2020). *Investigating the Role of Kisspeptin in Human Behaviour and Physiology* (Doctoral dissertation, Imperial College London).

Yang, Y., Song, S., Nie, Y., Chen, R., & Chen, P. (2022). Lentinan alleviates arsenic-induced hepatotoxicity in mice via downregulation of OX40/IL-17A and activation of Nrf2 signaling. *BMC Pharmacology and Toxicology*, *23*(1), 16.

Yi, T., Tan, K., Cho, S. G., Wang, Y., Luo, J., Zhang, W., ... & Liu, M. (2010). Regulation of embryonic kidney branching morphogenesis and glomerular development by KISS1 receptor (Gpr54) through NFAT2-and Sp1-mediated Bmp7 expression. *Journal of Biological Chemistry*, *285*(23), 17811-17820.

Yih, L. H., & Lee, T. C. (2000). Arsenite induces p53 accumulation through an ATM-dependent pathway in human fibroblasts. *Cancer Research*, *60*(22), 6346-6352.

Yoo, S. Z., No, M. H., Heo, J. W., Park, D. H., Kang, J. H., Kim, S. H., & Kwak, H. B. (2018). Role of exercise in age-related sarcopenia. *Journal of exercise rehabilitation*, *14*(4), 551.

Zafarullah, M., Li, W. Q., Sylvester, J., & Ahmad, M. (2003). Molecular mechanisms of N-acetylcysteine actions. *Cellular and Molecular Life Sciences CMLS*, *60*, 6-20.

Zaldivar, R. (1974). Arsenic contamination of drinking water and foodstuffs causing endemic chronic poisoning. *Beiträge zur Pathologie*, *151*(4), 384-400.

Zhang, D. D., & Hannink, M. (2003). Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. *Molecular and cellular biology*, *23*(22), 8137-8151.

Zhang, D., Li, J., Gao, J., & Huang, C. (2009). c-Jun/AP-1 pathway-mediated cyclin D1 expression participates in low dose arsenite-induced transformation in mouse epidermal JB6 Cl41 cells. *Toxicology and applied pharmacology*, *235*(1), 18-24.

Zhang, M., Xue, Y., Zheng, B., Li, L., Chu, X., Zhao, Y., ... & Chu, L. (2021). Liquiritigenin protects against arsenic trioxide-induced liver injury by inhibiting oxidative stress and enhancing mTOR-mediated autophagy. *Biomedicine & Pharmacotherapy*, *143*, 112167.

Zhang, X., Yan, L., Liu, J., Zhang, Z., & Tan, C. (2019). Removal of different kinds of heavy metals by novel PPG-nZVI beads and their application in simulated stormwater infiltration facility. *Applied Sciences*, *9*(20), 4213.

Zhao, H., Wang, Y., Liu, J., Guo, M., Fei, D., Yu, H., & Xing, M. (2019). The cardiotoxicity of the common carp (Cyprinus carpio) exposed to environmentally relevant concentrations of arsenic and subsequently relieved by zinc supplementation. *Environmental Pollution*, *253*, 741-748.

Zhao, J., Wang, H., Dong, L., Sun, S., & Li, L. (2019). miRNA-20b inhibits cerebral ischemia-induced inflammation through targeting NLRP3. *International Journal of Molecular Medicine*, *43*(3), 1167-1178.

Zhong, G., Wan, F., Wu, S., Jiang, X., Tang, Z., Zhang, X., ... & Hu, L. (2021). Arsenic or/and antimony induced mitophagy and apoptosis associated with metabolic abnormalities and oxidative stress in the liver of mice. *Science of the Total Environment*, *777*, 146082.

Zhou, Q., & Xi, S. (2018). A review on arsenic carcinogenesis: epidemiology, metabolism, genotoxicity and epigenetic changes. *Regulatory Toxicology and Pharmacology*, *99*, 78-88.

Zhu, J., Wang, H., Chen, F., Fu, J., Xu, Y., Hou, Y., ... & Pi, J. (2016). An overview of chemical inhibitors of the Nrf2-ARE signaling pathway and their potential applications in cancer therapy. *Free Radical Biology and Medicine*, *99*, 544-556.

Zuxiang, L., Xiaomin, C., & Xiaoliang, L. (2012). Effects of kisspeptin-10 on the onset of egg laying and cholesterol metabolism in quails. *J. Nanjing Agric. Univ*, *35*, 89-93.