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Toxopathological study of arsenic trioxide

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بسم الله الرحمن الرحيم

(فَتَعالى اللهُ المَلكُ الحَقُ ولا تعجَلْ بالقُرانِ مِنْ
قَبْلِ أنْ يُقضَى إليكَ وَحُيه وَقل رَبِ زدْني عِلْماً)

صدق الله العلي العظيم

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Dedication

To my parents

And to all my members of my family

To my Friends

I dedicate this work

Karrar

Acknowledgments

Firstly I would like to thank the Kind Merciful Allah for helping me in completing this work.

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Karrar

Abstract

Abstract

This study was performed to investigat on the toxicity of arsenic trioxide on the different body systems like nervous system, skin, respiratory system and liver through collection of previous studies which related with arsenic trioxide . also the broaching to the studies which performed on the arsenic trioxide through histroy of arsenic , chemical and physical properties and source of arsenic .

We concluded that arsenic trioxide is very common element in the nature, it present in the soil and ground water so that the human and animal exposed directly to it . also arsenic trioxide is almost highly toxic to all body system specially nervous system , respiratory system and skin ,and it cause acute and chronic toxicity.

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CHAPTER ONE

Introduction

Introduction

Arsenic (AS) is ubiquitous element in the environment. Weathering of rocks converts sulfides to arsenic trioxide, which enters the arsenic cycle as dust or by dissolution in rain, rivers, or ground water (Mandal and Suzuki, 2002). Arsenic is a very toxic metal, and also an environmental and industrial pollutant which is present in soil, water, air and food (Cullen, 1989).

This metal enters surface water from the industrial and found in soil by leaching of sewage sludge through soil (Antman, 2001). So, the population can be affected by arsenic through food consumption, drinking water and incidental ingestion of soil contaminated by arsenic (Celik *et al.*, 2008). It is used in foods preservatives, herbicides, insecticides and rodenticides (Akter *et al.*, 2005). Arsenic (As) is a widespread environmental toxin, it enters the organisms by dermal contact, inhalation, or ingestion of contaminated drinking water and affects nearly entire organ systems of the body (Ratnaike, 2003). Arsenic compounds are quite effectively incorporated from the gastrointestinal tract (Zielhuis and Wibowo, 1984). After absorption, It transports in the plasma is bound to albumin and accumulated mainly in kidney and liver. Arsenic occurs both organic and inorganic forms in nature but inorganic species of arsenic (AS^{III} and AS^{V}) represent a potential threat to the environment, human and animal health due to their carcinogenic and other effects (Singh *et al.*, 2004). Arsenic trioxide is a trivalent inorganic compound (Saxena *et al.*, 2008).

Arsenic can result in acute and chronic toxicity. The characteristics of chronic effects of arsenic toxicity are degenerative inflammatory and neoplastic changes of the skin, respiratory, haemopoitic, cardio vascular, nervous, hepatic, endocrine and renal system (Hughes, 2002). Also causes

an oxidative stress through lipid peroxidation and consumption of some antioxidant systems (Yamauchi *et al.*, 2004 ; Kitchen, 2001).

Previous studies showed that arsenic contamination may cause wide variety of diseases such as cancers (National research council, 2001), diabetes mellitus (Tseng *et al.*, 2000), cardiovascular disorders (Das *et al.*, 2004 ; Wang *et al.*, 2002), reproductive toxicity (Ahmed *et al.*, 2008) as well immunotoxicity (Patterson *et al.*, 2004 ; Soto-Pena *et al.*, 2006). Chronic exposure to arsenic, in addition to its general toxicity may affect lymphocyte, monocyte and macrophage activity in many mammals, resulting in immunosupperssion (Yang and Frenkel, 2002 ; Wu *et al.*, 2003; Duker *et al.*, 2005 ; Sakurai *et al.*, 2006).

CHAPTER TWO

Literatures of Review

Literatures Review

2-1-Arsenic trioxide review:

2-1-1- History of Arsenic :

It is unclear when and who discovered arsenic, historical records show that it was used by the ancient Greeks , Persians , and Chinese . It is best known as a poison , as a one of its earliest uses (Boffetta, 2004). It was almost the perfect poison difficult to detect because it lacks color , odor and taste (Hazardous substance fact sheet, 2010).

An early historical reference to arsenic as a poison is by a Greek physician named Discordia's who was in the court of Emperor Nero (54-68 AD) (Smith, 2005) . Arsenic has been used in medicine for more than 2400 years for a variety of ailments including ulcers, the plague and malaria (Waxman and Anderson, 2001).

Arsenic is known to mankind since the dawn of human civilization as an ideal homicidal poison and was used umpteen times with criminal intent (Arsenic poisoning, 2008). The poison was molded into a medicine in the 1700s, when Thomas Fowler developed a solution of arsenic trioxide in potassium bicarbonate (1%W/V) for the treatment of asthma, cholera, eczema, pemphigus, and psoriasis (Fowler's solution, 2008). It was also used empirically for the treatment of a variety of diseases , including leprosy, syphilis and yaws (Anonymous Arsenic, 2008). The art of arsenic therapy suffered a blow when this metalloid was identified as the culprit for major public health problem after exposure via drinking water in the early 1900s in Argentina , Chile, Mexico and Thaiwan (Zaldivar, 1974). In India the problem of arsenicosis due to ground water contamination was first identified in the state of west Bengal in 1984 , (Garai *et al.*, 1984 ; Saha, 1984) and a survey report in 2001 suggest that around 150 million people are at risk from arsenic–contaminated ground water in the combined areas

of west Bengal and its neighboring country, Bangladesh (Rahman *et al.*, 2001). Interestingly enough , the roller coaster of its effects and side effects is in operation even today . In spite of the pandemic of arsenic poisoning due to contaminated ground water in a very recent development, arsenic trioxide (trisenox) has been used in the treatment of patients with acute promyelocytic leukemia (Waxman *et al.*, 2001).

Commercial use of arsenic has been declining since in 1960s. Commercial products have included wood preservatives, pesticides, herbicides (weed killers ,defoliants), fungicides, cotton desiccants, cattle and sheep dips, paints and pigments, antifouling paints, leaded gasoline and fire salts (Neiger *et al.*, 2004). In the 5th century, William Withering, who discovered digitalis , was a strong proponent of arsenic-based therapies. He argued, "poisons in small doses are the best medicines; and the best medicines in too large doses are "poisonous" (Aronson, 1994). In 18th century, Thomas Fowler compounded a potassium bicarbonate-based solution of Arsenic trioxide (As_2O_3) that would his name, Following its introduction, Fowler's solution was used empirically to treat a variety of diseases during 18th, 19th and 20th centuries (Kwong and Todd, 1997), pharmacology texts of the 1880s describe the use of arsenical pastes for cancers of the skin and breast, and arsenous acid was used to treat hypertension , bleeding gastric ulcers, heartburn and chronic rheumatism. Arsenic's reputation as a therapeutic agent was enhanced in 1910 when Nobel laureate Paul Ehrlich developed salvarsan , an organic arsenical for treating syphilis and trypanosomiasis. However, as medicine evolved in the 20th century, enthusiasm for medicinal arsenic waned rapidly (Aronson, 1994).

In modern times, arsenic acquired a reputation as a toxic compound and a poison. Chronic arsenic exposure is a serious public health problem in some parts of the world (Gallagher, 1998). In the modern era, interest in arsenic as a chemotherapy was rekindled after it was identified as an active ingredient in traditional medicines in china (Wang and Chen, 2008). Researchers evaluated arsenic compounds for the treatment of various cancers and in 1992 published the

results of a trial in which intravenous administration of arsenic trioxide (As_2O_3) produced a complete response (CR) in 21 (66%) of 32 patients with acute promyelocytic leukemia (APL) (Wang and Chen, 2008 ; Sun *et al.* 1992). In two subsequent studies, Zhang *et al.* (1996), reported that (As_2O_3) induced a complete response in 22 (73%) of 30 newly diagnosed and 22 (52%) of 42 relapsed APL patients (Wang and Chen, 2008) and Shen *et al.* (1997) observed a complete response in nine (90%) of 10 relapsed APL patients. The use of arsenic trioxide As_2O_3 to treat APL began at the Harbin Medical University in the 1970s (Cyranoski, 2007). Recently , it has been reported that arsenic (mainly arsenic trioxide) in the treatment of leukemia has obvious curative effect through inducing tumor cell apoptosis (Ho *et al.*, 2011). The blockage of apoptosis plays a more important role not only in the cause of malignancy but also in the out of control of proliferation (Perkins *et al.*, 2000 ; Scholz *et al.*, 2005).

Rousselot *et al.*, (1999) found that arsenic has been used as an anticancer agent in the treatment of acute promyelocytic leukemia, and it's therapeutic action has been attributed to the induction of programmed cell death (apoptosis) in leukemia cells. Arsenical drugs are still used in treating certain tropical diseases , such as African sleeping sickness and amoebic dysentery, and in veterinary medicine to treat parasitic diseases, including filariasis in dogs and black head disease in turkeys and chickens (National Academy of Science, 1977).

2-1-2-Uses of Arsenic:

Arsenic is used in:

1- numerous pesticides, herbicides, insecticides and rodenticide though this practice is becoming less common as more of these products are banned (Hllinger, *et al.*, 1998)

2- Animal food to prevent disease and to promote growth specially in poultry and pigs Edmonds and francesconi, (1993).

3- Cattle and sheep dips to control lice and ticks (Reagor , 1973) .

4- Wood preserver because of its toxicity to insects, bacteria and fungi (U.S. Environmental Protection Agency, 2003).

5- The medical treatment of cancers such as acute promyelocytic leukemia (Mathews *et al.*, 2013).

6- Medical solutions such as (Fowler's solution) for psoriasis (Roy and Saha, 2002).

7- Preservative in animal hides (Christen, 2001)

8- Some Asian folk remedies that claim to relieve constipation during pregnancy, facilitate delivery in women, and relieve asthma in adults and children (Werner, 2001).

9- Metallic arsenic is for strengthening alloys of copper and lead to use in car batteries (Christen, 2001).

10- Semi conductive electronic devices.

2-1-3-Arsenic Forms and Mobility :

Arsenic has the chemical symbol (As), the atomic number 33, and an atomic weight of 74.92. It is present throughout the Earth crust (Hazardous substance fact sheet, 2010). Trivalent arsenic (As^{III}) in As_2O_3 or arsenite (As[OH]3) and pentavalent arsenic (As^{v}) in arsenate ($HASO_4^{-2}$) are the tow biologically significant forms of arsenic. Although As^{v} disrupts cellular processes as a phosphate (HPO_4^{-2}) mimic, the interaction of As^{III} with the thiol (or sulfhydryl) groups (-SH) of proteins with a high cysteine content is the base reaction that underlies the multiple mechanisms of action of this chemotherapeutic agent (Aposhian, 1989). In this reaction , the valence orbitals of arsenic (As) have a better overlap and energy match with those of sulfur (S) than with those of Oxygen (O), leading to formation of an As^{III}-thiolate bond and the release of water (as in fig. (2-1)), as demonstrated in the following equation (Spuches *et al.*, 2005):

$As^{III}\_OH + RSH \rightleftharpoons As^{III}\_SR + H2O$.

Arsenic has four main chemical forms having oxidation states,-3,0,+3,+5, but in natural water it's predominant forms are in organic oxyanions of trivalent arsenite (As^{III}) or pentavalent arsenate (As^{v}) (Smedley and Kinniburg, 2002). The toxicity of different arsenic forms varies in the order arsenite › arsenate › mono-methylarsenate › dimethylarsenite. Trivalent arsenic is about 60 times more toxic than arsenic in the oxidized pentavalent state, and inorganic arsenic compounds are about 100 times more toxic, than organic arsenic compounds (Jain and Ali, 2000). The organic forms of arsenic are quantitatively insignificant and are found mostly in surface waters or in areas severely affected by industrial pollution (Smedley and Kinniburg, 2002). The relative concentrations of (As^{III}) to (As^{v}) vary widely, depending on the redox condition in the geological environment (Jain and Ali, 2000).

2-1-4-Properties of Arsenic forms:

Arsenic is a semi-metallic element. Pure arsenic is seldomly found in nature; it exists in three allotropic forms: yellow (alpha), black (beta), and gray (gamma). Arsenic compounds generally occur in trivalent and pentavalent forms. Trivalent forms are arsenic trioxide and sodium arsenite, and pentavalent forms are arsenic pentaoxide and various arsenates (Özcan, 2010). Arsenic and arsenic compounds can occur in crystalline, powder amorphous or vitreous forms. Elemental arsenic has a specific gravity of 5.73, sublimes at 613^{5}C and has a very low vapor pressure of 1mmHg at 373^{5}C. Many of the organic arsenic compounds occur as white, odorless solids with a specific gravity ranging from 1.9 to 5 (ATSDR, 2010).

2-1-5-Description of Arsenic trioxide (ATO):

Arsenic trioxide is an odorless, colorless to white crystals or powder . It is used to make Arsenic compounds in pigments, ceramics, glass and medicines, and as an intermediate in insecticides, herbicides and fungicides (Özcan, 2010). Arsenic

trioxide can be found under various forms in the environment, but also in different forms in water and food consumed by humans. It is an odorless and tasteless semi-metals that has two oxidation states: (As^{III}) and (As^{V}) (Halliwell and Gutteridge, 1999), called respectively arsenite or trivalent arsenic , and arsenate or pentavalent arsenic. The most commonly used arsenical in cancer research is ATO (As_2O_3), where arsenic is in a trivalent state; this is also why it is commonly referred to as being arsenite . Before use , ATO powder is usually diluted in aqueous base (water and sodium hydroxide) to give the following reactions: (Harris, 2001)

$$As_2O_3 + 4OH^- \leftrightarrow 2HAsO_3^{-2} + H_2O$$

$$HAsO_3^{-2} + 2H^+ \leftrightarrow H_3AsO_3$$

H_3AsO_3 is called arsenous acid, or $As(OH)_3$. In this state, arsenic is in a trivalent form. However, arsenic is mostly found in nature in the form of arsenate (Marcoux, 2007).

2-1-6-Chemical and physical properties of Arsenic Trioxide :

The chemical and physical properties of arsenic trioxide can be mentioned by the following table (2-1) (Tobin, 2007):

Table (2-1): Chemical and Physical Properties

parameter	Value	Reference
Synonyms	Arsenic oxide; arsenious acid; arsenious oxide; white arsenic	ATSDR 2000
Chemical formula	As2O3 (As4O6)	ATSDR 2000
Molecular weight	197.84	ATSDR 2000
CAS Reg. No.	1327-53-3	ATSDR 2000
Physical state	Solid	ATSDR 2000
Solubility in water	37 g/L at 20 °C 115 g/L at 100 °C	ATSDR 2000
Vapor pressure	66.1 mmHg at 312 °C	
Vapor density (air = 1)	no data	
Liquid density (water = 1)	no data	
Melting point	312.3 °C	ATSDR 2000
Boiling point	613 °C sublimes	ATSDR 2000
Odor	Odorless	ATSDR 2000
Flammability	Nonflammable	ATSDR 2000
Explosive	no data	

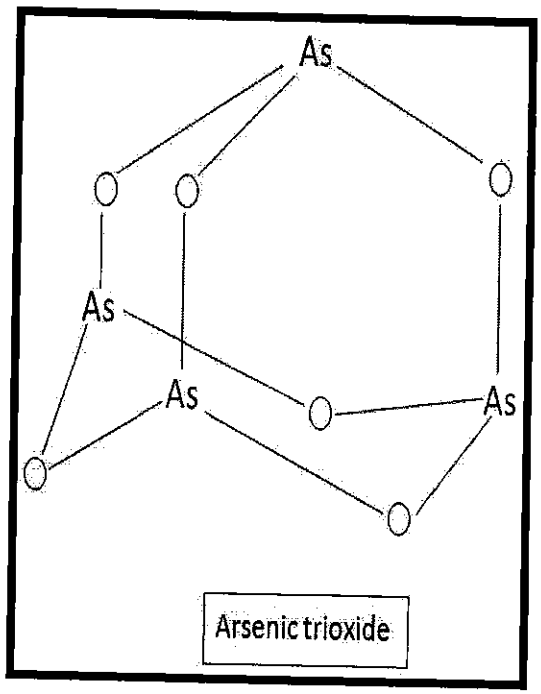

Figure (2-1) : Molecular structure of Arsenic Trioxide (Rochow, 1966)

2-1-7-Human and animal exposure to arsenic :

A large number of people are exposed to arsenic chronically throughout the world. Exposure to arsenic occurs via oral route (ingestion) , inhalation ,dermal contact ,and the parenteral route to some extent. Humans can be exposed to arsenic through the intake of air, food and water. Although food is usually the major source of arsenic exposure, most adverse effects have been associated with consumption of arsenic – contaminated drinking water (Patlolla *et al.*, 2005). Drinking water is derived from a variety of sources depending on local availability. These resources include surface water, ground water, and rainwater. The presence of arsenic varies in each source. Higher arsenic contamination is found in ground water as a result of the influence of water–rock interactions or where favorable physiological and geochemical conditions are present for arsenic mobilization and accumulation in the aquifer. In natural water arsenic is mostly found in the form of trivalent arsenite (As^{III}) or pentavalent arsenite (As^{V}) (Department of Environment Quality, 2003). Drinking water is the primary and

main route of exposure to arsenic. Maximum concentration level (MCL) is the standard concentration of arsenic in drinking water which is not hazardous is set by the EPA that is 10 µg/L (EPA, 2001) and the guideline value for concentration of arsenic in drinking water is recommended by the WHO is also 10 *mg*/L (WHO,1992). Millions of people are compelled to use the drinking water with higher arsenic level than MCL worldwide. West Bengal (India) and Bangladesh are the worst affected areas in the world from arsenicim. The standard of most developing countries is 50 µg/L which is several times higher than the MCL and more hazardous to the population (Tchounwou *et al.*, 1999).

Contamination of arsenic in ground water is the global problem and millions of people are at a risk of arsenicosis . Contaminated ground water is the main source of exposure to inorganic arsenic to the human population. Bangladesh, India , China , Taiwan , Thailand , Chili , Romania are the major affected countries where inorganic arsenic present in the ground water with high concentration. (National Research Council, 1999). Exposure of smokers to arsenic arises from the natural inorganic arsenic content of tobacco. This content is increased where tobacco plants have been treated with lead arsenate insecticide. Smelter workers, who have an elevated risk of developing lung cancer due to arsenic exposure, further increase their risk by smoking (WHO, 2000) ; IARC, 1987).

2-1-8-Sources of Arsenic Trioxide :-

Arsenic has two sources one of them is natural and the other is man made. Natural sources include; earth crust, soil, water, air and living organisms whereas anthrogenic sources inclued man-made ones such as fertilizers, drugs feed additives, insecticides, wood preservatives and herbicides. Significant anthrogenic sources include combustion of fossil fuels, leaching from mining wastes and landfills, mineraling, metal production, timber treatment, cattle and sheep dips and arsenical pesticides. Precipitation from the atmosphere and application of a range agricultural by products such as poultry manure, can also contribute to large quantities of arsenic contamination on land (Christen, 2001).

Arsenic (As) is metalloid that occurs naturally in water and soil. It is also released from a number of human activities including mining, petroleum and natural gas extraction, wood preservation and burning coal (Cullen and Reimer, 1989 ; Le, 2002). Although As is rare in nature as a pure element, both inorganic and organic forms of As are commonly found in a number of different oxidation states, of which two (+3 and +5) occur in soil, water and vegetation. Inorganic forms of As^{III} (e,g: the arsenic ion) may be found under reducing conditions; however, As(v) (e,g: the arsenate ion) predominates in surface water containing considerable dissolved oxygen (Cullen *et al.*,, 1984). Organic arsenical compounds have also been used as herbicides ,insecticides ,and drugs (Hllinger *et al.*, 1998).

2-1-8-1-Arsenic in Soil :

Arsenic contamination source in soil is the parent rock. As concentrations in soil ranges between 5-10 mg/kg; it may vary with the geological history of the region. Attrep and Anirudhan (1977) examined a wide range of soils and reported an average of 5-6 mg/kg for uncontaminated soils . Sandy soil and granites has the lowest concentration of arsenic . On the other hand alluvial and organic soils have higher concentration of As (Mandal and Suzuki, 2002 ; Smedly and Kinniburg, 2002).

2-1-8-2-Arsenic in water :

Arsenic is the 10^{th} most abundant element in seawater with average value of 2.0 µg/L. In general concentration of As is relatively stable in seawater, but some seasonal variations can occur due to biological uptake of surface seawater (Penrose *et al.*, 1977). In ground water, inorganic As commonly exists as (As^{V}) and (As^{III}), the latter is considered to be more mobile and toxic for living organisms. In aqueous environments prokaryotes biomethylate inorganic As to DMA and MMA reductively (Kramer and Allen, 1988) but the toxicity of these methylated forms is low. Biomethylation is a more subtle but persistent process and it may affect mobility and transports of As in ground water .

2-1-8-3-Arsenic in Atmosphere :

High temperature processes such as coal-fired power generation, smelting, burning vegetation and volcanism results in emission of arsenic into the atmosphere. Naturally occurring low temperature biomethylation of arsenic forms and microbial reduction process also release arsenic to the atmosphere. In this process, microorganisms forms volatile methylated derivatives of arsenic under both aerobic and anaerobic conditions (Diorio *et al.*, 1995) and also microorganism can reduce these methylated compounds to release arsenic gas. Arsenic is released to the atmosphere primarily as As_2O_3 or less frequently as one of several volatile organic compounds (Macur *et al.*, 2004).

2-1-8-4-Arsenic in Food :

Trace elements are important but they are more important in food. Because they can have adverse health effects. However arsenic present in food is in less harmful (organic) forms (Edmonds and francesconi, 1993). Inorganic arsenic forms found in food is no more than 1 to 3 percent of the total arsenic presents (Food Standards Agency, 2004).

2-1-9-Arsenic kinetics and toxicity

2-1-9-1-Arsenic kinetics

Both human and animal data suggest, that following ingestion, more than 90% of trivalent and pentavalent arsenics is absorbed by the gastrointestinal tract (Bettley and O'shea, 1975 ; Vahter and Norin, 1980 ; Marafante *et al.*, 1981). Both metabolites, MMA and DMA, are absorbed easily across the gastrointestinal tract (75-85%) (Buchet *et al.*, 1981). After absorption, arsenic is transported by the blood and bound to the sulfhydryl group (-SH) of thiol-containing protein such as glutathione (GSH), and then transported to other parts of the body. In humans, within 24hrs, arsenic is found mainly in the liver, kidneys, lungs, spleen, and skin (Kingston *et al.*, 1993). As (III) tends to accumulate in tissues, but As (V) and

organic arsenic are rapidly and almost completely eliminated via the kidneys (Hindmarsh and McCurdy, 1986). Several studies indicate that most drugs, absorption can occur via the gastrointestinal tract, lungs, skin or via special routes of administration (intraperitoneally, subcutaneous, intramuscular, or intravenous routes). In case of arsenic, in most studies involving human and animals, arsenic from the environment has been absorbed either via the gastrointestinal tract and or skin, while in the case of arsenic studied as an anti-cancer agent, arsenic is usually injected intravenously. At the cellular level (influx), a substance can enter cells by passive transport, via simple diffusion or filtration, or by special transport, either by active transport facilitated diffusion or by particles engulfing mechanisms, such as endocytosis or pinocytosis. In case of arsenic, the few studies available only report that, *in vitro* human intestinal epithelial cells line settings, AsIII intracellular transport is more effective than for As^{V} (Laparra *et al.*, 2005), and that $MMAs^{III}2$-(GSH) is the arsenic biotransformation intermediate that has the highest ability to enter rat heart microvessel epithelial cells, *in vitro* (Hirano *et al.*, 2004)

The distribution of a substance usually refers to which tissue the drug, toxin or toxicant will mainly localize and/or be stored after it's absorption. The commonest storage sites are the plasma proteins, the kidneys, the liver, fat tissues and bones, and the distribution of a chemical in these highly depends on its affinity for those tissues. In the case of arsenic, major distribution sites include the liver, lungs, kidneys, urinary bladder and blood, although several different studies report that distribution varies accordingly with the arsenic biotransformation intermediate studies (Adair *et al.*, 2007 ; Suzuki *et al.*, 2007). Distribution between erythrocytes and plasma components of blood depends upon the dose of As given, the species of animals, and the valence of administered As. In general plasma concentrations increase relative to red cell concentrations as the dose increases, and trivalent as has a higher affinity for erythrocytes than pentavalent As (As^{V}), resulting in slower clearance. Humans, rats, mice have higher

erythrocyte binding than other domestic mammals, also resulting in slower elimination (Kingston *et al.*, 1993).

The metabolism and excretion of As varies significantly between species and between genotypes within the human species (Abernathy *et al.*, 1999). Generally speaking, inorganic As is methylated *in vivo* via a series of sequential reduction and oxidative-methylation reactions. Inorganic (As^{V}) is reduced in a linked reaction with oxidation of reduced glutathione (GSH) to iAs III which is then methylated to MMA^{v} by reaction with S-adenysylmeth-ionine. Monomethylarsonic acid (MMA^{v}) is, in turn, reduced and methylated to DMA^{v} and so forth (Delnomdedieu *et al.*, 1994 ; Scott *et al.*, 1993). These metabolites are more readily excreted than inorganic As. In many, but not all, mammalian species, this process proceeds to DMA^{v} or the trimethyl arsenic metabolite trimethylarsine oxide (TMAO); however, in human beings significant amounts of MMA^{III} and DMA^{III} apparently escape methylation and react with critical tissue components. The rate of methylation and physiologic site of metabolic are thus important determinants of the rates of elimination and the potential for chronic effect of very low doses (Scott *et al.*, 1993).

The exact reactions occurring during arsenic's biotransformation have not been fully elucidated. One of the genes identified as involved in arsenic's detoxification process is the highly conserved arsenic (III) methyl transferees, (AS_3MT), which catalyzes the conversion of inorganic arsenic into methylated forms of arsenic. However, ever though this enzyme has been identified, data are still lacking to establish how exactly these arsenic methylations occur; so far, two major models have been proposed to explain the arsenic biotransformation pathway. In first model, inorganic arsenic is transformed into mono- , di-and finally tri-methylated forms of arsenic, in a linear scheme. Reducing equivalents and a methyl source are required in this conceptual model.

In the second model, the inorganic arsenic first gets bounded to three glutathione molecules, via a still undefined mechanism or reaction, and then these glutathione

moieties become the substrate for sequential methylations, transforming inorganic arsenic into mono- and dimethylated arsenic. A gamma-glutamyl transpeptidase (the enzyme that has the ability to break down GSH) deficient mice model supports this model as 60-70% of the inorganic arsenic they were fed was excreted as GSH-conjugates (Kala et al., 2004).

Arsenic's biotransformation is believed to occur mainly in the liver, as suggested by result obtained by injecting radioactive arsenic in rabbits (Marafante *et al.*, 1985) and rats (Suzuki *et al.*, 2004).

Excretion consists in disposing of the biotransformed drug, toxin or toxicant by eliminating it form the organism and returning it to the external environment. As for absorption, several different routes can be used for excretion, the most common being urinary excretion, fecal excretion is via the urinary tract. However, when urine analyses are preformed or by inhalation (Marcoux, 2007). The main route of arsenic excretion is via the urinary tract. However, when urine analyses are performed to detect arsenic traces, various arsenic biotransformation intermediates are usually detected (Sun *et al.*, 2007 ; Fukai *et al.*, 2006).

2-1-9-2-Arsenic Toxicity:

2-1-9-2-1-Chronic toxicity:

At present, despite convincing epidemiologic evidence that very low concentrations of arsenic in drinking water can cause chronic disease, especially cancer, in human beings (Kurokawa *et al.*, 1989). Chronic poisoning of the type (cancer, "black foot disease") that prompted lowering the human drinking water standard from 0.05 to 0.01mg/L (Kitchin, 2001). The mechanism(s) putatively involved in the pathogenesis of chronic damage in people i.e chemical attach by methylated AsIII metabolites on cellular macromolecules, do not appear to be relevant in livestock and wildlife. In domestic livestock, as opposed to people, most As is excreted via urine as DMA^{III} (Bertolero, 1987).

Chronic exposure to arsenic can cause skin, lung and bladder cancers. A small but measurable increase in the incidence of bladder cancer was associated with

exposure to concentration as low as 10ppm of inorganic arsenic. In addition to cancers, epidemiological studies have also established a strong correlation between chronic arsenic exposure and various non-cancer human diseases, such as hyperkeratosis, atherosclerosis, diabetes, and chronic obstructive pulmonary diseases (Jiang *et al.*, 2009). According to world health organization, the permissible limit of arsenic in drinking water is 0.01mg\L, which is equivalent to 10ppb (Prozialeck *et al.*, 2008 ; Walker and Fosbury, 2009). However, it has been reported that there is an increased risk of arsenic toxicity, even at the low and permissible dose of 10ppb (Karagas *et al.*, 2002). Chronic intake of arsenic is strongly associated with an increased risk of skin, lung, liver and other cancers, type 2 diabetes, cardiovascular diseases, neurological and cognitive defects, and reproductive and developmental problems (Steinmaus *et al.*, 2000 ; Bodwell *et al.*, 2006).

2-1-9-2-2-Cutaneous effect:

One of the hallmarks of chronic toxicity in humans from oral exposure of arsenic are skin lesions which are characterized by hyperpigmentation, hyperkeratosis and hypopigmentation (Yeh *et al.*, 1968 ; Cebrian *et al.*, 1983). Skin manifestation is the most common and initial sign of chronic arsenic exposure. Chronic ingestion of arsenic causes characteristic melanosis, keratosis, basal cell carcinoma and squamous cell carcinoma (Maloney, 1996). Presence of both melanosis and keratosis is the conformational sign of chronic arsenic toxicity. Prolonged ingestion of arsenic results in pigmentation, most intense on the trunk which can be diffuse hyperpigmentation/melanosis; (Tay, 1974 ; Saha, 1984) or localized (or patchy pigmentation), particularly affecting skin folds (Tay, 1974). Fine freckles of potted pigmentary changes are also seen, known as "rain-drop pigmentation" (A Field Guide for Detection, 2005). Sometime macular areas of depigmentation may appear on normal skin or hyperpegmented background producing the distinctive appearance of "leucomelanosis" (Saha, 1995). Blotchy pigmentation may also involve mucous membranes such as the undersurface of the tongue or buccal mucosa (Saha, 1995 ; A Field Guide for Detection, 2005).

Arsenical hyperkeratosis appears predominantly on the palms and soles, and it has been found that keratosis on the soles is the most sensitive marker for the detection of arsenicosis of an early stages (Kadono *et al.*, 2002). Keratosis are graded as mild, moderate and sever depending on the extent and severity (A Field Guide for Detection, 2005).

2-1-9-2-3-Respiratory system effect:

The respiratory symptoms resulting from arsenicosis have been shown to demonstrate dose-related symptoms, including cough, shortness of breath with the breath sounds revealing crepitation and\or rhonchi. These respiratory effects were most pronounced in individuals exposed to high concentrations of arsenic in water and who also had concomitant skin lesions (Mazumder *et al.*, 2000). An affected of inorganic arsenic in the form of airborne particles (mostly arsenic trioxide) on respiratory system mainly occurs in industrial area. Initially, the lesions of mucous membrane of respiratory system including the irritation of nasal mucosa, larynx, bronchi, and later perforation of nasal septum were observed (Hine *et al*, 1977). Exposure to inorganic arsenic in crude and refined form causes rhino-pharyngo-larynngitis, tracheobronchitis and pulmonary insufficiency due to emphysematous lesions (WHO, 1981). Exposure of arsenic through other routes instead of inhalation can also effect the respiratory system and cause a high rate of chronic cough and bronchopulmonary disease (Borgono *et al.*, 1977). Massive inhalation and swallowing of substantial amounts of crude arsenic dust (more than 80% As_2O_3) are responsible for the death, within several hours, of a worker. At autopsy, trachea and main bronchi shows widespread mucosal and submucosal hemorrhages and there is intense visceral congestion. Breathing inorganic arsenic increases the risk of lung cancer (Tahir, 2000).

2-1-9-2-4-Hepatic toxicity:

Liver is a major target organ in arsenic toxicity and carcinogenesis. Chronic exposure of experimental animals to inorganic arsenic had been shown to produce various liver lesions (Wu *et al.*, 2008). Also Liu *et al.* (1992) and Lu *et al.* (2001)

showed that liver injury is a major health problem in arsenicosis patients. Patients may develop hepatomegaly, jaundice, portal hypertension or pancreatitis caused by the direct effect of arsenic. However, Labadie *et al.*, (1990) suggested that arsenic induced hepatic injury is caused by vascular and not hepatocellular damage. Jaundice, is described after prolonged arsenical medication (Ishinishi *et al.*, 1986). No significant hepatotoxicity was reported in the pivoted trial in relapsed patients (Soignet *et al.*, 2001). Hu *et al.*,(2009) reported transient grade 1 or 2 liver dysfunction in 75% of patients during induction with all-trans-retinoic acid (ATRA) and As_2O_3; grade 3 or 4 toxicity was observed, and treatment was discontinued in any patient (44). Ravandi *et al.*, (2009) noted grade 3 elevations in liver enzymes in two of 82 patients (2%), but treatment was not discontinued. However, As_2O_3 treatment, resulted in elevated plasma liver transaminase levels in seven cases and two died of severe hepatic toxicity (Zhang *et al.*, 2001)

Chapter Three

Conclusions and Recommendations

Conclusion

Via the previous study, we conclude the following ;

1- Arsenic trioxide is highly common in the nature, it presents in the soil and ground water .
2- Arsenic trioxide have special chemical and physical properties which it make very toxic .
3- Arsenic trioxide is highly toxic to human , animals and plants .
4- Arsenic trioxide is very toxic to different body system specially nervous system , respiratory system , skin and livers .

Recommendation;-

1- Procedure study to investigate on the effect of arsenic trioxide on other system like male and female reproductive system , endocrine , digestive system .....ect .

2- Procedure molecular study to know the effect of arsenic trioxide on the oncogenes and know the role of arsenic trioxide in the induction of cancer.

3- Procedure immunohisto chemical study to know the role of arsenic trioxide in the immunity of the body .

4- Protection of the environment in Iraq through make the continuous measurements of arsenic trioxide and other heavy metals specially in the ground water .