EFFECTS OF AQUEOUS EXTRACTS OF ONION (*Allium cepa*) AND GARLIC (*Allium sativum*) ON ARSENIC-INDUCED TOXICITY IN RATS

BY

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CERTIFICATION

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DEDICATION

This piece of work is dedicated to God Almighty, the giver of life, for His faithfulness upon my life and family thus far. He alone is worthy to be glorified in my life.

And

To the memory of my late father, Mr Jonathan Kayode Oke, who could not wait to witness this achievement of his daughter in life.

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ABSTRACT

Human exposure to arsenic toxicity poses a health problem. *Allium cepa* (AC) and *Allium sativum* (AS) have the potential to ameliorate the effects of arsenic toxicity and are widely used as condiments in Nigeria. A proper understanding of these possible ameliorating effects of AC and AS in reducing arsenic toxicity is important. This study was designed to assess the effects of AC and AS on arsenic-induced toxicity in rats.

Seven weeks old male Wistar strain albino rats (*Rattus novergicus*) were acclimatized for 3 weeks. Lyophilized aqueous extracts of AC and AS were separately reconstituted in distilled water to give a concentration of 3 mg/L. Thereafter, the rats were randomly distributed into seven groups with five (5) rats in each group. Each group was given different treatments with dose equivalent of distilled water as follows: A (3 mg/L distilled water only, control), B (3 mg/L As_2O_3 only), C (3 mg/L AC extract only), D (3 mg/L AS extract only), E (3 mg/L each of AC and As_2O_3), F (3 mg/L each of AS and As_2O_3) and G (3 mg/L each of AC and AS extracts). Treatments were administered by gavage while maintaining the rats on commercial rat pellets and water *ad libitum* for 20 days. In addition to the baseline body weight, weights of rats, feed and water intake were recorded daily throughout the experiment. On day 20, the rats were sacrificed after which blood, liver, kidney, brain, testes, and spleen were removed for biochemical, haematological and histopathological examinations. Data were analysed using descriptive statistics, paired t-test and ANOVA at 5% level of significance.

Weight change for groups A to G was: 21.7±9.8 g, 7.6±12.1 g, 0.9±4.1 g, 8.0±12.0 g, 3.6±2.2 g, 2.2±1.0 g and 34.2±1.6 g respectively. The feed intake per day for group E (62.2+11.8 g) was the highest and significantly higher when compared with 61.8+18.4 g for group B. However, group C (53.8±11.8 g), D (56.7±24.2 g) were significantly lower when compared with 72.3+14.7 g of the feed intake for control. Packed cell volume (47.3+3.8%) was highest in F and significantly higher in comparison with 45.0+3.6% for group B. White blood cell count was highest in E (10567cell/mm^3) and significantly higher when compared with 7500 cell/mm^3 for control. Total protein (8.2+0.2 g/dl) for group F was higher than 7.8+0.7 g/dl for the control but not significantly different. Albumin production was significantly lower in G (4.1+0.1 g/dl) than the control (4.6+0.1 dl). Blood urea nitrogen production was significantly lower in group F (14.0+1.0 dl) than the control (15.3+0.6g/dl). Group B showed nasal discharge, fur removal, ocular lesion, cytoplasmic degeneration around renal tubules of the kidney, tissue necrosis of the liver, nuclear pleomorphism of the brain, and aggregation of inflammatory cells of the spleen than A,C,D and G. However, groups E and F showed none of these effects.

Based on the detoxifying effects of aqueous extracts of *Allium cepa* and *Allium sativum* on arsenic-induced toxicity in rats, their use as condiments among humans should be encouraged and possibly promoted.

Keywords: *Allium cepa, Allium sativum,* arsenic-induced toxicity

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GLOSSARY OF TECHNICAL TERMS AND ABBREVIATIONS

µm	-	Micrometers
AC	-	*Allium cepa* only
ALT	-	Alanine transaminase
ARS	-	Arsenic trioxide (As_2O_3) only
ASAT	-	*Allium sativum* only
AST	-	Aspartate transaminase
ATM	-	Atmosphere
ATSDR	-	Agency for Toxic Substances and Disease Registry
BUN	-	Blood urea nitrogen
CCA	-	chromated copper arsenate
CERCLA		-Comprehensive, Environmental, Response, Compensation and Liability Act
Control	-	Distilled water only
CrCl	-	Creatinine Clearance
CREAT	-	Creatinine
DAS	-	Diallyl sulfide
DAT	-	Diallyl trisulfide
DDS	-	Diallyl disulphide,
DMAIII	-	Dimethylarsinous acid
DMAV	-	Dimethylarsinic Acid
DMS	-	Diallyl monosulphide
DTS	-	Diallyl trisulfide

DTTS - Diallyl tetrasulfide

DV - Density Volume

EOSIN - Eosinophils.

EPA - Environmental Protection Agency

EVF - Erythrocyte Volume Fraction

FRIN - Forestry Research Institute of Nigeria

GFR - Glomerular Filtration Rate

GIT - Gastrointestinal Tract

GSH - Glutathione

Hb - Heamoglobin

HIV - Human Immunodeficiency Virus

Ht or HCT - Haematocrit

IARC - International Agency for Research on Cancer

IBD - Inflammatory Bowel Disease

IFN-gamma - Interferon gamma

IITA - International Institute of Tropical Agriculture

IL-8 - Interleukin-8

LED - Light-Emitting Diodes

LYMP - Lymphocytes,

MCSO - S-methyl-L-cysteine sulphoxide (+)-

MMAIII - Monomethylarsonous acid

MMAV - Monomethylarsonic Acid

MONO - Monocytes

NETs - Neutrophil Extracellular Traps

NEUT - Neutrophil

NIH - National Institutes of Health

PCV - Packed Cell Volume

PDH - Pyruvate dehydrogenase

PLAT - Platelets,

PMNs - Polymorphonuclear Leukocytes

PRENCSO - S-(1-propenyl)-L-cysteine sulfoxide

RBC - Red Blood Cells

RPM - Revolutions per minute

SAC - *S*-allylcysteine

SAM - *S*-adenosylmethionine

SIADH Syndrome of Inappropriate Anti-Diuretic Secretion

Tg.AC - Transgenic mouse model

TMA - Trimethylarsine oxide

TPA - 12-*O*-tetradecanoyl phorbol-13-acetate

WBC - White Blood Cells

CHAPTER ONE

INTRODUCTION

1.0 Background Information

Arsenic is a highly toxic, naturally occurring greyish- white heavy metal that is odourless, colourless, and tasteless in solubilized form. It is ubiquitous throughout our environment at low background levels. Its usage in insecticides, acarides and arsenical soap manufacture constitute a major risk factor when one is over exposed to these products. It is used most commonly in mining operations for smelting, in the agricultural field as an herbicide and pesticide, and in the electronics industry for semiconductors and lasers. Arsenic is a metalloid found in water, soil, and air from natural and anthropogenic sources. It exists in inorganic and organic forms and in different oxidation states (−3, 0, +3, +5). In the case of environmental exposure, toxicologists are primarily concerned with arsenic in the trivalent and pentavalent oxidation state. The more commonly known arsenic compounds, arsenate and arsenite, are the anionic forms of arsenic acid and arsenous acid, respectively. Arsenical exposure through drinking water is common in many areas of the world (Chatterjee *et al.,* 1993).

Arsenic poisoning is a medical condition caused by increased levels of the element arsenic in the body. It is a chronic illness resulting from drinking water with high levels of arsenic over a long period of time (such as from five to twenty years). Arsenic can either be produced naturally or anthropogenically. The natural sources of arsenic include ground water, air and soil through wind-blown dust and water run-off. Anthropogenic sources are much more which include burning of fossil fuels, industrial production of copper, lead and zinc, agricultural use of insecticides, pesticides and wood preservatives. Exposure to arsenicals, which is used as herbicides, fungicides and rodenticides, may cause soil, air and water pollution (Nickson *et al.*, 2003).

Garlic (*Allium sativum*) is arranged together as a bulb, which averages about 2 inches in height and diameter and consists of numerous small separate cloves. Both the cloves and the entire bulb are encased in paper-like sheathes that can be white, off-white, or have a pink/purple hue. Although garlic cloves have a firm texture, they can be easily cut or crushed.

Dietary factors play a key role in the development of various human diseases, including cardiovascular disease. Epidemiological studies have shown that diets rich in fruits, herbs and spices are associated with a low risk of cardiovascular disease. Garlic acquired a reputation in the folklore of many cultures over centuries as a formidable prophylactic and therapeutic medicinal agent. Garlic has attracted particular attention of modern medicine because of its widespread health use around the world, and the cherished belief that it helps in maintaining good health warding off illnesses and providing more vigour. To date, many favourable experimental and clinical effects of garlic preparations, including garlic extract, have been reported. These biological responses have been largely attributed to i) reduction of risk factors for cardiovascular diseases and cancer, ii) Stimulation of immune function, iii) enhanced detoxification of foreign compound. iv) hepato-protection, v) antimicrobial effect. vi) antioxidant effect (Eilat *et al*., 1995). This review has been made indicating an overall view of the efficacy of garlic in cardiovascular disease conditions both in human and animals. Garlic extracts have been used in the treatment of a wide range of disorders in the past. Sang *et al.* (1995) has also demonstrated that garlic oil is active against fat infiltration of the liver. Allicin, diallyldisulfide-oxide, an active ingredient released from garlic (alliin) is a systemic vasodilator (Sang *et al*., 1995). The chemistry of garlic is quite complex and likely developed as a self-protective mechanism against microorganisms.

Onion (*Allium cepa*), also known as the bulb onion, common onion and garden onion, is the most widely cultivated species of the genus Allium (Brewster,1994) and a very rich source of vitamin C, B1, B6, K, biotin, chromium, calcium, folic acid, and dietary fibre. Wide-ranging claims have been made for the effectiveness of onions against conditions ranging from the common cold to heart disease, diabetes, osteoporosis, and other diseases. They contain chemical compounds believed to have anti-inflammatory, anti-cholesterol, anticancer, and antioxidant properties, such as quercetin.

The onion is the richest dietary source of quercetin, a potent antioxidant (also in shallots, yellow and red onions only but not in white onions), which is specifically linked to inhibiting human stomach cancer. Quercitin in onions also thins the blood, lowers cholesterol, raises good-type HDL cholesterol (preferred dose: half a raw

onion a day), wards off blood clots, fight asthma, chronic bronchitis, hay fever, diabetes, atherosclerosis and infections.

As onions are sliced or eaten, cells are broken, allowing enzymes called alliinases to break down amino acid sulphoxides and generate sulphenic acids. A specific sulphenic acid, 1-propenesulfenic acid, formed when onions are cut, is rapidly rearranged by a second enzyme, called the lachrymatory factor synthase or LFS, giving syn-propanethial-S-oxide, a volatile gas known as the onion lachrymatory factor or LF (Eric, 2010). The LF gas diffuses through the air and eventually reaches the eye, where it activates sensory neurons, creating a stinging sensation. Tear glands produce tears to dilute and flush out the irritant.

1.1 Problem Statement

Arsenic poisoning from naturally occurring arsenic compounds in drinking water remains a problem in many parts of the world (Chatterjee *et al.*, 1993). Most reported arsenic poisoning are caused by one of arsenic compounds, also found in drinking water, arsenic trioxide (As_2O_3) which is 500 times more toxic than pure arsenic. Arsenic exposure can result in both acute and chronic toxicity in humans. Acute arsenic poisoning is relatively less common but has been documented after accidental ingestion of insecticides or pesticides, and attempted suicides or murders with arsenicals (Chatterjee *et al.*, 1993).

Arsenic poisoning (Arsenicosis) is a medical condition caused by increased levels of the element arsenic in the body. It is a chronic illness resulting from drinking water with high levels of arsenic over a long period of time (such as from five to twenty years). In spite of the awareness that alliums (including onions and garlic) are rich in antioxidant properties such as quercetin and allicin in onions and garlic respectively, there is however, inadequate information about the ameliorating effects of these alliums on arsenic toxicity when used as dietary supplement.

Few studies have been done on dietary intervention for heavy metal poisoning. This may be due to the cumbersomeness and wide range in the assessment of the chemical components in the food. Arsenic and its compounds, especially the trioxide, are used in the production of pesticides (treated wood products), herbicides,

and insecticides. Garlic and onions are extremely helpful to the body in as part of a natural detoxifier.

Two key areas are the interaction of trivalent arsenicals with sulphur in proteins and the ability of arsenic to generate oxidative stress. With advances in technology and the recent development of animal models for arsenic carcinogenicity, understanding of the toxicology of arsenic will continue to improve.

1.2 Justification/ Rationale of the Study

Several studies have been conducted on arsenic exposure and poisoning from different sources but few studies have been carried out on the detoxification of arsenic poisoning using dietary intervention and most importantly using the Alliums such as onions and garlic to ameliorate arsenic toxicity. All age groups of human beings are vulnerable to arsenic poisoning due to its natural occurrence in g'3roundwater. There is a need to establish a dietary intervention strategy in detoxifying arsenic poisoning. This justifies the use of crops in the Allium family since they are mostly eaten by humans as spices in food**.** Therefore, this research topic is worthy of study and may make a significant contribution to the body of already existing knowledge on food toxicology.

1.3 Public Health Significance of the Study

Arsenic is a natural contaminant of ground water, as well as drinking water, which translates into a public health issue worldwide. As the world population increases, one of the most fundamental resources for human survival, clean water, is decreasing. This study is of public health significance because all age groups of human beings are vulnerable to arsenic poisoning due to its natural occurrence in groundwater and various anthropogenic sources. The metalloid arsenic is a natural environmental contaminant to which humans are routinely exposed in food, water, air, and soil. There is therefore a need to establish a dietary intervention strategy in detoxifying arsenic poisoning. Onions and garlic both have antioxidant and anticancer properties among other properties they possess. Their dietary intervention in detoxifying arsenic poisoning is worthy of research.

1.4 Objectives

1.4.1 Broad Objective

The broad objective of this study is to assess the detoxifying effects of aqueous extracts of onion (*Allium cepa*) and garlic (*Allium sativum*) on induces arsenic toxicity in rats.

1.4.2 Specific Objectives

Specific Objectives are as follows: To:

i. Induce arsenic toxicity in rats
ii. Assess the efficacy of onions against arsenic toxicity in rats
iii. Assess the efficacy of garlic against arsenic toxicity in rats
iv. Use objectives i-iii to extrapolate on human, the ameliorating effects of onions and garlic against arsenic toxicity.

1.5 Limitation

i. The inability to estimate the various bioactive components present in *Allium cepa* and *Allium sativum*
ii. The inability to monitor the amount of arsenates excreted in the urine of experimental animals.
iii. The inability to follow up the mechanism os action of the arsenic trioxide in the experimental animals.

CHAPTER TWO

LITERATURE REVIEW

2.1 General Description of Arsenic

Arsenic is a highly poisonous metallic element and member of group Va of the periodic table, which combines readily with many elements. The Symbol is "As" and it has an atomic number of 33; atomic mass 74.9216. Arsenic is a naturally occurring element, widely distributed in the earth's crust. It is present in food, soil, air, and water, and all human populations are exposed to it in one form or another (WHO, 2009). The adult human body is thought to contain approximately 20mg – distributed in all tissue with higher concentrations in the skin, hair and nails (Katze, 2001). Food contains both organic and inorganic forms of arsenic, whereas drinking water contains primarily inorganic forms of arsenic. There has not been any established Recommended Daily Allowance (RDA) but an intake of 12 to 15 g per day is reported to be appropriate for adults (Katze, 2001). There is lack of any toxicity reports from dietary sources. However, toxicity reports from ingestion of concentrated arsenic from water as well as industrial exposure have been extensively documented (Gebel, 2000; Armienta *et al*., 1997).

The World Health Organization (WHO) advices a maximum concentration of 10 ppb. Although arsenic may be found in surface water, groundwater is the main source of arsenic in water. Consequentially, concentrations above 10 ppb may be found naturally in groundwater. Arsenic has a long history of use as a homicidal agent, but in the past 100 years arsenic, has been used as a pesticide, a chemotherapeutic agent and a constituent of consumer products. In some areas of the world, high levels of arsenic are naturally present in drinking water and are a toxicological concern. There are several structural forms and oxidation states of arsenic because it forms alloys with metals and covalent bonds with hydrogen, oxygen, carbon, and other elements. Environmentally relevant forms of arsenic are inorganic and organic existing in the trivalent or pentavalent state.

2.2 Properties of Arsenic

2.2.1 Physical properties

Arsenic forms colourless, odourless, crystalline oxides As_2O_3 ("white arsenic") and As_2O_5, which are hygroscopic and readily soluble in water to form acidic solutions. Arsenic (V) acid is a weak acid. Its salts are called arsenates (Chatterjee *et al.*, 1993).

2.2.2 Chemical Properties

Arsenic has a melting point of 817°C; sublimation point of 613°C; specific gravity 6.80 or 7.00. Its valency includes -3, 0, +3, or +5. Electronic configuration; $[Ar]3d^{10}4s^24p^3$. It appears in three allotropic forms, yellow, black, and grey. The stable form is a brittle, steel-grey hexagonal solid that oxidizes rapidly in air, and at high temperatures burns to form a white cloud of arsenic trioxide. Arsenic and some arsenic compounds sublime when heated and convert to gaseous form (Chatterjee *et al.*, 1993).

2.3 Occurrence and Production of Arsenic

Arsenic is an omnipresent element as arsenic minerals in the various lithosphere or fossil fuels including realgar, orpiment, and arsenopyrite. It is prepared commercially from arsenical pyrites (sulphide ores) by condensation of sublimed gas and by the reduction of white arsenic with carbon. This element contributes hardness and is used in preparing alloys for hard and corrosion-resistant properties. Arsenic is added to germanium to form gallium arsenide used in the production of semiconductor devices like integrated circuits and also used in laser and light-emitting diodes (LEDs) to convert electricity directly into light (Nickson *et al.*, 2003).

2.4 Human Exposure to Arsenic

Exposure can occur through the inhalation of air, through the ingestion of food and water, and via dermal absorption. Generally, the degree of non-occupational exposure to arsenic varies greatly, and this is dependent on the local geochemistry as well as the level of anthropogenic activity. The knowledge base of the exposure and

toxicological effects of arsenic has expanded greatly, particularly in the past 10–20 years. Exposure to arsenic for most people is an everyday occurrence because it is a natural component of the environment. The exposure pathways of arsenic to most people are dietary and drinking water and these exposures occur at relatively low levels. Data from Karagas *et al.* (2001) has suggested, especially among smokers, an increased risk of bladder and skin cancer is associated with toenail arsenic.

Other types of exposure can come from soil contaminated with arsenic, from its occupational use as a pesticide or a by-product of metal ore smelting, from its use as a chemotherapeutic agent, and what interests many people, but occurs rarely, as a homicidal agent. With increases in analytical technology, what most likely will occur is the discovery of presently unknown forms of arsenic (e.g., arsenolipids) that we are exposed to, particularly in our diet (EFSA, 2009).

2.5 Sources of Arsenic in the Environment

Since arsenic is found throughout the earth's crust in a variety of compounds coupled with its ubiquitous nature in the environment, human exposure to arsenic is inevitable. Therefore, exposure can occur through the inhalation of air, through the ingestion of food and water, and via dermal absorption. Generally, the degree of non-occupational exposure to arsenic varies greatly, and this is dependent on the local geochemistry as well as the level of anthropogenic activity. Given the above factors, it can be said that arsenic is a natural contaminant of ground water, as well as drinking water, which translates into a public health issue worldwide. As the world population increases, one of the most fundamental resources for human survival, clean water, is decreasing. The world water resource is diminishing, and one in every five persons does not have access to clean drinking water (BBC News, 2009). The rising demand for sanitary water cannot be met by surface water supplies, and this has resulted in dependence on underground water resources in many parts of the world.

In Nigeria and most other African countries, the use of underground water is on the rise as lakes and other surface waters dry up due to environmental degradation. The need to assess the water quality of the various sources of underground water cannot be overemphasized. It must be mentioned, however, that the risk of exposure via the three major pathways mentioned above are dependent on the bioavailability of the

element. Levels of arsenic in ambient air are generally low but higher levels (1000 ng/m3) have been seen in the vicinity of non-ferrous metal smelters (WHO, 2009). In air, arsenic exists predominantly as particulate matter, and is usually present as a mixture of arsenate and arsenite. The organic species (arsenic in combination with C and H) is of negligible importance, except in areas of arsenic pesticide application or biotic activity (Beavington and Cawse, 1978; Davidson *et al*., 1985). It is also worth mentioning the detection of arsenic in rain water. This is also an important source of exposure, especially in polluted areas where the mean concentrations range from 0.013 to 0.5 g/L, while near a North Sea gas platform, mean concentrations up to 45 g/L have been reported (Peirson *et al*., 1974; Andrea, 1980; Scudlark and Church, 1988). Background concentrations of arsenic have been recorded in non-contaminated soil and soil overlying arsenic rich geological deposits. The relative bioavailability of these is very low and based on rat model, bioavailability of arsenite and arsenate range from 1.0 to 9.9% and 0.3 to 3.0% respectively (WHO, 2002). Arsenic is widely distributed in surface water. Measured values below 10 g/dL are common (Smith *et al*., 1987; Welch *et al*., 1988). Nonetheless, the scientific literature shows areas having elevated ground water concentrations of arsenic, either naturally occurring or due to human activity, have been observed in major regions of the world.

2.5.1 Natural Sources

The natural sources of Arsenic include food, water, air and soil through wind-blown dust and water run-off. Vulcanoes release about 3000 tonnes per year and microorganisms release volatile methylarsines to the extent of 20,000 tonnes per year. The poultry is the major source of arsenic exposure to man through food.

2.5.2 Anthropogenic Sources

Anthropogenic sources are majorly occupational and are responsible for much more. 80,000 tonnes of arsenic per year are released by the burning of fossil fuels. Arsenic is also released to the environment during industrial production of copper, lead and zinc. Also, agricultural use of insecticides, acarides, pesticides, wood preservatives and feeding of poultry with antibiotics containing arsenic is another anthropogenic source of Arsenic. It is also released during arsenical soap manufacture and in the electronics industry for semiconductors and lasers (Peryea, 1998).

2.5.2.1 Sources of occupational exposures to arsenic may include:

i. Mining and processing: Arsenic is a constituent of many alloys, including those of copper, lead, zinc, silver and germanium. It is alloyed with lead in making, for example, lead shot, lead-based bearing metals and battery grids. Arsenic is often removed as an impurity during the smelting of copper, lead and zinc.
ii. Wood preservation: Wood fibres are impregnated under pressure with copper chrome arsenate.
iii. Herbicide: Monosodium methyl arsenate, disodium methyl arsenate and sodium arsenate are used extensively in agriculture.
iv. Pesticide: Lead arsenate is used to some extent in horticulture. Arsenic trioxide is used in termite control.
v. Glass making: Arsenic trioxide and pentoxide are used to produce clear glass, free from the green stain of iron impurity.
vi. Hide preservation: Arsenic trioxide and sodium arsenite are used as hide preservatives.
vii. Food additive: Derivatives of phenylarsenic acid are added to fowl and pig feed.
viii. Laboratory procedures: Arsenic trichloride is used in organo-arsenic chemistry and in chemical analysis procedures.

2.6 Uses of Arsenic

Arsenic compounds are used as agricultural pesticides (such as copper and lead arsenates), wood preservative, for glass making, in alloys, electronics, in indigo and calico printing, in tanning, in bronzing and pyrotechnics and in the manufacture of dyestuffs. Though some organic compounds of arsenic were used in medicine such as Salvarsan, used in the treatment of syphilis and yaws, Arsenic preparations for medical purpose are no longer recommended.

Arsenic has been used as a medicinal agent, a pigment, a pesticide, and an agent of criminal intent. In the form of chromated copper arsenate (CCA), it was used until recently as part of the treatment to render architectural wood immune to pest infestation. A great deal of the treated wood continues to exist in the form of decks and other structures exposed to the elements. Data suggest that a significant quantity of arsenic may leach out from such wood into landfills and into the interiors of

homes with existing CCA-treated decks. The durability of the CCA-treated wood suggests that such exposures may continue for decades. There is evidence in the rodent model that exposure to this compound, CCA, may produce significant renal pathology. Today, arsenic is primarily used in the production of glass and semiconductors.

2.7 Medicinal Uses of Arsenic

Despite its toxicity—or perhaps because of it—arsenic has been used beneficially to treat certain ailments. Documented cases of arsenic as a therapeutic agent date back to before 2000 BCE (Antman, 2001; Hyson, 2007). The Father of Medicine, Hippocrates, is thought to have used an arsenic paste to treat ulcers and abscesses (Riethmiller, 2005; Waxman and Anderson, 2001). Other pioneering physicians (e.g., Aristotle and Paracelsus) are also reported to have used arsenic medicinally (Jolliffe, 1993).

Although arsenic has been used throughout history, more detailed documentation of its use began in the late 18th century. Fowler's solution, which was discovered in 1786, is a 1% solution of potassium arsenite that was used in the treatment of various diseases, including malaria, syphilis, asthma, chorea, eczema, and psoriasis (Rohe, 1896; Scheindlin, 2005). In 1910, Paul Ehrlich introduced a new arsenic-based drug called Salvarsan, which became known as the "magic bullet" for treating syphilis and was used until the use of penicillin became more prevalent in the 1940s (Riethmiller, 2005).

Arsenic also has a rich history as a cancer chemotherapeutic. As reported by Antman (2001), pharmacology texts from the 1880s described the use of arsenical pastes for the treatment of skin and breast cancer. In 1878, it was found that Fowler's solution could be effective in lowering the white blood cell count in leukaemia patients (Antman, 2001). Although the use of Fowler's solution eventually declined over time due to its overt toxicity, a more detailed understanding of arsenic mechanism of action has allowed arsenic trioxide to emerge as an effective chemotherapeutic drug for treating acute promyelocytic leukemia (Rust and Soignet, 2001; Zhang *et al.*, 2001). With the success of this drug, the treatment of other cancers with arsenic trioxide is also being investigated (Murgo, 2001).

2.8 Arsenic as a Pesticide

Although it was recognized that the arsenic used in pigments could be toxic to humans, Paris Green was used as an insecticide from 1867 to 1900; it was effective in controlling Colorado potato beetles and mosquitoes (Peryea, 1998). In the late 1800s, another arsenic-based pesticide, lead arsenate, became extensively used; it was an effective pesticide but was less toxic to plants than Paris Green (Peryea, 1998). Up to the early 1900s, concerns about arsenic toxicity had focused on the acute effects of arsenic; the use of arsenic in insecticides furthered the understanding of how low levels of exposure over longer periods might affect public health. Studies in orchard workers were some of the first to propose a link between arsenic exposure and cancer (lung cancer in this case) (Mabuchi *et al.*, 1980; Tollestrup *et al.*, 1995). Also, because arsenic was released to the environment in concentrated amounts, the use of arsenic-based pesticides helped develop knowledge of arsenic's fate and transport (Peryea, 1998).

Some modern uses of arsenic-based pesticides still exist. Chromated copper arsenate (CCA) has been registered for use in the United States since the 1940s as a wood preservative, protecting wood from insects and microbial agents. In 2003, CCA manufacturers instituted a voluntary recall of residential uses of CCA-treated wood. CCA is still approved for use in nonresidential applications, such as in marine facilities (pilings and structures), utility poles, and sand highway structures (U.S. EPA, 2008; WPSC, 2008).

The use of organic arsenical pesticides began in the 1950s and has continued into the present day. Overall, organic arsenicals in the pentavalent oxidation state are much less toxic than inorganic arsenicals because, unlike inorganic arsenic, these ingested organic arsenicals are not readily taken up into cells and undergo limited metabolism (Cohen *et al.*, 2006).

2.9 Arsenic in the Environment

Arsenic can be found naturally on earth in small concentrations. It occurs in soil and minerals and it may enter air, water and land through wind-blown dust and water run-off. Arsenic in the atmosphere comes from various sources: vulcanoes release about 3000 tonnes per year and microorganisms release volatile methylarsines to the extent of 20.000 tonnes per year, but human activity is responsible for much more: 80000 tonnes of arsenic per year are released by the burning of fossil fuels. Despite its notoriety as a deadly poison, arsenic is an essential trace element for some animals, and maybe even for humans, although the necessary intake may be as low as 10 µg/day. Arsenic is a component that is extremely hard to convert to water-soluble or volatile products. The fact that arsenic is naturally a fairly a mobile component, basically means that large concentrations are not likely to appear on one specific site. This is a good thing, but the negative site to it is that arsenic pollution becomes a wider issue because it easily spreads. Arsenic cannot be mobilized easily when it is immobile. Due to human activities, mainly through mining and melting, naturally immobile arsenics have also mobilized and can now be found on many more places than where they existed naturally.

A little uncombined arsenic occurs naturally as microcrystalline masses, found in Siberia, Germany, France, Italy, Romania and in the USA. Most arsenic is found in conjunction with sulfur in minerals such as arsenopyrite (AsFeS), real gar, orpiment and enargite. None is mined as such because it is produced as a by-product of refining the ores of other metals, such as copper and lead. World production of arsenic, in the form of its oxide, is around 50.000 tonnes per year, far in excess of that required by industry. China is the chief exporting country, followed by Chile and Mexico. World resources of arsenic in copper and lead ores exceed 10 million tonnes.

Understanding the environmental levels that can cause a public health concern is a key area of research.

2.9.1 Arsenic in Drinking Water

Arsenic found in water is almost entirely in the inorganic form and can be stable as both arsenite and arsenate, trivalent and pentavalent inorganic arsenicals, respectively (Seike *et al.*, 2002). The U.S. Geological Survey estimates that the

median groundwater concentration is 1 µg/l or less, although some groundwater aquifers can contain much higher levels.

An extensive body of research studying the health effects associated with arsenic in drinking water both within and outside the United States has been published. It is through these studies that ingestion of arsenic has been definitively linked to increased incidence of cancer in lung, bladder, skin, kidney, liver, and potentially prostate. A number of non-cancer effects also are linked to exposure in drinking water, including skin lesions, cardiovascular disease, neurological effects, and diabetes (ATSDR, 2007b;NRC, 1999).

Arsenic can be removed from water in various ways. Examples of water purification techniques that may be applied are iron and aluminium coagulation, activated alumina adsorption, ion exchange and membrane filtration. AS-V can be removed easier than AS-III. AS-III can be removed when it is pre-oxidised to AS-V.

2.9.2 Arsenic in Diet

Arsenic is an essential diet and because it is also ubiquitous in the environment, diet is the largest source of both inorganic and organic arsenic for typical individuals. Estimates of dietary inorganic arsenic intakes vary. In the United States, Schott *et al.* (1993) estimated an average adult intake of 3.2 µg/day, with a range of 1–20 µg/day. Estimates for children were similar. Recently, the European Food Safety Authority (EFSA) estimated a higher intake level, although estimates depended on simplifying assumptions regarding the ratio of inorganic arsenic to total arsenic in food. The analysis estimated a typical intake of 0.13–0.56 µg/kg/day for average consumers (9.1–39.2 µg/day for a 70 kg adult) (EFSA, 2009).

Food also contains many organic arsenic compounds, which are generally considered to have low toxicity, although toxicity does vary among the individual compounds. Developing analytical methods to identify these compounds has been important for distinguishing these compounds from the more toxic inorganic forms. The key organic arsenic compounds that can be routinely found in food (depending on food type) include monomethylarsonic acid ($MMAs^V$), $DMAs^V$, arsenobetaine, arsenocholine, arsenosugars, and arsenolipids. $DMAs^V$ or $MMAs^V$ can be found in

various types of fin fish, crabs, and mollusks, but often at very low levels (Borak and Hosgood, 2007). Arsenobetaine is the major form of arsenic in marine animals, and, by all accounts, it is considered a compound that is nontoxic under conditions of human consumption (ATSDR, 2007b; EFSA, 2009). Although arsenobetaine is little studied, available information indicates it is not mutagenic, immunotoxic, or embryotoxic (Borak and Hosgood, 2007). Arsenocholine, which is mainly found in shrimp, is chemically similar to arsenobetaine, and is considered to be "essentially nontoxic" (ATSDR, 2007b).

Arsenosugars and arsenolipids have recently been identified. Exposure to these compounds and toxicological implications are currently being studied. Arsenosugars are detected mainly in seaweed but are also found to a lesser extent in marine mollusks (EFSA, 2009). Concerns about the potential toxicity of arsenosugars have been raised because there is evidence that arsenosugars are metabolized to $DMAs^{V}$ (Andrewes *et al.*, 2004). Studies addressing arsenosugar toxicity, however, have largely been limited to *in vitro* studies, which show that arsenosugars are significantly less toxic than both inorganic arsenic and trivalent methylated arsenic metabolites (Kaise *et al.*, 1996). Arsenolipids, which are a component of fish oil, have only been recently characterized; their toxicity has not been studied (Schmeisser *et al.*, 2006).

2.9.3 Arsenic in Soil

The natural content of arsenic in soils globally ranges from 0.01 to over 600 mg/kg, with an average of about 2–20 mg/kg (Roberts *et al.*, 2002)

Arsenic in soil is almost entirely in the inorganic form, except in areas with intentional organic arsenic application, where higher levels of organic compounds can be found (Saxe *et al.*, 2006). In soils, pentavalent arsenic predominates due to oxidation of trivalent arsenicals (Gong *et al.*, 2001).

Exposure to arsenic in soil can occur through multiple pathways. Incidental ingestion is typically the most significant exposure pathway for soil. Compared with the intake of naturally occurring arsenic from water and the diet, soil arsenic constitutes only a small fraction of intake (Petito Boyce *et al.*, 2008); this is a

reflection of the relatively small amounts of inorganic arsenic in soil that is typically ingested on a daily basis as well as the reduced bioavailability of arsenic in soil compared with water. Overall, a large number of studies have shown that the relative oral bioavailability of arsenic in soils to be less than 50% (Roberts *et al.*, 2002).

Other potential exposure pathways for soil arsenic include dermal absorption and inhalation of wind-blown soil particles (i.e., fugitive dust). However, arsenic is not readily absorbed through the skin from soil (U.S. EPA, 2008).

2.9.4 Arsenic in Air

Compared with arsenic exposure from food and water, exposure to arsenic in air, which is almost entirely as inorganic arsenic, is generally very low. The European Commission (EFSA, 2009) reports that levels of arsenic in air range 0–1 ng/m^3 in remote areas, 0.2–1.5 µg/m^3 in rural areas, 0.5–3 µg/m^3 in urban areas, and up to about 50 µg/m^3 in the vicinity of industrial sites. Based on these data, the European Commission (EFSA, 2009) estimated that in relation to food, cigarette smoking, water, and soil, air contributes less than 1% of total arsenic exposure, even when assuming an arsenic air exposure that is significantly above typical background (i.e., 20 µg/m^3).

2.10 Release to the Environment

Arsenic has a long history of being a poison, both intentional and unintentional, to humans. However, most laymen do not know or understand that we are constantly exposed to arsenic because it is naturally present in the environment; it is used in commercial products, and has medical applications. Although most typical environmental exposures to arsenic do not pose a health risk, several areas of the world contain arsenic from natural or anthropogenic sources at levels that create a toxicological concern. Many of these areas have been identified, and efforts are being made to either remediate these areas or limit access to them.

Arsenic ends up in the environment through industrial production of copper, lead and zinc, and through insecticide applications on farmland. Additionally, it is an ingredient of wood preservatives. A long-term uptake of large quantities of drinking

water that contains arsenic may cause skin conditions and certain cancers, such as skin cancer and lung cancer.

Arsenic is the number one substance in the most recent Comprehensive, Environmental, Response, Compensation and Liability Act (CERCLA) Priority List of Hazardous Substances published by the Agency for Toxic Substances and Disease Registry (ATSDR, 2007). This list is comprised of substances found at hazardous waste sites on the National Priorities List. The substances are ranked on frequency or occurrence, toxicity, and potential for human exposure.

2.11 Human Exposure to Arsenic

Arsenic is well known for its carcinogenic properties, but interestingly, this metalloid is also used to treat a specific form of cancer. Arsenic trioxide (which solubilizes to arsenite in water) is a treatment for cancer patients with all trans-retinoic acid-resistant acute promyelocytic leukemia (Bode and Dong, 2002). Thus, the leukemic cells undergo arsenic-induced apoptosis and the patients enter a state of remission to the cancer. However, the cancer treatment with arsenic needs to be carefully monitored because of its acute toxicological effects.

2.11.1 The Skin

The skin is a target organ in humans exposed to inorganic arsenic. Dermal effects of arsenic in humans include altered pigmentation, hyperkeratosis, and cancer. The transgenic mouse model (Tg.AC), contains the *v-Ha ras* oncogene. This strain has the characteristics of genetically initiated skin with a predisposition to papillomas. In an experiment conducted in the late 1990s, such transgenic mice were exposed to arsenite (200 ppm) in drinking water for 4 weeks. Then the mice were treated topically (two times per week for 2 weeks) with the promoter 12-*O*-tetradecanoyl phorbol-13-acetate (TPA). The number of papillomas increased in the arsenite plus TPA-treated Tg.AC mice (Germolec *et al.*, 1997).

2.12 Arsenic Toxicity

The more commonly known arsenic compounds, arsenate and arsenite, are the anionic forms of arsenic acid and arsenous acid, respectively. Monomethylarsonic

acid (MMAV) and dimethylarsinic acid (DMAV) are stable methylated mammalian metabolites of inorganic arsenic and are primarily excreted in the urine. An item of interest is that DMAV and the sodium salts of MMAV have been used as herbicides. Arsenic is an environmental chemical of toxicological concern today. The metabolism of this metalloid has an important role in its toxicological effect.

2.12.1 Metabolism of Arsenic

The metabolism of arsenic has an important role in its toxic effects. Many, but not all, mammalian species methylate inorganic arsenic (Vahter, 1994). There is also variation between species and among human populations in the rate and extent of methylation of inorganic arsenic (Vahter, 2000). Inorganic arsenic is metabolized by a sequential process involving a two-electron reduction of pentavalent arsenic to trivalent arsenic, followed by oxidative methylation to pentavalent organic arsenic (reviewed in Thomas *et al*., 2001). The reduction can occur nonenzymatically in the presence of a thiol such as glutathione (GSH) (Delnomdedieu *et al*., 1994b; Scott *et al*., 1993). However, human liver arsenate (Radabaugh and Aposhian, 2000) and MMAV (Zakharyan *et al.* 2001) reductases have been partially purified and the latter enzyme appears to be a glutathione-*S*-transferase (omega). The methylation of arsenic is enzymatic, requiring *S*adenosylmethionine (SAM) and a methyltransferase. The predominant metabolite of inorganic arsenic, dimethylarsinic acid ($(CH_3)2As_5O(OH)$), is rapidly excreted by most mammals. Trimethylarsine oxide (TMAO, (CH3)3AsVO)) is the final product in this scheme, but is found in very low amounts in urine, if at all, after exposure to inorganic arsenic. For many years, monomethylarsonous acid (MMAIII) and dimethylarsinous acid (DMAIII) have been proposed intermediates in the metabolism of arsenic. Recently, MMAIII and DMAIII have been detected in the urine of humans chronically exposed to inorganic arsenic in their drinking water (Aposhian *et al*., 2000; Del Razo *et al.*, 2001b) and in the bile of rats administered arsenite intravenously (Gregus *et al*., 2000).

$As_5O(OH)_3+2e^- \longrightarrow As_3(OH)_3+CH_3^+ \longrightarrow CH_3As_5O(OH)_2+2e^- \longrightarrow CH_3As_3(OH)_2+$
$CH3^+ \longrightarrow (CH_3)2As_5O(OH)+2e^- \longrightarrow (CH_3)2As_3OH+CH_3^+ \longrightarrow (CH_3)_3As_5O$

2.12.2 Acute Toxicity

The most visible symptoms of acute arsenic poisoning—nausea, vomiting, diarrhea, and abdominal pain—could be easily confused with other common diseases at the time (e.g., cholera and pneumonia) (ATSDR, 2007b). Immediate symptoms on an acute poisoning typically include vomiting, esophageal and abdominal pain, and bloody "rice-water" diarrhea (WHO, 2002). The symptoms and signs of arsenic toxicity differ between individual population groups and geographic regions.

The acute toxicity of arsenic is related to its chemical form and oxidation state. A basic tenet is that the acute toxicity of trivalent arsenic is greater than pentavalent arsenic. For example, in the mouse, the oral LD50 of arsenic trioxide is more than 36-fold lower than that of MMAV. In the human adult, the lethal range of inorganic arsenic is estimated at a dose of 1–3 mg As/kg. The characteristics of severe acute arsenic toxicity in humans include gastrointestinal discomfort, vomiting, diarrhoea, bloody urine, anuria, shock, convulsions, coma, and death. For many years it was believed that the acute toxicity of inorganic arsenic was greater than organic arsenic and hence, the methylation of inorganic arsenic was a detoxification reaction. This dogma was held because DMAV, the primary excreted metabolite of inorganic arsenic, is less acutely toxic than inorganic arsenic. However, Cullen *et al*. (1989) found that a derivative of MMAIII is more toxic than arsenite to the mi-croorganism *Candida humicola* in vitro. Human cells are also more sensitive to the cytotoxic effects of MMAIII than arsenite (Petrick *et al*., 2000; Styblo *et al.*, 1999). DMAIII is at least as cytotoxic as arsenite in several human cell types (Styblo *et al*., 2000). Recently Petrick *et al.* (2001) reported that MMAIII has a lower LD_{50} than arsenite in the hamster. The greater acute toxicity of the methylated trivalent intermediates of arsenic suggests that the methylation of arsenic is not solely a detoxification mechanism. These results suggest that gastrointestinal symptoms, leucopenia, and hepatic and urinary injury are predominant in the initial phase of subacute arsenic poisoning. Peripheral neuropathy is the most frequent manifestation after the initial phase. The biomethylation of arsenic decreases in a dose rate–dependent manner.

2.12.3 Chronic Toxicity

Long-term exposures to arsenic via drinking water are known to cause cancer of the skin, lungs, urinary bladder and the kidney. Skin changes including pigmentation and thickening (hyperkeratosis) have also been recorded (Mandal and Suzuki, 2002; WHO, 2009). Increased risk of lung and bladder cancers and of arsenicassociated skin lesions has been associated with drinking water, in concentrations of as low as

0.05 mg/L. It is important to mention here that absorption of arsenic through the skin is minimal, therefore, hand-washing, bathing and laundry, etc. with arsenic containing water does not pose a significant human health risk. Arsenic has been implicated in causing health problems such as hypertension, diabetes, and reproductive disorders and endocrine disruption (Mandal and Suzuki, 2002; Tseng, 2004; Navas-Acien *et al*., 2008; WHO, 2009).

Many different systems within the body are affected by chronic exposure to inorganic arsenic. Some of these systems and their associated toxic effects from chronic arsenic exposure are listed in Table 2.1 below. One of the hallmarks of chronic toxicity in humans from oral exposure to arsenic is skin lesions, which are characterized by hyperpigmentation, hyperkeratosis, and hypopigmentation (Yeh *et al*., 1968; Cebrian *et al*., 1983). In Taiwan, Blackfoot disease, a vaso-occlusive disease which leads to gangrene of the extremities, is also observed in individuals chronically exposed to arsenic in their drinking water (Tseng, 1977).

Table 2.1: Effects observed in humans and laboratory animals after chronic arsenic exposure

System	Effects
Skin	Skin lesions
Cardiovascular	Blackfoot disease
Nervous	Peripheral neuropathy, encephalopathy
Hepatological	Hepatomegaly, cirrhosis, altered heme metabolism
Haematological	Bone marrow depression
Endocrine	Diabetes
Renal	Proximal tubule degeneration, papillary and cortical necrosis

(Mandal and Suzuki, 2002)

2.12.4 Mechanism of Trivalent Arsenic Toxicity

Specific functional groups within enzymes, receptors or coenzymes, such as thiols or vicinal sulfhydryls, have a major role in the activity of these molecules. Trivalent arsenicals readily react in vitro with thiol-containing molecules such as GSH and cysteine (Scott *et al.*, 1993; Delnomdedieu *et al*., 1994b). Binding of MMAIII and DMAIII to protein in vitro occurs to a greater extent than with the pentavalent organic forms (Styblo *et al*., 1995). Arsenite has a higher affinity for dithiols than monothiols, as shown by the highly favoured transfer of arsenite from a (GSH) 3-arsenic complex to the dithiol 2, 3-dimercaptosuccinic acid (Delnomdedieu *et al*., 1993). The binding of trivalent arsenic to critical thiol groups may inhibit important biochemical events which could lead to toxicity. However, binding of arsenite at nonessential sites in proteins may be a detoxification mechanism.

Pyruvate dehydrogenase (PDH) is a multi subunit complex that requires the cofactor lipoic acid, a dithiol, for enzymatic activity. Arsenite inhibits PDH (Peters, 1955; Szinicz and Forth, 1988; Hu *et al*., 1998), perhaps by binding to the lipoic acid moiety. Petrick *et al*. (2001) has shown that MMAIII is a more potent inhibitor of PDH than arsenite. PDH oxidizes pyruvate to acetyl– CoA, a precursor to intermediates of the citric acid cycle. The citric acid cycle degrades the intermediates, and this provides reducing equivalents to the electron transport system for ATP production.

2.12.5 Arsenic Carcinogenicity

Inorganic arsenic is classified by the International Agency for Research on Cancer (IARC, 1980 & 1987) and the US Environmental Protection Agency (EPA, 1988) as a known human carcinogen. This classification is based on several epidemiological studies which show an association of exposure to arsenic and the development of cancer. Cancer has developed in individuals exposed to arsenic through medical treatment with Fowler's solution (potassium arsenite) (Fierz, 1965), occupational exposure via inhalation at copper smelters (Lee-Feldstein, 1983, 1986) or naturally contaminated drinking water (Cebrian *et al*., 1983). Tumors that develop after inhalation of arsenic are observed primarily in the lung (Lee-Feldstein, 1983, 1986),

whereas they are initially observed in the skin after oral exposure to arsenic (Tseng *et al*., 1968 Cebrian *et al*., 1983). However, additional studies indicate that cancer of internal organs occurs in individuals who chronically consume arsenic-contaminated drinking water. Tumor sites include bladder, liver, and kidney (Smith *et al*., 1992; Bates *et al.*, 1995).

Crude garlic extract administered before exposure to arsenite reduces its clastogenic l3effect (Das *et al.*, 1993; Roy- Choudhury *et al*., 1996). Garlic extract is composed of several sulphur-containing chemicals which may interact with trivalent arsenic and inhibit its toxic effect.

2.12.6 Tumor Promotion

Arsenite administered in drinking water does not promote tumors in the skin of wild-type mice topically treated with 3-methylcholanthrene (Milner, 1969) or in Tg.AC mice, a transgenic mouse strain with genetically initiated skin (Germolec *et al*., 1997). However, DMAV, a metabolite of inorganic arsenic, is a tumor promoter (Yamamoto *et al*. 1995; Yamanaka *et al*., 1996; Wanibuchi *et al*., 1996). This has been observed in both rats and mice in multiple organs (rat-urinary bladder, kidney, liver, and thyroid gland; mice-lung). Lung tumors in the rat, however, are not promoted by DMAV (Seike *et al*., 2002). The basic protocol in these studies is that the rodents are administered an initiator(s), followed by exposure to DMAV in drinking water (2–400 ppm). Tumors begin to be promoted at levels of 10 ppm. However, it is questionable whether the levels of DMA used in the promotion studies would be attained in vivo after exposure to inorganic arsenic at doses that are not acutely toxic.

2.12.7 Arsenic as an Intentional Homicidal and Suicidal Poison

Arsenic is a naturally occurring element that an individual typically encounters every day in food, water, soil, and air. While understanding how environmental exposures may affect human health, especially at low levels, is currently an active area of research, humans have known on some level about the toxicity of arsenic for centuries.

In the Middle Ages, arsenic gained notoriety as an effective homicidal and suicidal agent, both because of the frequency of its use and because of its involvement in

many high-profile murders. In fact, arsenic is often referred to as the "king of poisons" and the "poison of kings" because of its potency and the discreetness, by which it could be administered, particularly with the intent of removing members of the ruling class during the middle Ages and Renaissance (Vahidnia *et al.*, 2007). For example, it is well documented that arsenic was among the poisons used by the Medici and Borgia families to eradicate rivals (Cullen, 2008). Arsenic continued to enjoy its reputation as a high-profile poison and was implicated in several other prominent murder cases, most famously in the death of Napoleon Bonaparte in 1851, which some conspiracy theorists claim was a political assassination (Cullen, 2008).

Up until the mid-1850s, arsenic remained a popular poison for several reasons. Arsenic was readily available and because it is odourless and tasteless, it was undetectable in food or beverages (Bartrip, 1992).

2.13 Garlic

Garlic (*Allium sativum*) is a frost-resistant perennial herb. The plant is between 50 and 80 inches high and is one of the first plants cultivated by man. The bulb grows underground and consists of several small cloves, each encased by thin papery husks in several layers. Garlic contains the active ingredient alliin which is basic for the active ingredient allicin, including some enzymes, proteins, fats, potassium, zinc, vitamin A and C, calcium, magnesium, sodium, iron, B complex vitamins and amino acids. Garlic is one of the most important herbs for centuries as food, as a seasoning and as a natural medicine. Garlic is well known as a folk remedy for a variety of ailments since ancient times, however, very few studies are available suggesting its beneficial role against arsenic toxicity pertaining to its ability to eliminate arsenic from the blood and soft tissues and in reversal of arsenic-induced oxidative stress in affected tissues.

Recent trends in controlling and treating diseases favour natural antioxidants. The human diet, which contains many natural compounds, is essential in protecting the body against the development of diseases, and garlic (Allium sativum Linn.) has a broad spectrum of activities: antibacterial, anticarcinogenic, hypolipidemic, hypoglycemic, antifungal, antiatherosclerotic, and antioxidant (Hassan *et al.*, 2009). Garlic is used widely in foodstuffs and medicines. Its use is cost effective and so it is suitable for economically disadvantaged people or societies. The antioxidant activity

of garlic is attributed to biologically active lipophilic sulphur-bearing compounds such as allicin, S-allyl-cysteine (SAC), diallyl-di-sulphide (DADS), and diallyl-sulphide (DAS). Processed garlic preparations typically contain different sulphur compounds. One can expect that the composition of processed garlic products depend on the processing method. Preclinical and clinical studies reveal a close relationship between dietary habits and the occurrence of disease. Diets high in fat may increase the risk of heart disease and some forms of cancer. On the contrary, increased intake of fruits, vegetables, herbs and some of their constituents reduces risks and may even prevent some diseases. Alliums such as garlic have been studied extensively for their health benefits. Several of the allium foods have been shown to reduce risks and/or modulate metabolism to favour the prevention of diseases. Garlic, in particular, is considered to be one of the best disease-preventive foods because of its potent and widespread effects. Although some studies have cast doubt on the benefits of garlic extract, careful examination of such data emphasizes the need to clarify the influence of processing on the benefits of garlic.

2.13.1 Health benefits of garlic

Garlic is known to have antioxidant properties which scavenge free radicals that are produced in the body during metabolic reactions. It has a vitalizing effect on the body and gives more energy. Garlic is also a rich source of highly bioavailable selenium, which is thought to account, in part, for garlic's antioxidant and cancer preventive effects; some growers add selenium to the soil to enhance garlic's selenium content (Ip and Lisk, 1995).

The potency of garlic (*Allium sativum*) has been acknowledged for >5000 years. In ancient times, the Babylonians, Egyptians, Phoenicians, Vikings, Chinese, Greeks, Romans and Hindus used garlic frequently (Block, 1985). They took garlic as a remedy for intestinal disorders, flatulence, worms, respiratory infections, skin diseases, wounds, symptoms of aging and many other ailments. The use of garlic to treat wounds surfaced repeatedly through the middle ages into World War II, when garlic was used to treat the wounds of soldiers (Essman, 1984). Garlic was ground or sliced and was applied directly to wounds to inhibit the spread of infections. Figure 2.1 shows the picture of raw garlic before crushing.

Garlic thus acquired a reputation in the folklore of many cultures over the centuries as a formidable prophylactic and therapeutic medicinal agent. To date, >3000 publications from all over the world have gradually confirmed the traditionally recognized health benefits of garlic. Many favourable experimental and clinical effects of the consumption of garlic preparations, including garlic extract, have been reported. These biological responses include reduction of risk factors for cardiovascular diseases and cancer, a stimulation of immune function, enhanced foreign compound detoxification, radioprotection, restoration of physical strength, resistance to various stresses and potential anti-aging effects.

It has long been known that extraction of a food can increase its potency and eliminate unpleasant characteristics. The irritating, acidic and oxidizing compounds in raw garlic can be eliminated or modified by extraction. In fact, in some cultures, garlic is soaked or extracted with alcohol, wine, milk or vinegar before use. Many adverse reactions to garlic can be attributed to an excess of oil-soluble organosulphur constituents. For example, the lipid-lowering effects of some oil-soluble sulphur compounds in hepatocytes coincide with cytotoxicity, as revealed by increased lactate dehydrogenase release from cells (Liu and Yeh, 1999). Water-soluble sulphur compounds, on the other hand, although effective at reducing cholesterol, were not cytotoxic. An array of water-soluble constituents, including *S*-allylcysteine (SAC), may account for the reduced toxicity of the hydroalcoholic extracts of garlic compared with raw preparations. (Kanezawa *et al*., 1984, Nakagawa *et al*., 1980, 1984a and 1984b, Sumiyoshi *et al.*, 1984, Yoshida *et al*., 1984).

Figure 2.1: Garlic (*Allium sativum)*

2.13.2 Chemistry of Garlic

The chemistry of garlic is quite complex and likely developed as a self-protective mechanism against microorganisms and other insults. The primary sulphur-containing constituents in whole, intact garlic are the γ-glutamyl-*S*-alk(en)yl-L-cysteines and *S*-alk(en)yl-L-cysteine sulfoxides, including alliin. The γ-glutamyl peptides are biosynthetic intermediates for corresponding cysteine sulfoxides(Lancaster and Shaw 1989). Whole garlic typically contains ~1% alliin, together with (+)-*S*-methyl-L- cysteine sulfoxide (methiin) and (+)-*S*-(trans-1-propenyl)-L-cysteine sulfoxide. *S*-(2-Carboxypropyl)glutathione, γ-glutamyl-*S*-allyl-L-cysteine, γ-glutamyl-*S*-(trans-1-propenyl)-L-cysteine and γ-glutamyl-*S*-allyl-mercapto-L-cysteine are also present in garlic cloves (Fenwick and Hanley 1985, Sugii *et al*., 1964). During storage of garlic bulbs at cool temperatures, alliin accumulates naturally. On average, a garlic bulb contains up to 0.9% γ-glutamylcysteines and up to 1.8% alliin. In addition to these main sulphur compounds, intact garlic bulbs also contain a small amount of SAC, but no allicin. SAC is formed from γ-glutamyl cysteine catabolism and has been reported to contribute to the health benefits of some garlic preparations.

Intact garlic bulbs contain high amounts of γ-glutamylcysteines. These reserve compounds can be hydrolyzed and oxidized to form alliin, which accumulates naturally during storage of garlic bulbs at cool temperatures. After processing, such as cutting, crushing, chewing or dehydration, the vacuolar enzyme, alliinase, rapidly lyses the cytosolic cysteine sulfoxides (alliin) to form the cytotoxic and odoriferous alkyl alkane-thiosulphinates such as allicin. Allicin and other thiosulphinates instantly decompose to other compounds, such as diallyl sulphide (DAS), diallyl disulphide (DADS) and diallyl trisulphide (DAT), dithiins and ajoene. At the same time, γ-glutamylcysteines are converted to *S*-allylcysteine (SAC) via a pathway other than the alliin/allicin pathway. SAC contributes heavily to the health benefits of garlic.

Garlic is famous for its characteristic odour, arising from allicin and other oil-soluble sulphur components. Typical volatiles in crushed garlic and garlic essential

oil include diallyl sulphide (DAS), diallyl disulphide (DADS), diallyl trisulphide, methyl allyl disulphide, methyl allyl trisulphide, 2-vinyl-1,3-dithiin, 3-vinyl-1,2-dithiin (Fenwick and Hanley, 1985) and *E,Z*-ajoene (Block *et al*., 1984). Once garlic is processed by cutting or crushing, compounds in the intact garlic are converted into hundreds of organosulphur compounds in a short period of time. When garlic is "damaged," i.e., attacked by a microbe, crushed, cut or chewed, or when it is dehydrated, pulverized and then exposed to water, the vacuolar enzyme, alliinase, rapidly lyses the cytosolic cysteine sulphoxides (alliin) to form the cytotoxic and odiferous alkyl alkane-thiosulphinates . The transiently formed compound, allicin, an oily, colourless liquid, comprises 70–80% of the thiosulphinates. Typically, alliin is converted to allicin by alliinase.

Allicin is an odorous and extremely unstable compound that decomposes to sulphides, including ajoene and dithiins. Allicin is sometimes mis-labeled as "garlic oil" because it is not present in intact garlic or garlic products (Freeman and Kodera, 1995). Although allicin has been shown to be an effective antimicrobial agent in vitro, its effects in vivo are questionable. Recent studies reveal that the bioavailability of allicin is poor (Lawson *et al.*, 1992). Allicin was actually discovered to be a component of garlic by Cavallito and Bailey (1944). At that time, the use of antibiotics to treat infectious diseases was just being discovered. The discovery of allicin in garlic was so sensational that garlic was patented in the United States for its antibiotic and antifungal effects. However, the plan of medicinal or antiseptic use of allicin soon faded because of its instability. Within a few minutes after adding allicin to blood, it can no longer be detected (Freeman and Kodera, 1995). Allicin cannot be detected in the blood or urine after the ingestion of raw garlic or pure allicin (Lawson *et al.* 1992). Although freshly crushed garlic may contain limited amounts of allicin, no commercially available processed garlic preparations contain allicin. The acidity of the stomach would be expected to prevent the conversion of allium to allicin (Freeman and Kodera, 1995). Freshly crushed garlic is chemically unstable and has been shown to cause undesirable side effects, such as stomach disorders (Desai *et al*., 1990, Nakagawa *et al.*, 1980) and allergic reactions (Lybarger *et al*., 1982).

2.13.3 History of Garlic

Roman and Greek healers used garlic in treating respiratory tract infections, ulcers and warts, In the Middle Ages and after then people used garlic primarily to protect against infectious diseases. Late nineteenth century Louis Pasteur discovered that garlic destroyed certain harmful bacteria and Albert Schweizer used garlic compounds in the fight against ameobedysenterie. During World War II Russian soldiers put cloves of garlic in their pockets, to disinfect their wounds. In the twentieth century, they studied the effects of garlic. The use of garlic had positive effects on blood circulation and managed the fight of infections effectively. But there was also a downside: the (prolonged) use of raw garlic in therapeutic doses (one dose of four cloves of raw garlic) led to anaemia, irritation of stomach and intestinal mucosa, poor liver function and so on.

2.13.4 Allicin

Allicin is an organosulphur compound obtained from garlic. Allicin is garlic's defence mechanism against attacks by pests. Allicin is the most powerful medicinal compound derived from garlic and provides the greatest reputed health benefits.

Allicin does not occur in "ordinary" garlic, it is produced when garlic is finely chopped or crushed. The finer the chopping and the more intensive the crushing, the more allicin is generated and the stronger the medicinal effect.

As well as having antibiotic properties, allicin is an excellent anti-fungal and garlic preparations have been used in folk medicine to treat skin infections such as athlete's foot. Be cautious: too much contact with crushed garlic can result in skin blistering.

2.13.5 History of Allicin

Processed garlic contains a variety of sulphur-containing compounds other than those found naturally in intact garlic cloves, depending on the conditions applied (Fenwick and Hanley, 1985, Lawson 1993). Sulphur-containing compounds in commercial garlic preparations vary, depending on their manufacturing processes. In addition to odouriferous oil-soluble compounds, less odourous water-soluble

organosulphur compounds have been shown to be biologically active in various areas. SAC has an array of biological effects including a reduction in carcinogen bioactivity and a depression in oxidative damage (Amagase and Milner, 1993, Numagami *et al.*, 1996).

The non-volatile sulphur-containing compounds, SAC and SAMC are present in several garlic preparations, although the content varies considerably (Imai *et al.*, 1994, Lawson, 1993).

Additional constituents of intact garlic include the following: steroidal glycosides (Matsuura *et al.*, 1988), lectins (Kaku *et al.*, 1992), prostaglandins, fructan, pectin, essential oil, adenosine, vitamins B-1, B-2, B-6, C and E, biotin, nicotinic acid, fatty acids, glycolipids, phospholipids, anthocyanins, flavonoids, phenolics and essential amino acids (Fenwick and Hanley, 1985). The importance of the constituents in explaining the health benefits of garlic remains to be resolved.

2.13.6 Potential Health Benefits of Allicin

Several animal studies published between 1995 and 2005 indicate that allicin may: reduce atherosclerosis and fat deposition (Eliat *et al.*, 1995, Abramovitz *et al.*, 1999), normalize the lipoprotein balance, decrease blood pressure (Silagy and Neil, 1994, Elkayam *et al.*, 2003), have anti-thrombotic (Srivastava, 1986) and anti-inflammatory activities, and function as an antioxidant to some extent (Sela *et al.*, 2004: Lindsey, 2005 and Bautista, 2005). Other studies have shown a strong oxidative effect in the gut that can damage intestinal cells (Lawson *et al.*, 1992). A randomized clinical trial funded by the National Institutes of Health (NIH) in the United States and published in the Archives of Internal Medicine in 2007 found that the consumption of garlic in any form did not reduce blood cholesterol levels in patients with moderately high baseline cholesterol levels (Gardner *et al.*, 2007). The fresh garlic used in this study contained substantial levels of allicin so this study casts doubt on the ability of allicin when taken orally to reduce blood cholesterol levels in human subjects.

In 2009, Vaidya, Ingold, and Pratt have clarified the mechanism of the antioxidant activity of garlic.works to produce its medicinal effects, such as trapping damaging

radicals. When allicin decomposes, it forms 2-propenesulfenic acid, and this compound is what binds to the free-radicals (Vaidya *et al.*, 2009). The 2-propenesulfenic formed when garlic is cut or crushed has a lifetime of less than one second (Block *et al*., 2010).

2.13.7 Structure of Allicin

Allicin begins to break down quickly, expecially if heated. Conversely its breakdown can be slowed by refrigeration.

When allicin degrades it produces various diallyl sulphides, the most common of which is diallyl dishulphide

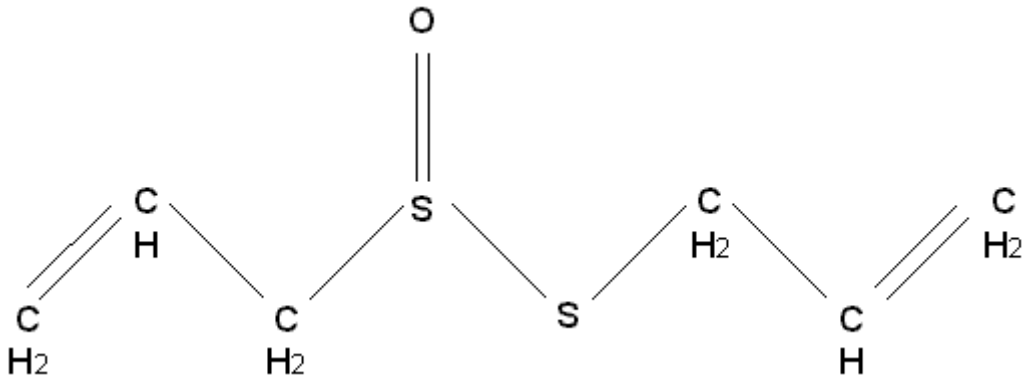

Figure 2.2 : Structure of Allicin

2.13.8 Chemistry of Allicin

Allicin features the thiosulfinate functional group, R-S(O)-S-R. The compound is not present in garlic unless tissue damage occurs,(Eric, 1985) and is formed by the action of the enzyme alliinase on alliin.Allicin is chiral but occurs naturally only. The racemic form can also be generated by oxidation of diallyl disulfide (Cremlyn, 1996).

$(SCH_2CH{=}CH_2)_2 + RCO_3H \rightarrow CH_2{=}CHCH_2S(O)SCH_2CH{=}CH_2 + RCO_2H$

Alliinase is irreversibly deactivated below a pH of 3; as such, allicin is generally not produced in the body from the consumption of fresh or powdered garlic. Furthermore, allicin can be unstable, breaking down within 16 h at 23 °C (Hahn *et al*., 1996)

Allicin has been found to have numerous antimicrobial properties, and has been studied in relation to both its effects and its biochemical interactions. One potential application is in the treatment of Methicillin-resistant Staphylococcus aureus (MRSA), an increasingly prevalent concern in hospitals. A screening of allicin against 30 strains of MRSA found high level of antimicrobial activitity, including against strains that are resistant to other chemical agents (Cutler and Wilson, 2004).

2.14 ONIONS (*Allium cepa*)

The onion, known scientifically as *Allium cepa*, is, on the surface, a humble brown, white or red, paper-thin skinned bulb; yet, despite its plain looks, it has an intense flavour and is a beloved part of the cuisine of almost every region of the world. The word onion comes from the Latin word *unio*, which means "single," or "one" reflecting of the onion plant producing a single bulb, unlike its cousin, the garlic, that produces many small bulbs. The name also describes the onion bulb when cut down the middle; it is a union (also from *unio*) of many separate, concentrically arranged layers. They generally have a more pungent flavour and are usually named by their colour: white, yellow or purple (Figure 2.3).

Onions range in size, colour, and taste depending upon their variety. There are generally two types of large, globe-shaped onions, classified as spring/summer or storage onions. The former class includes those that are grown in warm weather climates and have characteristic mild or sweet tastes. Included in this group are the Maui Sweet Onion (in season April through June), Vidalia (in season May through June) and Walla Walla (in season July and August). Storage onions are grown in colder weather climates and, after harvesting, are dried out for a period of several months, which allows them to attain dry, crisp skins. Figure 3.1 shows purple onions.

Figure 2.3: Purple Onions (*Allium cepa*)

2.14.1 Composition of Onions

Onions are a very good source of vitamin C, B6, biotin, chromium, calcium and dietary fibre. In addition, onions contain good amounts of folic acid and vitamin B1 and K. They also contain flavonoids. Like garlic, onions also have the enzyme alliinase, which is released when an onion is cut or crushed and it causes your eyes to water. Onions are a major source of polyphenols in general, and also of flavonoids (a very important subdivision of polyphenols) which are pigments that give vegetables their colour. These compounds act as antioxidants, have a direct antitumor effect and have immune-enhancing properties.

They can also vary greatly in their polyphenol and flavonoid content. In general, red onions are higher in total flavonoids than white onions, (with yellow onions falling somewhere in between). The total polyphenol content of onions is very high. (Polyphenols are one of the largest categories of phytonutrients in food. This category includes all flavonoids as well as tannins). The total polyphenol content of onion is not only higher than its fellow allium vegetables, garlic and leeks, but also higher than tomatoes, carrots, and red bell pepper. In the French diet, only six vegetables (artichoke heart, parsley, Brussels sprouts, shallot, broccoli, and celery) have higher polyphenol content than onion. Since the French diet has been of special interest to researchers in terms of disease prevention, onion's strong polyphenol contributions will very likely lead to follow-up studies that pay closer attention to this unique allium vegetable.

The flavonoid content of onions can vary widely, depending on the exact variety and growing conditions. Although the average onion is likely to contain less than 100 milligrams of quercetin per 3-1/2 ounces, some onions do provide this amount. And while 100 milligrams may not sound like a lot, in the United States, moderate vegetable eaters average only twice this amount for *all* flavonoids (not just quercetin) from *all* vegetables per day.

When onions are simmered to make soup, their quercetin does not get degraded. It simply gets transferred into the water part of the soup. By using a low-heat method for preparing onion soup, you can preserve the health benefits of onion that are associated with this key flavonoid.

2.14.2 Nutritional Components of Onions

In Nigeria the most – producing onion state is Kebbi. Onions are native to Asia and the Middle East and have been cultivated for over five thousand years. Figure 2.4 below shows the nutritional components of onions

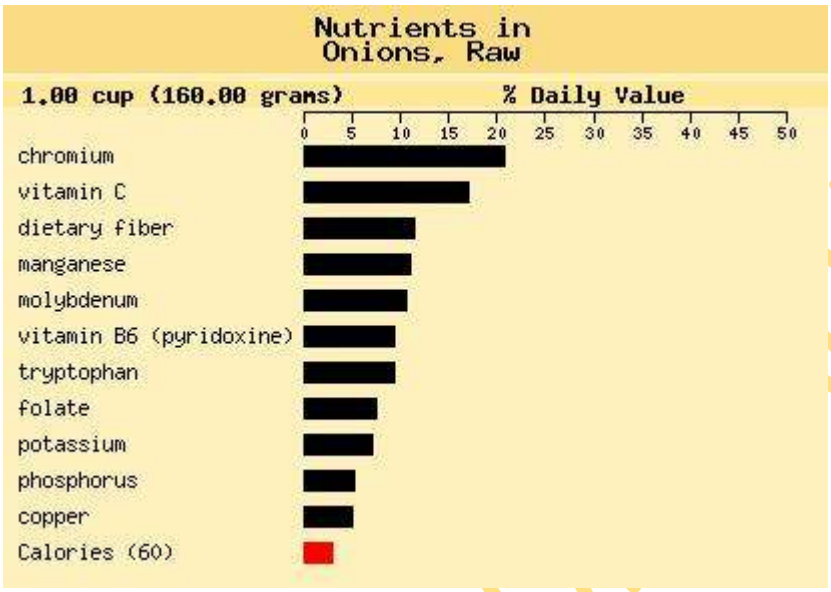

Figure 2.4: Nutritional Components of Onions (Brewster, 1994)

2.14.3 Health Benefits of Onions

Onions, like garlic, are members of the Allium family, and both are rich in sulphur-containing compounds that are responsible for their pungent odours and for many of their health-promoting effects. A wide variety of allyl sulphides are found in onion, including the four major diallyl sulphides: DMS (diallyl monosulphide), DDS (diallyl disulphide), DTS (diallyl trisulphide), and DTTS (diallyl tetrasulphide). Also present are a wide variety of sulphoxides, including (+) S-methyl-L-cysteine sulphoxide (MCSO), (+)-S-(1-propenyl)-L-cysteine sulphoxide (PRENCSO), S-methyl-l-cysteine sulphoxide, S-propyl-l-cysteine sulphoxide, and S-propenyl-l-cysteine sulphoxide. Onions are an outstanding source of polyphenols, including the flavonoid polyphenols. Within this flavonoid category, onions are a standout source of quercetin.

2.14.3.1 Cardiovascular Benefits

Unlike the research on garlic and its cardiovascular benefits, research specifically focused on onion has mostly been conducted on animals rather than humans. In animal studies, there is evidence that onion's sulphur compounds may work in an anti-clotting capacity and help prevent the unwanted clumping together of blood platelet cells. There is also evidence showing that sulphur compounds in onion can lower blood levels of cholesterol and triglycerides, and also improve cell membrane function in red blood cells.

In human studies, most of the cardiovascular benefits have been demonstrated in the form of overall diet. Multiple studies show onion to be a food that provides protection for the heart and blood vessels when consumed in a diet that is rich in other vegetables and fruits especially flavonoid-containing vegetables and fruits. The benefits of onion in this overall dietary context extend to prevention of heart attack. In virtually all of these diet-based studies, participants with the greatest intake of vegetables (including onions) gain the most protection. The outstanding flavonoid content of onions supports these research findings. It's also interesting to note that onion is most commonly consumed in relatively small amounts along with other foods rather than by itself. For this reason, it can be more difficult to study in large-

scale dietary research studies that involve thousands of participants and rely on diet diaries to determine onion consumption.

2.14.3.2 Support for Bone and Connective Tissue

Human studies have shown that onion can help increase our bone density and may be of special benefit to women of menopausal age who are experiencing loss of bone density. In addition, there is evidence that women who have passed the age of menopause may be able to lower their risk of hip fracture through frequent consumption of onions. "Frequent" in this context means onion consumption on a daily basis! In this research on bone density in older women, very sporadic eating of onion (once a month or less) did not provide much benefit. That finding, of course, was very expected. But less expected was the finding that it took daily consumption of onion to show robust benefits for bone density. Just as in the cancer-related onion research, the take-away message here is clear: you don't want to skimp on onions when you are incorporating them into your meal plan.

In and of itself, the high sulphur content of onions may provide direct benefits to our connective tissue. Many of our connective tissue components require sulphur for their formation. For example, with the exception of hyaluronic acid, all glycosaminoglycans (GAGS) are sulfated. (GAGS are the premiere family of molecules found in the ground substance of our connective tissue.)

2.14.3.3 Anti-Inflammatory Benefits

While onion is not as well researched as garlic in terms of specific inflammatory health problems like rheumatoid arthritis or allergic airway inflammation, this allium vegetable has nevertheless been shown to provide important anti-inflammatory benefits. Onionin, a unique sulphur molecule in onion that is found in the bulb portion of the plant has been shown to inhibit the activity of macrophages, specialized white blood cells that play a key role in our body's immune defence system, and one of their defence activities involves the triggering of large-scale inflammatory responses. While macrophage activity is typically a good thing, inhibition of their activity can sometimes be critical in getting chronic unwanted inflammation under control.

Onion's antioxidants including its hallmark flavonoid antioxidant, quercetin also provides us with anti-inflammatory benefits. These antioxidants help prevent the oxidation of fatty acids in our body. When we have lower levels of oxidized fatty acids, our body produces fewer pro-inflammatory messaging molecules, and our level of inflammation is kept in check.

2.14.3.4 Cancer Protection

Onion has repeatedly been shown to lower our risk of several cancers, even when we consume it in only moderate amounts. "Moderate" generally means 1-2 times per week, even though in some studies it has been used to mean up to 5-6 times per week. Colorectal cancer, laryngeal cancer, and ovarian cancer are the cancer types for which risk is reduced along with moderate amounts of dietary onion. For other cancer types, however, moderate intake of onion has not been enough to show significant risk reduction. For these cancer types including oesophageal cancer and cancers of the mouth daily intake of onion is required before research results show significant risk reduction.

Exposure to steam for 10 full minutes can affect some of onion's health benefits. For these reasons, special care may be needed in the storage, handling, and cooking of this allium vegetable.

Table 2.2: In-Depth Nutritional Profile for Onions

Nutrient	Amount	DV (%)	Nutrient Density	World's Healthiest Foods Rating
Chromium	24.80µg	20.7	6.1	very good
Vitamin C	10.24 mg	17.1	5.1	very good
Dietary fibre	2.88 g	11.5	3.4	very good
Manganese	0.22 mg	11.0	3.3	good
Molybdenum	8.00 µg	10.7	3.2	good
Vitamin B6 (pyridoxine)	0.19 mg	9.5	2.8	good
Tryptophan	0.03 g	9.4	2.8	good
Folate	30.40 µg	7.6	2.2	good
Potassium	251.20 mg	7.2	2.1	good
Phosphorus	52.80 mg	5.3	1.6	good

2.15 Lyophilization (Freeze-Drying)

Freeze-drying, also known as lyophilization, or cryodesiccation, is a dehydration process typically used to preserve a perishable material. .Freeze-drying is the complete removal of water from food material, while leaving the basic structure and composition of the material intact. Freeze-drying works by freezing the material and then reducing the surrounding pressure to allow the frozen water in the material to sublimate directly from the solid phase to the gas phase. Lyophilisation is done to keep food from spoiling for a long period of time i.e to extend the shelf life of a food product, to preserve a product, to stabilize a product, making it easier to transport and less sensitive to temperature and to produce a product that can be fully reconstituted such as for injection. Lyophilization also reduces the total weight of the food. Dehydration only removes 90 to 95 percent of the water, which will certainly slow down bacteria and enzyme activity, but won't stop it completely. Also, the heat involved in the evaporation process significantly changes the shape, texture, taste, smell, appearance and composition of the food molecules. The basic idea of Freeze-drying is to lock in the composition and structure of the food material by drying it without applying the heat necessary for the evaporation process.

The fundamental principle of Freeze-drying is sublimation. The process converts solid water (ice) directly into water vapour, skipping the liquid phase entirely. Sublimation occurs when a molecule gains enough energy to break free from the molecules around it. A typical Freeze-drying machine consist of a freeze-drying chamber with several shelves attached to heating units, a freezing coil connected to a refrigerator compressor, and a vacuum pump.

2.15.1 The Freeze-Drying Process

There are four stages in the complete drying process: pre-treatment, freezing, primary drying, and secondary drying.

2.15.1.1 Pre-treatment

Pre-treatment includes any method of treating the product prior to freezing. This may include concentrating the product, formulation revision (i.e., addition of components to increase stability and/or improve processing), decreasing a high vapour pressure solvent or increasing the surface area. In many instances the

decision to pre-treat a product is based on theoretical knowledge of freeze-drying and its requirements, or is demanded by cycle time or product quality considerations. Methods of pre-treatment include: Freeze concentration, Solution phase concentration, Formulation to Preserve Product Appearance, Formulation to Stabilize Reactive Products, Formulation to Increase the Surface Area, and Decreasing High Vapour Pressure Solvents.

2.15.1.2 Freezing

In a lab, this is often done by placing the material in a freeze-drying flask and rotating the flask in a bath, called a shell freezer, which is cooled by mechanical refrigeration, dry ice and methanol, or liquid nitrogen. On a larger scale, freezing is usually done using a freeze-drying machine. In this step, it is important to cool the material below its triple point, the lowest temperature at which the solid and liquid phases of the material can coexist. This ensures that sublimation rather than melting will occur in the following steps. Larger crystals are easier to freeze-dry. To produce larger crystals, the product should be frozen slowly or can be cycled up and down in temperature. This cycling process is called annealing. However, in the case of food, or objects with formerly-living cells, large ice crystals will break the cell walls (a problem discovered, and solved, by Clarence Birdseye), resulting in the destruction of more cells, which can result in increasingly poor texture and nutritive content. In this case, the freezing is done rapidly, in order to lower the material to below its eutectic point quickly, thus avoiding the formation of ice crystals. Usually, the freezing temperatures are between −50 °C and −80 °C. The freezing phase is the most critical in the whole freeze-drying process, because the product can be spoiled if badly done.

Amorphous materials do not have a eutectic point, but they do have a critical point, below which the product must be maintained to prevent melt-back or collapse during primary and secondary drying.

2.15.1.3 Primary drying

During the primary drying phase, the pressure is lowered (to the range of a few millibars), and enough heat is supplied to the material for the water to sublime. The amount of heat necessary can be calculated using the sublimating molecules' latent heat of sublimation. In this initial drying phase, about 95% of the water in the material is sublimated. This phase may be slow (can be several days in the industry), because, if too much heat is added, the material's structure could be altered.

In this phase, pressure is controlled through the application of partial vacuum. The vacuum speeds up the sublimation, making it useful as a deliberate drying process. Furthermore, a cold condenser chamber and/or condenser plates provide a surface(s) for the water vapour to re-solidify on. This condenser plays no role in keeping the material frozen; rather, it prevents water vapour from reaching the vacuum pump, which could degrade the pump's performance. Condenser temperatures are typically below −50 °C (−60 °F).

It is important to note that, in this range of pressure, the heat is brought mainly by conduction or radiation; the convection effect is negligible, due to the low air density.

2.15.1.4 Secondary drying

The secondary drying phase aims to remove unfrozen water molecules, since the ice was removed in the primary drying phase. This part of the freeze-drying process is governed by the material's adsorption isotherms. In this phase, the temperature is raised higher than in the primary drying phase, and can even be above 0 °C, to break any physico-chemical interactions that have formed between the water molecules and the frozen material. Usually the pressure is also lowered in this stage to encourage desorption (typically in the range of microbars, or fractions of a Pascal). However, there are products that benefit from increased pressure as well. After the freeze-drying process is complete, the vacuum is usually broken with an inert gas, such as nitrogen, before the material is sealed. At the end of the operation, the final residual water content in the product is extremely low, around 1% to 4%.

2.16 Haematological Indices of Tissue Damage and Toxicity

Haematological indices include Packed Cell Volume (PCV), Red Blood Cells (RBC), White Blood Cells (WBC), Platelets, Haemoglobin (Hb), Lymphocytes, Neutrophil, Monocytes and Eosinophils. Each of these is further explained below.

2.16.1 Packed Cell Volume (PCV)

Packed cell volume is the total volume of blood plasma, i.e., the extracellular fluid volume of the vascular space. Essentially, the PCV measures the red cells that have settled to the bottom of a micro-capillary tube after this has been centrifuged.

PCV or Packed Cell Volume describes the volume that is occupied by a cell pellet after centrifugation. A PCV of 0,1% means that 1 ml of cell pellet is expected per 1 liter of cell culture, respectively 1µl per 1 ml sample (1 µl cell pellet = 1 mg wet biomass).

The volume of the cell pellet is measured in µl. This value can be directly read from the calibrated capillary. It depends on the cell density and the sample volume. Dividing it by the sample volume yields % PCV. For example reading 4 µl for a 400 µl sample corresponds to 1% PCV (4µl /400µl = 1 %). The % PCV value linearly correlates with the cell density.

The packed cell volume (PCV) can be determined by centrifugingheparinized blood in a capillary tube (also known as a micro haematocrit tube) at 10,000 RPM for five minutes.This separates the blood into layers. The volume of packed red blood cells divided by the total volume of the blood sample gives the PCV. Because a tube is used, this can be calculated by measuring the lengths of the layers.

The haematocrit (Ht or HCT), also known as erythrocyte volume fraction (EVF), is the volume percentage (%) of red blood cells in blood. It is normally about 45% for men and 40% for women (Purves et al, 2004) It is considered an integral part of a person's complete blood count results, along with hemoglobin concentration, white blood cell count, and platelet count.

2.16.2 Red Blood Cells (RBC)

These are the blood cells that carry oxygen. Red cells contain haemoglobin and it is the haemoglobin which permits them to transport oxygen (and carbon dioxide). Haemoglobin, aside from being a transport molecule, is a pigment. It gives the cells their red colour. Red blood cells are sometime simply called red cells. They are also called erythrocytes or, rarely today, red blood corpuscles.

2.16.3 White Blood Cells (WBC)

White blood cells help protect the body against diseases and fight infections. There are several types of white blood cells (leukocytes). The two most common types are the lymphocytes and neutrophils (also called polymorphonuclear leukocytes, PMNs, or "polys").The white blood cell count is done by counting the number of white blood cells in a sample of blood. A normal WBC is in the range of 4,000 to 11,000 cells per microliter. A low WBC is called leukopenia. A high WBC is termed leukocytosis (Purves *et al*., 2004)

2.16.4 Lymphocytes

A lymphocyte is a type of white blood cell present in the blood. Lymphocyte comes from the Latin word "lympha" meaning "spring water," and the Greek word "kytos" meaning "cell". They are also known as lymph cells, lymphocysts, and lympholeukocytes.

Lymphocytes are small white blood cells, usually 7 to 8 micrometers in length. Larger forms of lymphocytes are usually about 10 to 20 micrometers in length.
The nucleus (central structure) of a lymphocyte is made of large groupings of thin threads known as chromatin. The nucleus of a lymphocyte stains dark purple/blue when exposed to a stain known as Wright's stain. Unlike other types of white blood cells, such as basophils and eosinophils, the cytoplasm of lymphocytes usually do not contain large, rough-looking, grain-like particles. However, larger forms of lymphocytes may have a lot of cytoplasm that contain several bright reddish/purplish, rough-looking, grain-like particles. Unlike some other types of cells, the granules of lymphocytes do not turn a blue colour when exposed to certain

types of chemical used in laboratory tests. Lymphocytes are formed in lymphatic tissue throughout the body. Lymphatic tissues are a network of fibers and cells that help protect the body against disease. Examples of places in the body where lymphocytes are made that are made of lymphatic tissue include the spleen, thymus, tonsils, and lymph nodes. The spleen is an organ next to the stomach that helps fight infection and removes and destroys worn-out red blood cells. Red blood cells are cells that help carry oxygen in the blood. The thymus gland is an organ located in the upper part of the chest and is very important in producing substances that protect the body against disease. The tonsils are a pair of oval masses at the back of the throat. Lymph nodes are small egg shaped structures in the body that help fight against infection. Mammalian stem cellsdifferentiate into several kinds of blood cell within the bone marrow (Abbas and Lichtman, 2003).This process is called haematopoiesis. All lymphocytes originate, during this process, from a common lymphoid progenitor before differentiating into their distinct lymphocyte types. The differentiation of lymphocytes follows various pathways in a hierarchical fashion as well as in a more plastic fashion. The formation of lymphocytes is known as lymphopoiesis..

There are two types of lymphocytes. One type of lymphocyte is known as a T cell. The other type is known as a B cell. T cells and B cells differ in function and the molecules that are on their surface also differ. T cells (also known as T lymphocytes) are types of lymphocyte that circulate through the thymus gland and have turned into cells known as thymocytes (cells that have developed in the thymus gland). The thymus gland is an organ located in the upper part of the chest and is very important in producing substances that protect the body against disease. When thymocytes are exposed to antigens (substances in the body, such as those present on the surface of bacteria, that can produce a defensive reaction by the body), they rapidly divide and produce large numbers of new T cells that are sensitive to that type of antigen. More than 80% of lymphocytes in the circulating blood are T cells. There are two main groups of T cells. One group of T cells are called " Natural killer cells" (also known as cytotoxic T cells) because they produce chemical substances known as lymphokines that are essential in helping the B cells destroy foreign substances. Like B cells (Janeway *et al*, 2001) T cells are sensitized and stimulated to respond to certain antigens present on invading microorganisms or abnormal cells. Another group of T cells are called helper T cells. Helper T cells assist the killer T

cells in performing their activities and help protect the body against diseases in other ways. T cells also appear to play an important role in the body's response to the spreading of cancer cells. Cancer is a group of diseases in which symptoms are due to an abnormal and excessive growth of cells in one of the body organs or tissues. The process of the T cells protecting the body is known as cellular or cell-mediated immunity.

T cells reproduce through a process known as mitosis, in which the cells split in two. In mitosis, each cell contains an exact copy of the chromosomes in the original cell. Chromosomes are structures in a person's cells that contain proteins and a substance known as DNA (an abbreviation for deoxyribonucleic acid). DNA is a chain of many connected genes. Genes are units of material contained in a person's cells that contain coded instructions as for how certain bodily characteristics (such as eye colour) will develop.

B cells (also known as B lymphocytes) are types of lymphocytes that circulate in the blood in an immature (not fully developed) form. About 10% of lymphocytes that circulate in the blood are B cells. B cells produce proteins known as antibodies that are then inserted into the area that immediately surround the cytoplasm (a gooey substance that fills up a cell). Antibodies attach to foreign proteins in the body known as antigens that are found on the surface on certain microorganisms. A microorganism is a tiny organism made of one cell that is usually too small to be seen without using a microscope. Table 2.3 below shows recognition markers for lymphocytes.

Table 2.3: Typical Recognition Markers For Lymphocytes (Berrington *et al.*, 2005)

CLASS	FUNCTION	PROPORTION	PHENOTYPIC MARKER(S)
NK cells	Lysis of virally infected cells and tumour cells	7% (2-13%)	CD16CD56 but not CD3
Helper T cells	Release cytokines and growth factors that regulate other immune cells	46% (28-59%)	TCRαβ, CD3 and CD4
Cytotoxic T cells	Lysis of virally infected cells, tumour cells and allografts	19% (13-32%)	TCRαβ, CD3 and CD8
γδ T cells	Immunoregulation and cytotoxicity	5% (2%-8%)	TCRγδ and CD3
B cells	Secretion of antibodies	23% (18-47%)	MHC class II, CD19 and CD21

In the circulatory system they move from lymph node to lymph node. This contrasts with macrophages, which are rather stationary in the nodes. A lymphocyte count is usually part of a peripheral complete blood cell count and is expressed as percentage of lymphocytes to total white blood cells counted. A general increase in the number of lymphocytes is known as lymphocytosis whereas a decrease is lymphocytopenia. An increase in lymphocyte concentration is usually a sign of a viral infection (in some rare case, leukemias are found through an abnormally raised lymphocyte count in an otherwise normal person). A high lymphocyte count with a low neutrophil count might be caused by lymphoma. A low normal to low absolute lymphocyte concentration is associated with increased rates of infection after surgery or trauma.

One basis for low T cell lymphocytes occurs when the human immunodeficiency virus (HIV) infects and destroys T cells (specifically, the $CD4^+$ subgroup of T lymphocytes). Without the key defence that these T cells provide, the body becomes susceptible to opportunistic infections that otherwise would not affect healthy people. The extent of HIV progression is typically determined by measuring the percentage of $CD4^+$ T cells in the patient's blood. The effects of other viruses or lymphocyte disorders can also often be estimated by counting the numbers of lymphocytes present in the blood. In order to study the function of a lymphocyte by virtue of the proteins it generates, other scientific techniques like the ELISPOT or secretion assay techniques can be used (Janeway, 2001). A CD4 count of $<200 \times 10^6$ cells/μL is very likely if the EDALC is $<950 \times 10^6$ cells/μL and less likely if the ALC is $>1{,}700 \times 10^6$ cells/μL" (Napoli *et al.*, 2011)

2.16.5 Neutrophil

Neutrophils, which are produced in the bone marrow and circulate in the blood, are a type of white blood cell. Neutrophils are abundant and make up about 50% to 75% of white blood cells. Neutrophils respond to infection and attack bacteria and other foreign invaders directly.

Neutrophils are the first type of immune cell to respond to and arrive at the site of infection, often within an hour. Neutrophils are recruited to the site of injury within minutes following trauma and are the hallmark of acute inflammation Cohen *et al.*, 2002). Neutrophils are normally found in the blood stream. During the beginning

(acute) phase of inflammation, particularly as a result of bacterial infection, environmental exposure (Jacobs, *et al.*, 2010) and some cancers (Waugh, 2008), neutrophils are one of the first-responders of inflammatory cells to migrate towards the site of inflammation. They migrate through the blood vessels, then through interstitial tissue, following chemical signals such as Interleukin-8 (IL-8), in a process called chemotaxis. They are the predominant cells in pus, accounting for its whitish/yellowish appearance. (De Larco *et al.*, 2004). Neutrophil granulocytes have an average diameter of 12–15 micrometers (µm) in peripheral blood smears. When analyzing a pure neutrophil suspension on an automated cell counter, neutrophils have an average diameter of 8–9 µm. Neutrophils are the most abundant white blood cells in humans (approximately 10^{11} are produced daily); they account for approximately 50-70% of all white blood cells (leukocytes). The stated normal range for human blood counts varies between laboratories, but a neutrophil count of 2.5–7.5 x 10^9/L is a standard normal range. People of African and Middle Eastern descent may have lower counts, which are still normal. A report may divide neutrophils into segmented neutrophils and bands. When circulating in the bloodstream and unactivated, neutrophils are spherical. Once activated, they change shape and become more amorphous or amoeba-like and can extend pseudopods as they hunt for antigens (Edwards and Steven, 1994). The average lifespan of (non-activated human) neutrophils in the circulation is about 5.4 days (Pillay *et al.*, 2010) Upon activation, they marginate (position themselves adjacent to the blood vessel endothelium), and undergo selectin-dependent capture followed by integrin-dependent adhesion in most cases, after which they migrate into tissues, where they survive for 1–2 days (Wheater *et al.*, 2002).

Neutrophils are much more numerous than the longer-lived monocyte/macrophage phagocytes. A pathogen (disease-causing microorganism or virus) is likely to first encounter a neutrophil. Some experts hypothesize that the short lifetime of neutrophils is an evolutionary adaptation. The short lifetime of neutrophils minimizes propagation of those pathogens that parasitize phagocytes because the more time such parasites spend outside a host cell, the more likely they will be destroyed by some component of the body's defence. Also, because neutrophil antimicrobial products can also damage host tissues, their short life limits damage to the host during inflammation (Wheater *et al.*, 2002).

Neutrophils undergo a process called chemotaxis, which allows them to migrate toward sites of infection or inflammation. Neutrophils have a variety of specific receptors, including complement receptors, cytokine receptors for interleukins and interferon gamma (IFN-gamma), receptors for chemokines, receptors to detect and adhere to endothelium, receptors for leptins and proteins, and Fc receptors for opsonin (Charles *et al.*, 2010).

Being highly motile, neutrophils quickly congregate at a focus of infection, attracted by cytokines expressed by activated endothelium, mast cells, and macrophages. Neutrophils express and release cytokines, which in turn amplify inflammatory reactions by several other cell types (Ear and McDonald, 2008).

In addition to recruiting and activating other cells of the immune system, neutrophils play a key role in the front-line defence against invading pathogens. Neutrophils have three strategies for directly attacking micro-organisms: phagocytosis (ingestion), release of soluble anti-microbials (including granule proteins), and generation of neutrophil extracellular traps (NETs) (Hickey and Kubes, 2009).

Neutrophils are phagocytes, capable of ingesting microorganisms or particles. For targets to be recognised, they must be coated in opsonins—a process known as antibody opsonization (Segal, 2005). They can internalize and kill many microbes, each phagocytic event resulting in the formation of a phagosome into which reactive oxygen species and hydrolytic enzymes are secreted. Neutrophils also release an assortment of proteins in three types of granules by a process called degranulation. The contents of these granules have antimicrobial properties, and help combat infection.

In 2004, Brinkmann and colleagues described a striking observation that activation of neutrophils causes the release of web-like structures of DNA; this represents a third mechanism for killing bacteria (Brinkmann, *et al.*, 2007). These NETs comprise a web of fibers composed of chromatin and serine proteases that trap and kill microbes extracellularly. It is suggested that NETs provide a high local concentration of antimicrobial components and bind, disarm, and kill microbes independent of phagocytic uptake. In addition to their possible antimicrobial properties, NETs may serve as a physical barrier that prevents further spread of

pathogens. Trapping of bacteria may be a particularly important role for NETs in sepsis, where NET are formed within blood vessels (Clark *et al*., 2007).

An abnormally high number of neutrophils circulating in the blood is called neutrophilia. This condition is typically associated with acute inflammation, though it may result from chronic myelogenous leukemia, a cancer of the blood-forming tissues. An abnormally low number of neutrophils is called neutropenia. This condition can be caused by various inherited disorders that affect the immune system as well as by a number of acquired diseases, including certain disorders that arise from exposure to harmful chemicals. Neutropenia significantly increases the risk of life-threatening bacterial infection.

Low neutrophil counts are termed neutropenia. This can be congenital (genetic disorder) or it can develop later, as in the case of aplastic anaemia or some kinds of leukemia. It can also be a side-effect of medication, most prominently chemotherapy. Neutropenia makes an individual highly susceptible to infections. Neutropenia can be the result of colonization by intracellular neutrophilic parasites.

In alpha 1-antitrypsin deficiency, the important neutrophil enzyme elastase is not adequately inhibited by alpha 1-antitrypsin, leading to excessive tissue damage in the presence of inflammation – the most prominent one being pulmonary emphysema.

2.16.6 Platelets

The blood platelets, also called thrombocytes are tiny, irregular cell fragments without nucleus. They are fewer in number and smaller in size than the red blood cells, i.e., 250,000 – 400,000 per mm^2 of human blood (Michael, 2008). Platelets assist in blood clotting. During normal blood clotting, the platelets clump together (aggregate). Although platelets are often classed as blood cells, they are actually fragments of large bone marrow cells called megakaryocytes.

2.16.7 Eosinophils

Eosinophils are produced in the bone marrow. They are usually seen in fewer numbers than neutrophils. They are normally about 1% to 4% of the white blood

cells. Eosinophils can increase in response to allergic disorders, inflammation of the skin and parasitic infections. They can also increase in response to bone marrow disorders. Decreased levels of eosinophils can occur as result of stress, steroid pressure and anything that may suppress white blood cell production.

2.16.8 Monocytes

Monocytes have the ability to engulf foreign materials such as infectious organisms. Often times, they are also called macrophages. They develop and are stored in the spleen and bone marrow. Monocytes are also known to secrete various protein molecules that help in the cleanup of inflamed tissues. Their level can increase in response to infection and inflammatory disorders.

2.17 Biochemical Parameters

The biochemical parameters include: total protein, albumin, globulin, aspartate amino-transferase, alanine amino-transferase, blood urea nitrogen and creatinine

2.17.1 Total Protein

Proteins are important building blocks of all cells and tissues; they are important for body growth, development, and health. They form the structural part of most organs and make up enzymes and hormones that regulate body functions. This test measures the total amount of the various types of proteins in the plasma portion of your blood (Burtis *et al*., 2006).

There are two classes of proteins, albumin and globulin, found in the blood. Albumin is a carrier of many small molecules, but its main purpose is to keep fluid from leaking out of blood vessels through osmotic pressure. Globulin proteins include enzymes, antibodies, and more than 500 other proteins. The ratio of albumin to globulin (A/G ratio) is calculated from values obtained by direct measurement of total protein and albumin. It represents the relative amounts of albumin and globulins.

Total protein measurements can reflect nutritional status and may be used to screen for and help diagnose kidney disease, liver disease, and many other conditions.

Sometimes conditions are first detected with routine testing before symptoms have begun to appear. If total protein is abnormal, further tests must be performed to identify which specific protein is abnormally low or high so that a specific diagnosis can be made.

Low total protein levels can suggest a liver disorder, a kidney disorder, or a disorder in which protein is not digested or absorbed properly. Low levels may be seen in severe malnutrition and with conditions that cause malabsorption, such as Celiac disease or inflammatory bowel disease (IBD).

High total protein levels may be seen with chronic inflammation or infections such as viral hepatitis or HIV. They may be caused by bone marrow disorders such as multiple myeloma (Clarke and Dufour, 2006).

2.17.2 Albumin

The albumins (formed from Latin: albumen "(egg) white; dried egg white") are a family of globular proteins, the most common of which is serum albumin. The albumin family consists of all proteins that are water-soluble, are moderately soluble in concentrated salt solutions, and experience heat denaturation. Albumins are commonly found in blood plasma, and are unique from other blood proteins in that they are not glycosylated. Substances containing albumins, such as egg white, are called albuminoids. A number of blood transport proteins are evolutionarily related, including serum albumin, alpha-fetoprotein, vitamin D-binding protein and afamin (Lichenctein *et al.*, 1994).

Albumin is the main protein of human plasma.It binds water, cations (such as Ca^{2+}, Na^{+} and K^{+}), fatty acids, hormones, bilirubin, thyroxine (T4) and drugs (including barbiturates) - its main function is to regulate the colloidalosmotic pressure of blood. Alpha-fetoprotein (alpha-fetoglobulin) is a fetal plasma protein that binds various cations, fatty acids and bilirubin. Vitamin D-binding protein binds to vitamin D and its metabolites, as well as to fatty acids. The biological role of afamin (alpha-albumin) has not yet been characterised. Serum albumin is the most abundant blood plasma protein and is produced in the liver and forms a large proportion of all

plasma protein. The human version is human serum albumin, and it normally constitutes about 50% of human plasma protein (Farrugia, 2010).

Serum albumins are important in regulating blood volume by maintaining the osmotic pressure (also known as colloid osmotic pressure) of the blood compartment. They also serve as carriers for molecules of low water solubility this way isolating their hydrophobic nature, including lipid soluble hormones, bile salts, unconjugated bilirubin, free fatty acids (apoprotein), calcium, ions (transferrin), and some drugs like warfarin, phenobutazone, clofibrate & phenytoin. For this reason, it's sometimes referred as a molecular "taxi". Competition between drugs for albumin binding sites may cause drug interaction by increasing the free fraction of one of the drugs, thereby affecting potency.

Specific types include:

- human serum albumin
- bovine serum albumin (cattle serum albumin) or BSA, often used in medical and molecular biology labs.

Low albumin (hypoalbuminemia) may be caused by liver disease, nephrotic syndrome, burns, protein-losing enteropathy, malabsorption, malnutrition, late pregnancy, artefact, genetic variations and malignancy.

High albumin (hyperalbuminemia) is almost always caused by dehydration. In some cases of retinol (Vitamin A) deficiency the albumin level can be elevated to high-normal values (e.g., 4.9 g/dL). This is because retinol causes cells to swell with water (this is also the reason too much Vitamin A is toxic) (Gaull *et al*., 1984). In lab experiments it has been shown that All-trans retinoic acid down regulates human albumin production. Normal range of human serum albumin in adults is 3.5 to 5 g/dL. For children less than three years of age, the normal range is broader, 2.9-5.5 g/dL (Suzuki *et al*., 2006).

2.17.3 Globulin

Globulin is one of the major classifications of proteins, which may be further divided into the eu-globulins and the pseudo-globulins. The former group is

insoluble in water but soluble in saline solutions and may be precipitated in water that has been half-saturated with a salt such as ammonium sulphate. The latter group is soluble in water and has properties that resemble those of the true globulins. Globulins are an important source of protein in seed plants and are found in minute amounts in cereals. Globulins found in animal fluids are enzymes, antibodies, and fibrous and contractile proteins usually contained in the blood plasma.

Globulins can also be measured quantitively and qualitatively with electrophoresis. Radial immuno-diffusion is used for accurate quantification of immuno-globulins and has also replaced immune-electophoresis for determining the immuno-globulin comprising a monoclonal gammopathy.

Three types of globulin have been identified—alpha, beta, and gamma. Alpha and beta globulins are transport proteins, serve as substrates upon which other substances are formed, and perform other diverse functions. Gamma globulins have a vital role in natural and acquired immunity to infection. Non-human globulin proteins exist as well, such as cucurbitin from squashes and vicilin and legumin from legumes and peas, functioning as protein storage within seeds. These proteins can cause allergic reactions if they bind with human IgE antibodies (Sanchez-Monge *et al*, 2004).

2.17.4 Aspartate transaminase (AST)

Aspartate aminotransferase, also called Aspartate transaminase or serum glutamic oxaloacetic transaminase (SGOT), is a pyridoxal phosphate (PLP)-dependent transaminase enzyme). AST catalyzes the reversible transfer of an α-amino group between aspartate and glutamate and, as such, is an important enzyme in amino acid metabolism. AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells, and it is commonly measured clinically as a marker for liver health.

Aspartate transaminase, as with all transaminases, operates via dual substrate recognition; that is, it is able to recognize and selectively bind two amino acids (Asp and Glu) with different side-chains (Hirotsu *et al*., 2005). AST is similar to alanine transaminase (ALT) in that both enzymes are associated with liver parenchymal

cells. The difference is that ALT is found predominantly in the liver, with clinically negligible quantities found in the kidneys, heart, and skeletal muscle, while AST is found in the liver, heart (cardiac muscle), skeletal muscle, kidneys, brain, and red blood cells. As a result, ALT is a more specific indicator of liver inflammation than AST, as AST may be elevated also in diseases affecting other organs, such as myocardial infarction, acute pancreatitis, acute hemolytic anemia, severe burns, acute renal disease, musculoskeletal diseases, and trauma.

AST was defined as a biochemical marker for the diagnosis of acute myocardial infarction in 1954. However, the use of AST for such a diagnosis is now redundant and has been superseded by the cardiac troponins (Gaze, 2007). AST (SGOT) is commonly measured clinically as a part of diagnostic liver function tests, to determine liver health. Female range is 6 – 34 IU/L and the male range is 8 – 40 IU/L

2.17.5 Alanine transaminase (ALT)

Alanine transaminase (ALT) is an enzyme found primarily in the liver and kidney. It was originally referred to as serum glutamic pyruvic transaminase (SGPT). ALT was formerly called serum glutamic pyruvic transaminase (SGPT). The ALT test measures the amount of alanine aminotransferase (an enzyme found in liver cells) in blood. This test is used to evaluate and manage diseases or injury to the liver (Prati *et al.*, 2002). The reference range for ALT is 20-60 IU/L. Normally, a low level of ALT exists in the serum. ALT is increased with liver damage and is used to screen for and/or monitor liver disease. Alanine aminotransferase (ALT) is usually measured concurrently with AST as part of a liver function panel to determine the source of organ damage

ALT is measured to see if the liver is damaged or diseased. Low levels of ALT are normally found in the blood. But when the liver is damaged or diseased, it releases ALT into the bloodstream, which makes ALT levels go up. Most increases in ALT levels are caused by liver damage. The ALT test is often done along with other tests that check for liver damage, including aspartate aminotransferase (AST), alkaline phosphatase, lactate dehydrogenase (LDH), and bilirubin. Both ALT and AST levels are reliable tests for liver damage.

2.17.6 Blood Urea Nitrogen (BUN)

Blood urea nitrogen (BUN) measures the amount of urea nitrogen, a waste product of protein metabolism, in the blood. Urea is formed by the liver and carried by the blood to the kidneys for excretion. Because urea is cleared from the bloodstream by the kidneys, a test measuring how much urea nitrogen remains in the blood can be used as a test of renal function. However, there are many factors besides renal disease that can cause BUN alterations, including protein breakdown, hydration status, and liver failure.

Reference values for BUN are:

- Adult: 7-20 mg/100 ml; men may have slightly higher values than women
- Pregnancy: values decrease about 25%
- Newborn: values slightly lower than adult ranges
- Elderly: values may be slightly increased due to lack of renal concentration

An increase in the BUN level is known as azotemia. An elevated BUN may be caused by impaired renal function, congestive heart failure as a result of poor renal perfusion, dehydration, shock, haemorrhage into the gastrointestinal tract, acute myocardial infarction, stress and excessive protein intake or protein catabolism.

Diseased or damaged kidneys cause an elevated BUN because the kidneys are less able to clear urea from the bloodstream. In conditions in which renal perfusion is decreased, such as hypovolemic shock or congestive heart failure, BUN levels rise. A patient who is severely dehydrated may also have a high BUN due to the lack of fluid volume to excrete waste products. Because urea is an end product of protein metabolism, a diet high in protein, such as high-protein tube feeding, may also cause the BUN to increase. Extensive bleeding into the gastrointestinal tract (GIT) will also cause an elevated BUN because digested blood is a source of urea. For example, a haemorrhage of one litter of blood into the GIT may elevate the BUN up to 40 mg/ml.

A decreased BUN may be seen in liver failure malnutrition, anabolic steroid use, over hydration, which can result from prolonged intravenous fluids pregnancy (due

to increased plasma volume), impaired nutrient absorption, Syndrome of Inappropriate Anti-Diuretic Secretion (SIADH).

Because urea is synthesized by the liver, severe liver failure causes a reduction of urea in the blood. Just as dehydration may cause an elevated BUN, over hydration causes a decreased BUN. When a person has "syndrome of inappropriate anti-diuretic secretion" (SIADH), the anti-diuretic hormone responsible for stimulating the kidney to conserve water causes excess water to be retained in the bloodstream rather than being excreted into the urine. SIADH can cause the BUN level, along with other important substances, to decrease because the fluid volume of the bloodstream may significantly increase.

2.17.7 Creatinine

Serum creatinine (a blood measurement) is an important indicator of renal health because it is an easily-measured by-product of muscle metabolism. Creatinine itself is an important biomolecule because it is a major by-product of energy usage in muscle, via a biological system involving creatine, phosphocreatine (also known as creatine phosphate), and adenosine triphosphate (ATP, the body's immediate energy supply).

Creatinine is primarily synthesized in the liver from the methylation of glycocyamine (guanidino acetate, synthesized in the kidney from the amino acidsarginine, glycine, and methionine) by S-Adenosyl methionine. It is then transported through blood to the other organs, muscle, and brain where, through phosphorylation, it becomes the high energy compound phosphocreatine. During the reaction Creatine, phosphocreatine, catalyzed by Creatine kinase, spontaneous conversion to creatinine may occur(Allen, Patricia J.,2012)

Creatinine is chiefly filtered out of the blood by the kidneys (glomerular filtration and proximal tubular secretion). There is little or no tubular reabsorption of creatinine. If the filtering of the kidney is deficient, creatinine blood levels rise. Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance (CrCl), which reflects the glomerular filtration rate (GFR). The GFR is clinically important because it is a measurement of renal function. However,

in cases of severe renal dysfunction, the creatinine clearance rate will be "overestimated" because active secretion of creatinine from the proximal tubule will account for a larger fraction of the total creatinine cleared. Ketoacids, cimetidine and trimethoprim reduce creatinine tubular secretion and therefore increase the accuracy of the GFR estimate, particularly in severe renal dysfunction. (In the absence of secretion, creatinine behaves like insulin).

A more complete estimation of renal function can be made when interpreting the blood (plasma) concentration of creatinine along with that of urea. BUN-to-creatinine ratio (the ratio of blood urea nitrogen to creatinine) can indicate other problems besides those intrinsic to the kidney; for example, a urea level raised out of proportion to the creatinine may indicate a pre-renal problem such as volume depletion. One-percent to two-percent of muscle creatinine is converted to creatinine each day. Men tend to have higher levels of creatinine than women because they generally have a greater mass of skeletal muscle. Increased dietary intake of creatinine or eating a lot of meat can increase daily creatinine excretion (Taylor, 1989).

A rise in blood creatinine level is observed only with marked damage to functioning nephrons. Therefore, this test is unsuitable for detecting early-stage kidney disease. A better estimation of kidney function is given by the creatinine clearance (CrCl) test. Creatinine clearance can be accurately calculated using serum creatinine concentration and some or all of the following variables: sex, age, weight and race, as suggested by the American Diabetes Association without a 24-hour urine collection (Gross *et al*., 2005). Creatinine concentration is also checked during standard urine drug tests. Normal creatinine levels indicate the test sample is undiluted, whereas low amounts of creatinine in the urine indicate either a manipulated test or low individual baseline creatinine levels. Test samples considered manipulated due to low creatinine are not tested, and the test is sometimes considered failed.

Diluted samples may not always be due to a conscious effort of subversion,and diluted samples cannot be proved to be intentional, but are only assumed to be. Random urine creatinine levels have no standard reference ranges. They are usually used with other tests to reference levels of other substances measured in the urine.

CHAPTER THREE

METHODOLOGY

3.1 Purchase of the Plants

Onion bulbs and cloves of garlic were purchased from a well-known store at Bodija Market, Ibadan North Local Government, Ibadan, Oyo State. They were cultivated without fertilizers. They were taken to The Department of Botany for identification. The onion bulbs and garlic cloves were respectively identified as:

1. *Allium cepa*
2. *Allium sativum*

3.2 Identification and Authentication of Plant Samples

Cloves of garlic and bulbs of onions were planted as shown in Plate 3.1, to observe their floral parts. These plants were then taken to Forestry Research Institute of Nigeria (FRIN), Ibadan in Oyo State, together with fresh cloves of garlic and onion bulbs for their identification and virtual number. The virtual Number given to *Allium cepa* was 109539 while *Allium sativum* was given 109538.

Plate 3.1 *Allium cepa* and *Allium sativum* Plants

3.3 Extraction Process

The extraction process was done at the Institute of Medical Research and Training (IMRAT), College of Medicine, University of Ibadan, Ibadan. One kilogram (1kg) each of selected onion bulbs and garlic cloves were weighed using the laboratory scale. The alliums were peeled and crushed differently using one litre of distilled water to squeeze out the juice. After crushing, two different muslin clothes were used (one for each sample) to filter the residue from the filtrate. The filtrates were collected into two separate white plastic containers and then taken to the International Institute of Tropical Agriculture (IITA), Ibadan for freeze-drying.

3.4 Lyophilization

Freeze-drying or lyophilisation was done at the International Institute of Tropical Agriculture (IITA), Ibadan. The samples were taken to IITA in liquid form after the extraction process. A litre was made each from the crush of garlic and onion samples.

The aqueous extract of onions and garlic were placed on the shelves when they were still unfrozen. When the chamber was sealed and the Freeze-drying process began, the machine ran the compressors to lower the temperature in the chamber. Next, the machine turned the vacuum pump to force air out of the chamber, lowering the atmospheric pressure below 0.06 atm. The heating units applied a small amount of heat to the shelves, causing the ice to change phase. Since the pressure is so low, the ice turned directly into water vapour. The water vapour was released out of the Freeze-drying chamber, past the freezing coil. The water vapour condensed onto the freezing coil into solid ice form, in the same way water condenses as frost on a cold day. This continued for several days while the onion and garlic extracts gradually dried out. Additionally, accelerating the sublimation process could produce more water vapour in a period of time when the pumping system can remove from the chamber, thereby degrading the quality of the lyophilized products.

Once the aqueous extracts were dried sufficiently, they were sealed differently in a moisture-free package with an oxygen-absorbing material. This process prepared the onions and garlic extract for stock preparation to be administered to the rats.

3.5 Preparation of the Stock and Working Concentration

3.5.1 Stock Preparation

Arsenic trioxide (As_2O_3), onion and garlic extracts (30 mg each) were dissolved differently in 1000ml, that is, one litre of distilled water. From this preparation, 100 ml was measured to make 1:10 dilution. This made the final stock preparation to be 3 mg/100ml each of the toxin (As_2O_3) and both extracts. These preparations were made separately in plastic bottles and test tubes. This was done to avoid cross reaction with glass bottles and test tubes. Also, the plastic bottle containing As_2O_3 was wrapped using aluminium foil paper to prevent the penetration of white light into the solution which can initiate various chemical reactions before the administration of the drugs to the animals.

3.5.2 Working Concentration

From the stock, working concentration of the solutions were prepared daily using the 1:10 dilution. This was achieved by measuring 1 ml of the stock preparation of As_2O_3, onion and garlic extract in a sterile plastic bottle already containing 9 ml distilled water.

Before the daily administration of drugs, the rats were weighed. Their individual weight determined the concentration to be administered to each rat. A total of 1ml of the solution was administered to each rat. This 1ml was achieved by giving the rats equivalent volume of their daily weight and equivalent amount of distilled water. For example, a rat weighing 200 g was administered 200 µml of As_2O_3, onion or garlic extract and 800 µml of distilled water. That is for a 200 g rat, 200 µml and 800 µml, making 1000 µml (1 ml) was administered.

3.6 Experimental Animals

Three weeks old Wistar strain male albino rats were obtained from the animal house of the Zoology Department, University of Ibadan. A total of thirty-five (35) rats were randomly selected and kept in cages in the quarantine section at IMRAT building. The rats acclimatized for a period of three weeks before the commencement of the experiment. The rats were distributed randomly into seven groups with five (5) rats each and were fed with commercial rat pellet (ad-libitum)

and distilled water to acclimatise to the environment, they were kept in a metal cage with treated wood at room temperature. The experiment was a dose-toxicity study in which the aqueous extracts of onion and garlic were prepared in different concentrations and administered to the rats orally. Treatments were administered by gavage while maintaining the rats on commercial rat pellets and water *ad libitum* for 20 days. In addition to the baseline body weight, weights of the rats, feed and water intake were recorded daily throughout the experiment. On day 20, the rats were sacrificed after which blood, liver, kidney, brain, testes and spleen were removed for biochemical, haematological and histopathological examinations.

Plate 3.2: Animal Cage Showing Five Rats in a Group

3.7 Administration of Arsenic Trioxide and the Extracts

The materials used included the following:

i. Plastic test tubes
ii. Single pipette aid
iii. Test tube rack/holder
iv. Beaker
v. Conical flask
vi. 1ml syringe
vii. Cannula
viii. Distilled water
ix. Aluminium foil paper
x. Cotton wool

In summary, the treatment was given to each group as follows:

GROUP 1: Rats were administered with distilled water only. This will serve as the positive control.

GROUP 2: Rats were given 3 mg/kg/day of Arsenic trioxide only with dose equivalent amount of distilled water.

GROUP 3: Rats were given 3 mg/kg/day of aqueous onion extract with dose equivalent amount of distilled water.

GROUP 4: Rats were given 3 mg/kg/day aqueous garlic extract with dose equivalent amount of distilled water.

GROUP 5: Rats were given 3 mgkg/day of aqueous onion extract and Arsenic trioxide with dose equivalent amount of distilled water.

GROUP 6: Rats were given 3 mgkg/day of aqueous garlic extract and Arsenic trioxide with dose equivalent amount of distilled water.

GROUP 7: Rats were given 3 mgkg/day of aqueous onion and garlic extract with dose equivalent amount of distilled water.

3.8 Identification Operation Strategy

Each rat across the groups were marked at easily notable parts of the body, using an indelible liquid (Picric acid) for ease of identification.

3.9 Experimental Procedure

3.9.1 Procedure for the Administration of Arsenic Trioxide and Allium Extracts

The animals were grabbed from the tail and the flesh of the back neck was held tight with the left hand and turned upwards with its limbs hanging up and the tail tucked between the hollow of the left hand. This is shown in Plate 3.3. A clear passage to the throat was sought before the drug was administered. Volume and concentration of As_2O_3 and plant extracts administered were based on the weight of individual rat. Before the daily drug administration, physical (i.e. average change in body weight, feed intake and water intake) and physiological parameters (i.e. agility, fur colour, nasal discharge and ocular lesion) were observed and noted. This procedure was followed for 19 days. The labelling was as follows:

Group 1: Control

Group 2: AS only

Group 3: AC only

Group 4: ASAT only

Group 5: AC + AS

Group 6: ASAT + AS

Group 7: AC + ASAT

Where Control: Distilled water only

ARS: Arsenic trioxide (As_2O_3)

AC: *Allium cepa*

ASAT: *Allium sativum*

Plate 3.3: Administration of Treatments by the Researcher

3.9.2 Observations

Various observations of the rats in each experimental group was made each day to record changes in

1. Body weight
2. Amount of feed intake
3. volume of water intake
4. Physical characteristics

3.10 Collection of Blood Samples

The rats were fasted for 24 hours before the blood samples were collected. Using capillary tubes, blood samples were collected from each lively rat separately into EDTA bottles using the ocular puncture method. The samples were taken for haematological and biochemical analysis.

3.11 Collection of Tissues

After the collection of blood samples, the animals were sacrificed. This was carried out through the Cervical Dislocation Method. This was achieved by holding the rats at the neck and tail and stretched until the spine dislocates from the neck. Each rat was dissected and five organs were removed from each rat. The organs were kidney, liver, brain, testes and spleen. Histopathological examinations were carried out on these organs.

3.12 Determination of Haematological Parameters

The haematological parameters included Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC), Platelet, Haemoglobin, lymphocytes, Neutrophils, monocytes and Eosinophil.

3.12.1 Preparation of RBC Dilution

RBC diluting fluid (4 ml) was placed into a Bijou bottle. The blood sample was mixed by inverting approximately 20 times (vigorous agitation was avoided). 20 µl pipette having a disposable tip was used to draw the blood and expelled into the bottle containing 4 ml of diluting fluid. The bottle contents were mixed by inversion thereby having a dilution of the blood sample to be 1:200 (Maff, 1984).

3.12.2 Preparation of WBC Dilution

0.95 ml of WBC diluting fluid was placed into a Bijou bottle. The blood sample was mixed by inverting approximately 20 times (vigorous agitation was avoided). 50 µl pipette having a disposable tip was used to draw the blood and expelled into the bottle containing 4ml of diluting fluid. The bottle contents were mixed by inversion thereby having a dilution of the blood sample to be 1:20 (MAFF, 1984)

3.12.3 Method for Counting Blood Cells.

The haemocytometer and cover glass were thoroughly cleaned, ensuring that both are free of grease. The haemocytometer was placed on a flat horizontal surface, and using a firm pressure, the cover-glass was placed over the ruled counting areas. A rainbow effect (Newton's rings) was observed on both sides. The counting chamber was placed on the microscope stage and allowed 2 minutes to elapse before commencing the count; this allowed the cells to settle. Using a high dry objective and the *10 eyepiece, the center square millimetre (ABCD) of the ruled area was focused on and all the cells contained in 80 of the 400 small squares were counted (MAFF, 1984)

RBC Calculation was given as follows:

Number of cells counted * depth *dilution *area

WBC Calculation was given as follows:

Average number of cells counted per square mm * depth * dilution

3.12.4 Determination of Packed Cell Volume (PCV)

Blood sample was thoroughly mixed by inverting about 20 times. Using a capillary pipette, the Wintrobe Haematocrit was filled to the 10 mark. This was centrifuged at 3000 r. p. m. for 30 minutes. The blood sample was removed from the centrifuge and the height of the red cell column was noted. The tube was divided into 100 divisions; the height of the column of red cells was read off and was expressed as a fraction of whole blood.

3.12.5 Determination of Haemoglobin Using Cyanmethaemoglobin Method.

The blood sample was mixed by inverting approximately 20 times (vigorous agitation was avoided). Using 0.02 ml pipette, 0.02 ml of blood was removed and the tip of the pipette was wiped neatly. This was washed into 5 ml Drabkin's solution and mixed well. After standing for 10 minutes, the absorbance was read using a colorimeter at 540nm with a tube of Drabkin's solution as a blank. (Maff, 1984). The standard solution of cyanmethaemoglobin.the Haemoglobin was calculated as follows:

(Reading of test/reading of Standard) * Concentration of standard * dilution = g/dl

3.12.6 Platelet Counts

The platelet count fluctuates several times a day, while exercise, fatigue, and temperature all affect the count, resulting in thrombocytopenia (or thrombocytosis).

The diluent solution prepared contained 3.8 g Sodium citrate, 0.2 ml of 40% formaldehyde, 0.05g Brilliant cresol blue and 100 ml distilled water.

The blood sample was collected with a syringe and transferred to an EDTA tube. Blood (20 µl)was diluted using 1.98 ml diluent. After thorough mixture, the suspension was left for several hours but mixed at least 2 minutes. A counting chamber was filled with a Pasteur pipette and placed in a moist chamber and left for at least 20 minutes to allow the platelets to settle (MAFF, 1984).

3.13 Determination of Biochemical Parameters

The biochemical parameters such as total protein, albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), BUN and creatinine were determined using the spectrophotometer (SM 23A). Other materials used included, centrifuge, micro haematocrit reader, Neubaur Counting Chamber, Capillary tubes, microscope slides, cover slip, cotton wool, beaker, conical flasks and tap water.

3.13.1 Total Protein

The reagents used were 45.0g Potassium sodium tartrate, 15.0g Cupric sulphate, 5.0g Potassium iodide and 0.2M Sodium hydroxide. The tartrate was dissolved in approximately 400ml of 0.2 M Sodium hydroxide. While stirring, the cupric sulphate was added. Potassium iodide was added and dissolved into 1000ml of 0.2ml sodium hydroxide. 200µl, 5.0ml each of saline, Biuret reagent and Blank diluent were pipetted into test tubes. All tubes were mixed and placed in a water bath at 37^0C for 15 minutes. The Optical Density (O.D) of the samples were measured against the reagent blank using the Spectrophotometer at 565 nanometer (Weichselbaum, 1946).

Total Protein Concentration (g/l):

(O. D. of Sample/O. D. Standard) * Concentration of Standard *10

3.13.2 Alanine transaminase (ALT)

Alanine Aminotransferase was measured by monitoring the concentration of pyruvate hydrazine formed with 2, 4-dinitrophenylhydrazine (Reitman *et al*., 1957). The reagents used were phosphate buffer (R1) and 2, 4-dinitrophenylhydrazine (R2) 0.1ml 0f the serum, 0.5ml of R1 and 0.1ml of distilled water were transferred into test tubes. After proper mixing, the mixture was incubated for exactly 30 minutes at 37^0C. 0.5ml of R2 was added, mixed and allowed to stand for exactly 20 minutes at 20^0C. 5.0ml Sodium hydroxide was then added. After 5 minutes, the absorbance of the sample was read against the blank using the Spectrophotometer at 505 nanometer (Schmidt and Schmidt, 1963).

3.13.3 Creatinine (CREAT)

Creatinine is derived from creatine and creatine phosphate in muscle tissue and may be defined as a nitrogenous waste product. Creatinine is not reutilised but is excreted from the body in the urine via the kidney. It is produced and excreted at a constant rate which is proportional to the body muscle mass. As a consequence of the way in which creatinine is excreted by the kidney, creatinine measurement is used almost exclusively in the assignment of kidney function. Creatinine is regarded as the most useful endogenous marker in the diagnosis and treatment of kidney disease.

Creatinine is measured primarily to assess kidney function and has certain advantages over the measurement of urea. The plasma level of creatinine is relatively independent of protein ingestion, water intake, rate of urine production and exercise. Since its rate of production is constant, elevation of plasma creatinine is indicative of under-excretion, suggesting kidney impairment. Depressed levels of plasma creatinine are rear and not clinically significant.

In this research, the reagents used were Picric acid, Sodium Hydroxide and the standard. 2.0ml of the working reagent, 0.2 ml of standard solution and 0.2 ml of the sample was pipetted into the cuvette. After 30 seconds, the absorbance of the sample and standard was read at 540 nanometer

Concentration of Creatinine

(Sample/Standard) *Standard concentration (µmol/l)

3.13.4 Aspartate transaminase (AST)

AST was measured by monitoring the concentration of oxaloacetate hydrazine formed with 2, 4-dinitrophenylhydrazine. The reagents used were buffer (R1) and 2, 4 dinitrophenylhydrazine (R2). 0.1 ml of blood serum, 0.5ml of R1 and 0.1ml of distilled water were pipetted in test tubes, mixed and incubated for exactly 30 minutes at 37^0C. R2 was later added mixed and allowed to stand for 20 minutes at 20^0C. 5.0ml of Sodium Hydroxide was added and after 5 minutes, the absorbance of the sample was read against blank at 505 nanometer (Schmidt and Schmidt, 1963).

3.13.5 Albumin (ALB)

Albumin is the most abundant serum protein representing 55 – 65% of the total protein. It is synthesized in the liver and has a half-life of 2 to 3 weeks. The main biological functions of albumin are to maintain the water balance in serum and plasma and to transport and store a wide variety of ligands e.g. fatty acids, calcium, bilirubin and hormones such as thyroxine. Albumin also provides an endogenous source of amino acids. Hypoalbuminaemia is associated with the following conditions: analbuminaemia; impaired albumin synthesis in the liver; liver disease; malnutrition or malabsorption; generalised shock, burns and dermatitis;kidney

disease and intestinal disease. Hyperalbiminaemia has little diagnostic relevance except, perhaps in dehydration (Grant, 1987).

The reagents involved were BCG Concentrate and Albumin Standard. 0.01ml of distilled water, Standard and serum were pipetted into test tubes with 3.00ml of BCG reagent. The mixture was incubated for 5 minutes at 25^0C. The absorbance of the sample and of the standard were measured against the reagent blank at 640 nanaometer.

The Albumin Concentration was calculated in g/l as (Sample/standard) * Concentration of Standard.

3.14 Determination of Histopathological Parameters

Histopathology is the examination of various body organs to determine the damage done by the toxins to the tissues. Histopathological examination was done on five organs taken from each rat. The organs were kidney, liver, brain, spleen and testes. The processes involved were, grossing (macroscopy), sectioning and trimming of tissues, tissue processing and embedding. Rotary Microtonne (Histoline Laboratories, Model, MR 2258) was used for the sectioning and trimming of tissues. SAKURA Rotary Tissue Tek II Tissue Processor (Model Number: 4634, Serial Number: 99070931) was used for Tissue Processing while the tissue embedding was done using embedding molds, paraffin wax, gas, burner and forceps (Avwioro, 2002).

The purpose of processing the tissues was to provide a solid support medium for tissue during section cutting. Paraffin wax was used as embedding medium which was made up of a mixture of solid hydrocarbons. The removal of water from the tissues was achieved through a process called dehydration and graded solutions of alcohol were used. The tissues had to be passed through low to high concentrations of alcohol. This was because when water molecules mixed with absolute alcohol, there was usually turbulence at point of contact, leading to the distortion of tissue constituents. Clearing is a process of removing absolute alcohol from tissue and replacing it with a solvent. Infilteration (impregnation) was another process involved in histopathology. It involved replacing a clearing agent or antemedium with molten paraffin wax. The paraffin wax clompletely displaced the clearing agent from the

tissue. Embedding was the next step which involved burying a tissue in molten paraffin wax. The paraffin wax formed a firm support medium for the tissues during microtomy. Embedding moulds were used during this process (Avwioro, 2002).

3.15 Data and Statistical Analysis

Throughout the period of this research, a daily record of body weight, feed intake and water intake were taken. The mean difference of each group between day 0 and day 19 of the administration was calculated so as to determine the weight gain in each treatment group. Also, the data from haematological and biochemical study were obtained. These data were subjected to statistical analysis using the one way ANOVA followed by the post hoc test (LSD and Duncan) for comparing control and various groups. The level of significance was taken as $p<0.05$.

CHAPTER FOUR

4.0 Results

This chapter gives a comprehensive report of the effects of the various treatment exposures on the physical parameters of the rats (such as body weight change, feed intake and water intake), biochemical parameters, haematological parameters and histopathological parameters such as cytoplasmic degeneration of the vital organs including the aggregation of inflammatory cells in the tissues.

4.1 Effects of treatment exposure on average body weight feed and water consumption

All the rats in the group administered As_2O_3 (Group 2) survived to the end of the study. When compared with the control group, the other six groups of rat showed a significant decrease in average body weight, feed and water consumption ($P< 0.05$). Rats on As_2O_3 treatment significantly had reduced average body weight change when compared with the other groups ($P< 0.05$). Table 4.1 shows the average body weight (g), feed consumption (g) and water consumption (ml) of the seven groups of rats. Table 4.2 shows the body weight change of each group while Figures 4.1 – 4.6 shows the trend in weight between Group 1 (control) and other groups.

Table 4.1: **Results of Physical Parameters**

Serial Number	Treatment Group	Weight change(g)	Feed Intake (g)	Water Intake (ml)
1	**Control**	159.86± 17.9^{e}	72.29 ± 14.7^{b}	139.44 ± 34.6^{b}
2	**AS Only**	145.40 ± 25.3^{c} *	61.80 ± 18.4^{b}	103.89 ± 44.3^{a}
3	**AC Only**	140.47 ± 29.0^{b} *	53.83 ±11.8^{a} *	96.39 ± 34.3^{a} *
4	**ASAT Only**	138.61 ±15.2^{a} *	56.66 ± 24.2^{a} *	100.56 ± 53.2^{a} *
5	**AC + AS**	151.89 ±15.6^{d} *	62.15 ± 11.8^{b}	97.50 ± 46.7a *
6	**ASAT + AS**	135.90 ±17.9^{a} *	53.53 ± 16.0^{a} *	117.78 ± 30.3^{b}
7	**AC + AS**	147.36 ± 22.3^{d} *	62.40 ± 20.7^{b}	102.78 ± 39.5^{a}

All data were mean and standard deviation of (five replicates) determinants. a, b, c, d and e are means from least to the highest. Means of the same alphabet are not significantly different.

Mean within the same column with different alphabets are significantly different at $P < 0.05$ (Duncan)

* indicates significant difference when compared with the control group (LSD)

Note: Check glossary for full meaning of abbreviations

Table 4.2: Body Weight Change (g)

Serial Number	Group	Mean Initial Weight	Mean Final Weight	Weight Change	Standard Deviation
1	**Control**	152.96±11.8	174.68±21.7	21.72	±9.9
2	**AS Only**	146.50±18.5	154.14±30.7	7.64	±12.2
3	**AC Only**	140.54±33.1	139.64±29.0	0.90	±4.1
4	**ASAT Only**	129.26±7.3	137.28±19.3	8.02	±12.0
5	**AC + AS**	148.46±18.6	152.06±13.7	3.60	±4.9
6	**ASAT+ ARS**	138.76±22.2	140.98±21.7	2.22	±0.5
7	**AC + AS**	124.56±17.5	158.80±21.1	34.24	±3.6

All data were mean and standard deviation of two replicates.

Note: Check glossary for full meaning of abbreviations.

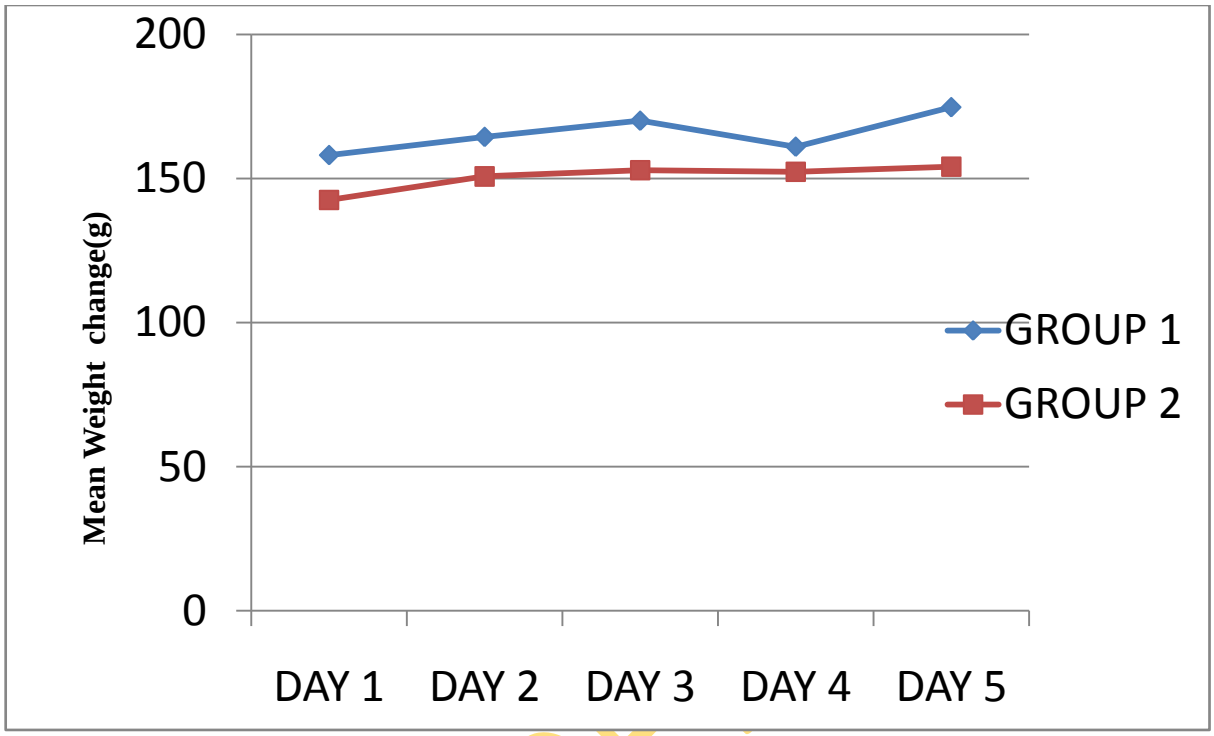

Figure 4.1: Trend in weight between group 1 and 2 within the last five days of treatment

Group 1: Rats treated with distilled water only

Group 2: Rats treated with Arsenic trioxide only

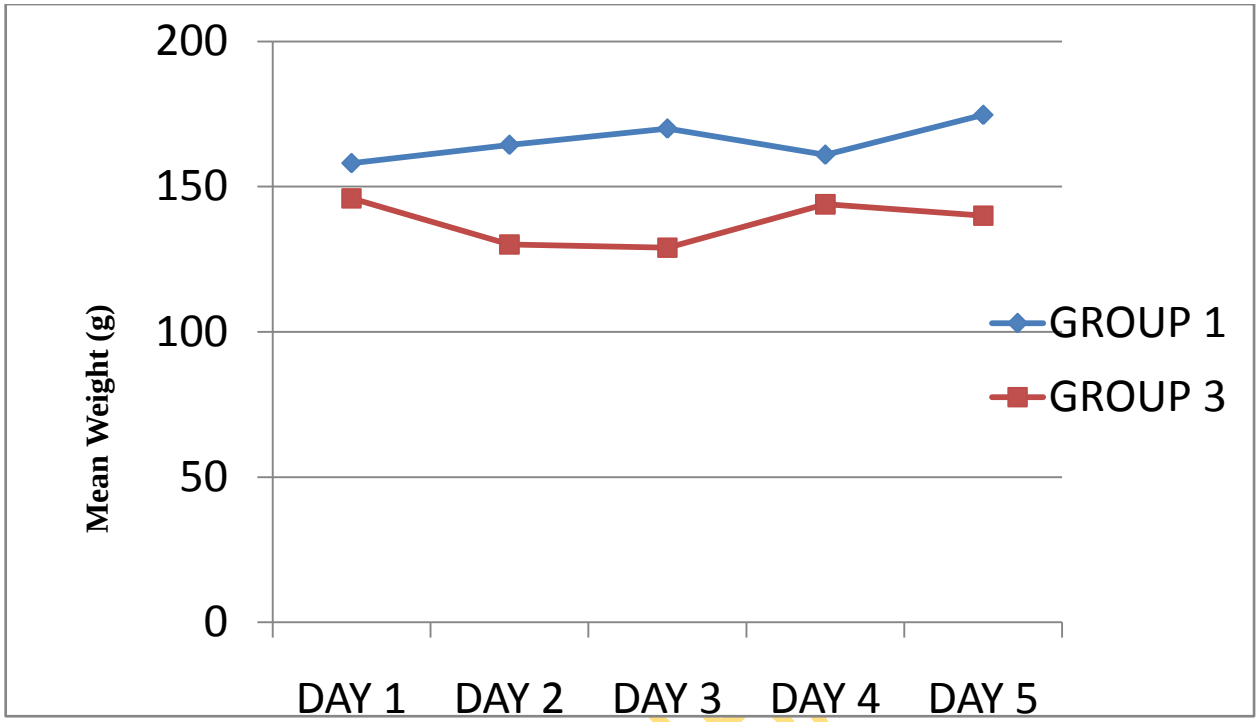

Figure 4.2: Trend in weight between group 1 and 3 within the last five days of treatment

Group 1: Rats treated with distilled water only

Group 3: Rats treated with *Allium cepa* only

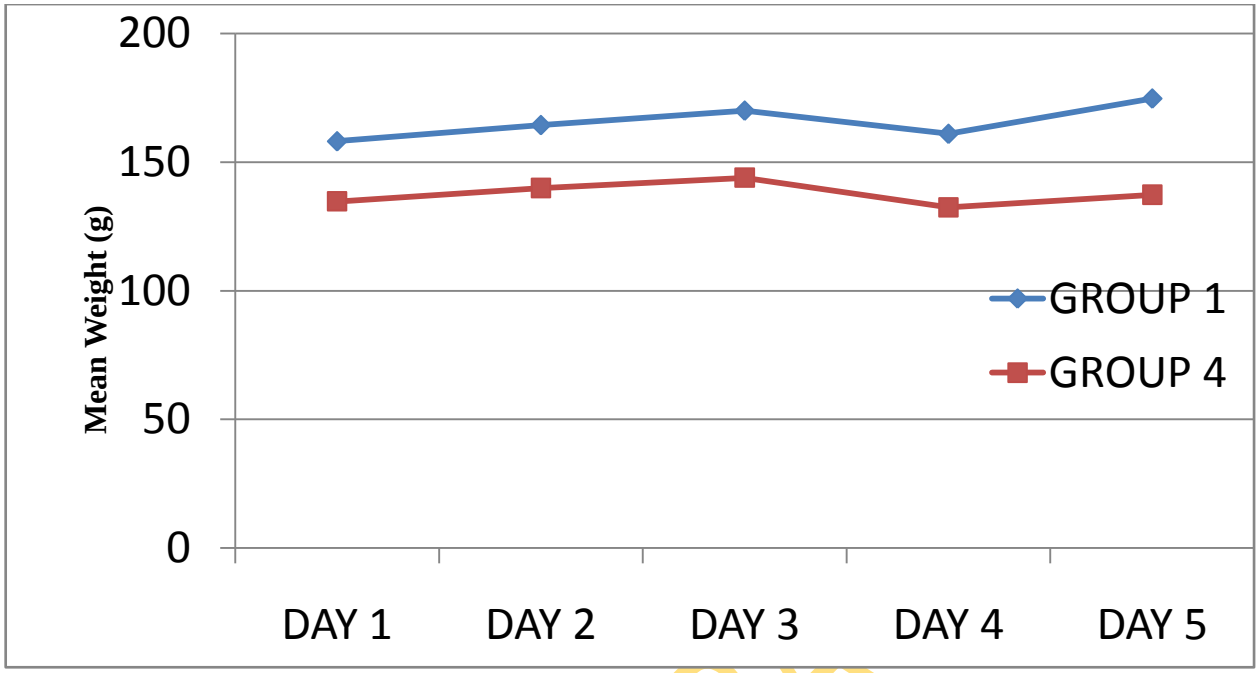

Figure 4.3: Trend in weight between group 1 and 4 within the last five days of treatment

Group 1: Rats treated with distilled water only

Group 4: Rats treated with *Allium sativum* only

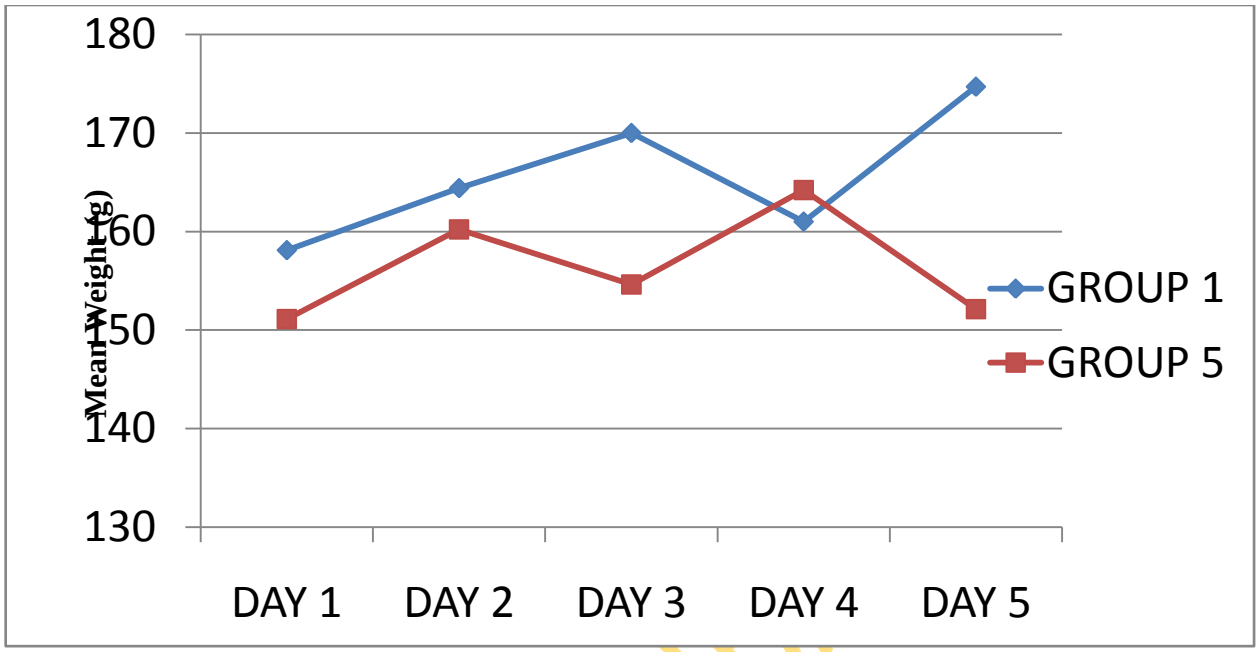

Figure 4.4: Trend in weight between group 1 and 5 within the last five days of Treatment

Group 1: Rats treated with distilled water only

Group 5: Rats treated with *Allium cepa* and Arsenic trioxide

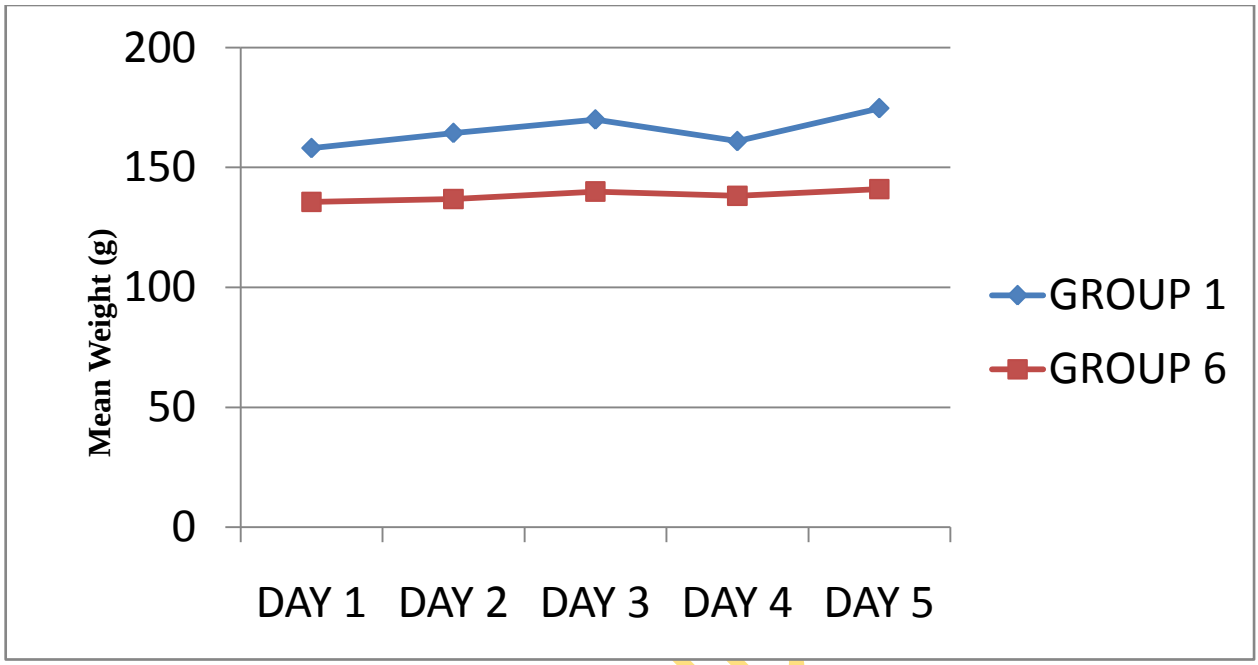

Figure 4.5: Trend in weight between group 1 and 6 within the last five days of treatment

Group 1: Rats treated with distilled water only

Group 6: Rats treated with *Allium sativum* and Arsenic trioxide

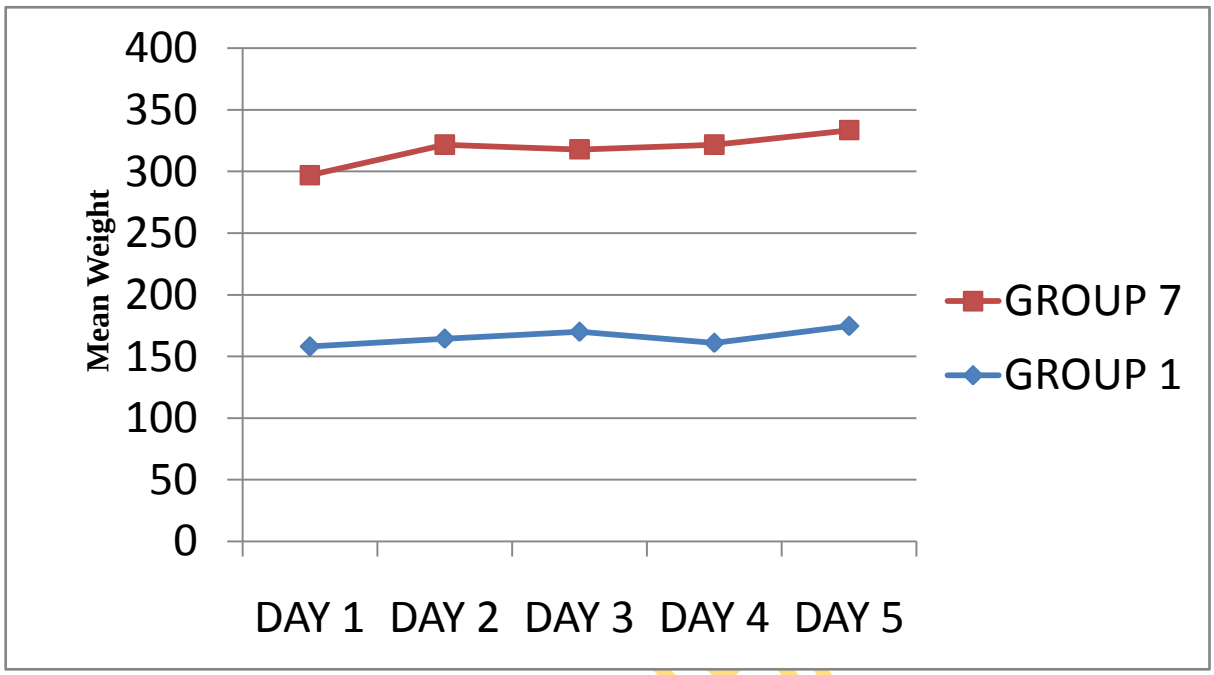

Figure 4.6: Trend in weight between group 1 and 7 within the last five days of Treatment

Group 1: Rats treated with distilled water only

Group 7: Rats treated with *Allium cepa* and *Allium sativum*

4.2 Results Obtained from Haematological Analysis

Haematological analysis was carried out on the blood samples collected from the sacrificed rats. The haematological parameters include Packed Cell Volume (PCV), Red Blood Cells (RBC), White Blood Cells (WBC), Platelets, Haemoglobin (Hb), Lymphocytes, Neutrophil, Monocytes and Eosinophils. Table 4.3 shows the result of the haematological analysis.

Table 4.3: Haematological Parameters in Blood of Rats after 19 Days

SN	Treatment group	PCV (%)	Hb (g/dl	Lymph (Cell/mm^3)	RBC (Cell/mm^3)	WBC (Cell/mm^3)	Platelet (Cells/ml)	Neutrophil (%)	Eosinophil (%)	Monocytes (%)
1	Control	47.00 ± 2.0^a	15.00 ± 0.6^a	72.33 ± 7.4^b	7.64 ± 0.3^b	7500 ± 2128.4^b	134670 ± 31628.0^a	24.67 ± 8.3^a	1.33 ± 0.6^a	1.67 ± 0.6^a
2	ARS Only	45.00 ± 3.6^a	14.33 ± 1.7^a	59.00 ± 3.6^b	7.55 ± 0.7^b	8433 ± 275.4^b	142330 ± 7094.6^a	37.33 ± 6.1^b	1.00 ± 1.0^a	2.00 ± 1.7^a
3	AC Only	47.67 ± 3.5^a	15.50 ± 1.6^a	53.67 ± 12.1^a *	7.99 ± 0.6^b	9433 ± 1373.3^b	155330 ± 68391.0^a	44.00 ± 12.5^b *	0.67 ± 1.2^a	1.67 ± 0.6^a
4	AS Only	43.67 ± 2.3^a	13.93 ± 1.2^a	65.33 ± 9.9^b	7.14 ± 0.3^a	9283 ± 2664.7^b	147670 ± 53724.6^a	31.00 ± 8.7^b	2.00 ± 1.0^a	1.67 ± 1.5^a
5	AC + ARS	45.33 ± 1.5^a	15.03 ± 0.8^a	73.33 ± 7.6^b	7.43 ± 0.2^b	10567 ± 671.4^b	$189000 \pm 24248..7^a$	23.33 ± 6.1^a	1.33 ± 1.5^a	2.00 ± 0.0^a
6	AS + ARS	47.33 ± 3.8^a	15.30 ± 1.5^a	64.00 ± 4.4^b	8.05 ± 0.6^b	8267 ± 862.2^b	139000 ± 15874.5^a	32.67 ± 5.8^b	1.67 ± 1.2^a	1.67 ± 0.6^a
7	AC + AS	44.33 ± 3.1^a	14.33 ± 1.5^a	67.67 ± 9.6^b	7.40 ± 0.2^b	6533 ± 3402.0^a	131330 ± 56923.9^a	30.33 ± 9.3^b	0.33 ± 0.6^a	1.67 v 0.6^a

All data were mean and standard deviation of (five replicates) determinants. a and b are means from least to the highest. Means of the same alphabet are not significantly different.

Mean within the same row with different alphabets are significantly different at $p < 0.05$ (Duncan)

* indicates significant difference when compared with the control group (LSD)

Note: Check glossary for full meaning of abbreviations

4.3 Results Obtained from Biochemical Analysis

Biochemical analysis was carried out on the blood samples collected from the sacrificed rats. The biochemical analysis included total protein, albumin, globulin, aspartate amino-transferase, alanine amino-transferase, blood urea nitrogen and Creatinine. Table 4.4 shows the result of the biochemical analysis.

Table 4.4: Biochemical Parameters in Blood of Rats after 19 Days

SN	Treatment group	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio	AST (µl)	ALT (µl)	BUN (mg/dl)	Creatinine (mg/dl)
1	Control	7.77 ± 0.7^{b}	4.57 ± 0.4^{b}	3.20 ± 0.3^{b}	0.67 ± 0.1^{a}	48.00 ± 1.0^{b}	29.67 ± 2.5^{a}	15.33 ± 0.6^{c}	1.07 ± 0.1^{a}
2	ARS Only	7.83 ± 0.3^{b}	4.47 ± 0.2^{b}	3.37 ± 0.2^{b}	0.73 ± 0.1^{a}	43.33 ± 3.1^{a}*	30.00 ± 3.0^{a}	15.33 ± 0.6^{b}	1.03 ± 0.2^{a}
3	AC Only	7.53 v 0.5^{b}	4.47 ± 0.3^{b}	3.50 ± 0.4^{b}	0.73 ± 0.2^{a}	46.00 ± 2.6^{b}	32.00 v 2.6^{a}	14.33 ± 0.6^{c}	1.23 ± 0.1^{a}
4	AS Only	7.30 ± 0.4^{a}	4.27 ± 0.3^{b}	3.07 ± 0.3^{a}	0.67 ± 0.1^{a}	47.33 ± 0.6^{b}	30.67 ± 4.5^{a}	15.67 ± 0.6^{c}	1.20 ± 0.3^{a}
5	AC + ARS	7.73 ± 0.2^{b}	4.63 ± 0.1^{b}	3.07 ± 0.3^{a}	0.67 ± 0.1^{a}	46.00 ± 3.1^{b}	33.00 ± 2.6^{a}	15.33 ± 0.6^{c}	1-10 ± 0.1^{a}
6	AS + ARS	8.17 ± 0.2^{b}	4.53 ± 0.3^{b}	3.63 ± 0.7^{b} *	0.77 ± 0.1^{a}	46.00 ± 2.6^{b}	29.33 ± 3.2^{a}	14.00 ± 1.0^{a}*	0.93 ± 0.3^{a}
7	AC + AS	7.53 ± 0.1^{b}	4.13 ± 0.1^{a} *	3.40 ± 0.2^{b}	0.77 ± 0.1^{a}	46.57 ± 1.7^{b}	31.33 ± 1.5^{a}	15.67 ± 0.6^{c}	1.03 ± 0.2^{a}

All data were mean and standard deviation of (three replicates) determinants. a, b and c are means from least to the highest. Means of the same alphabet are not significantly different.

Mean within the same row with different alphabets are significantly different at $p < 0.05$ (Duncan)

* indicates significant difference when compared with the control group

Note: Check glossary for full meaning of abbreviations

4.4 Histopathological Parameters

Histopathological examinations were carried out on the five organs that were removed from the sacrificed rats. The organs were kidney, liver, brain, testes and spleen. Tables 4.5 – 4.11 shows the result of the histopathological analysis of the rats in all the seven groups. Plate 4.1 also shows a rat from the group administered arsenic trioxide only (Group 2), with bloody nasal discharge.

Table 4.5: Group 1 Control (Distilled water only)

	Kidney	Liver	Brain	Testes	Spleen
Rat 1	NVPL	NVPL	NVPL	NVPL	NVPL
Rat 2	NVPL	NVPL	NVPL	NVPL	NVPL
Rat 3	NVPL	NVPL	NVPL	NVPL	NVPL

Where NVPL means: No Visible Pathological Lesions

Table 4.6: Group 2: Arsenic trioxide only

	Kidney	Liver	Brain	Testes	Spleen
Rat 1	Necrosis (Nuclear and cytoplasmic degeneration)	Cytoplasmic degeneration	Nuclear pleomorphism	Necrotic degeneration of seminiferous tubules	Dilated sinuses
Rat 2	Cytoplasmic degeneration	Necrosis	Neurones and glial cells show nuclear pleomorphism	Necrotic degeneration of seminiferous tubules	Aggregation of inflammatory cells
Rat 3	Necrotic degeneration around renal tubules	Tissue necrosis within hepatic portal vein	Vacuoles contain eosinophilic materials	Necrotic degeneration of seminiferous tubules	Aggregation of inflammatory cells

Table 4.7: Group 3: Rats treated with *Allium cepa* only

	Kidney	Liver	Brain	Testes	Spleen
Rat 1	NVPL	NVPL	NVPL	NVPL	NVPL
Rat 2	NVPL	NVPL	NVPL	Focal areas of Cytoplasmic degeneration	NVPL
Rat 3	Focal areas of necrosis seen	NVPL	NVPL	NVPL	NVPL

Where NVPL: No Visible Pathological Lesions

Table 4.8: Group 4: Rats treated with *Allium sativum* only

	Kidney	Liver	Brain	Testes	Spleen
Rat 1	Mild Nuclear pleomorphism	Enlarged hepatic portal vein	NVPL	NVPL	Necrosis and Fibrosis
Rat 2	NVPL	NVPL	NVPL	NVPL	NVPL
Rat 3	Cytoplasmic degeneration	Cytoplasmic degeneration	Cytoplasmic degeneration	NVPL	Cytoplasmic degeneration

Where NVPL: No Visible Pathological Lesions

Table 4.9: Group 5: Rats treated with *Allium cepa* and Arsenic trioxide

	Kidney	Liver	Brain	Testes	Spleen
Rat 1	Mild necrosis	Cytoplasmic degeneration	Cytoplasmic disruption	NVPL	Necrosis
Rat 2	Necrosis	NVPL	NVPL	Necrotic patches	Dilated sinusoids
Rat 3	Necrosis	Cytoplasmic degeneration	Cytoplasmic disruption	NVPL	Dilated sinusoids

Where NVPL: No Visible Pathological Lesions

Table 4.10: Group 6: Rats Treated with *Allium sativum* and Arsenic Trioxide

	Kidney	Liver	Brain	Testes	Spleen
Rat 1	NVPL	Mild necrosis	Mild Cytoplasmic degeneration	Mild necrosis	Dilated sinusoids
Rat 2	Mild fibrosis	Enlarged sinusoids	Cytoplasmic disruption	Nuclear disruption	Cytoplasmic disruption
Rat 3	NVPL	Dilated sinusoids	Cytoplasmic degeneration	NVPL	Cytoplasmic degeneration

Where NVPL: No Visible Pathological Lesions

Table 4.11:Group 7: Rats treated with *Allium cepa* and *Allium sativum*

	Kidney	**Liver**	**Brain**	**Testes**	**Spleen**
Rat 1	Extensive septa formation	NVPL	NVPL	NVPL	NVPL
Rat 2	NVPL	Cytoplasmic disruption	NVPL	NVPL	Aggregation of inflammatory cells and septa formation
Rat 3	NVPL	NVPL	NVPL	NVPL	NVPL

Where NVPL: No Visible Pathological Lesions

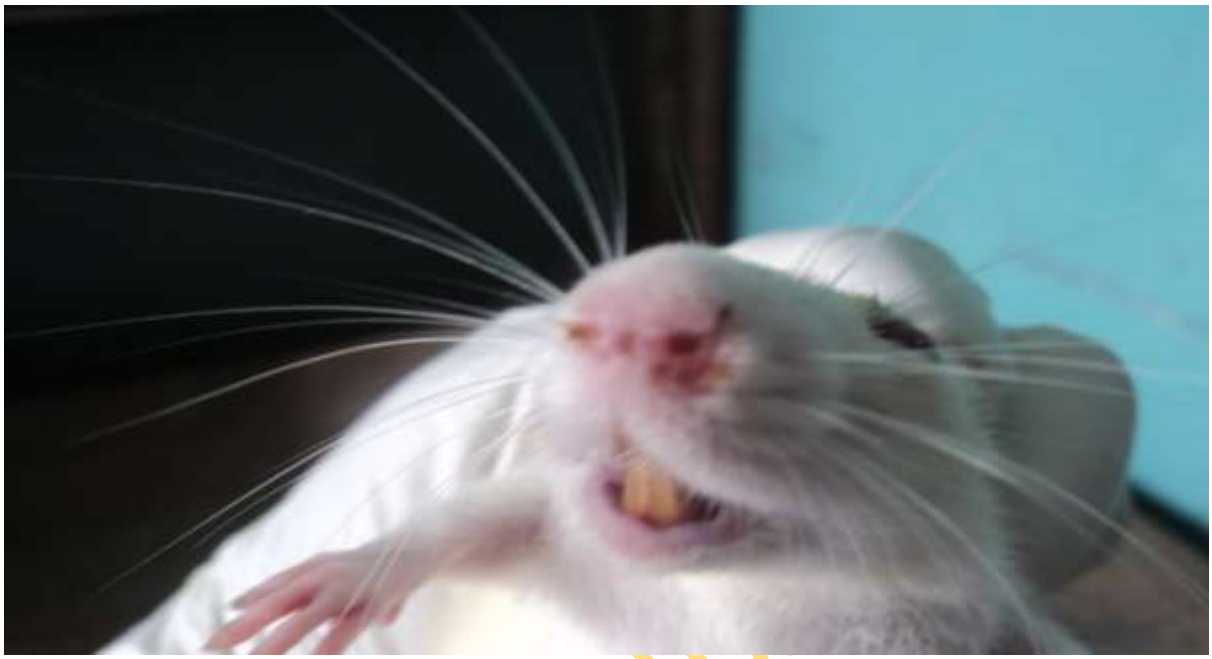

Plate 4.1: A Rat (from group 2) with Bloody Nasal Discharge

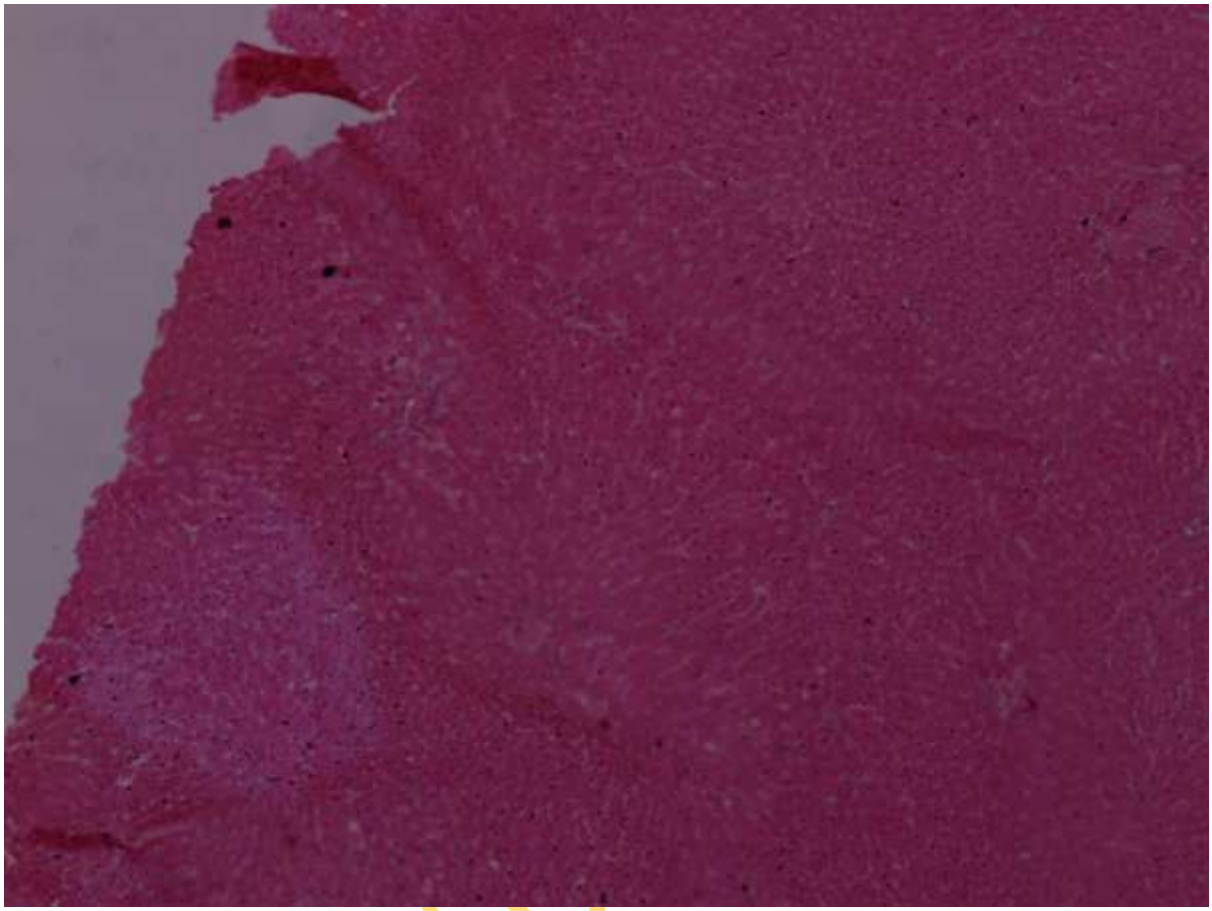

Plate 4.2. Transverse section of liver of rat in the control Group (Group 1). It shows normal arrangement of hepatocytes and Kupffer cells. HE staining (X 800).

There was no visible pathological lesion.

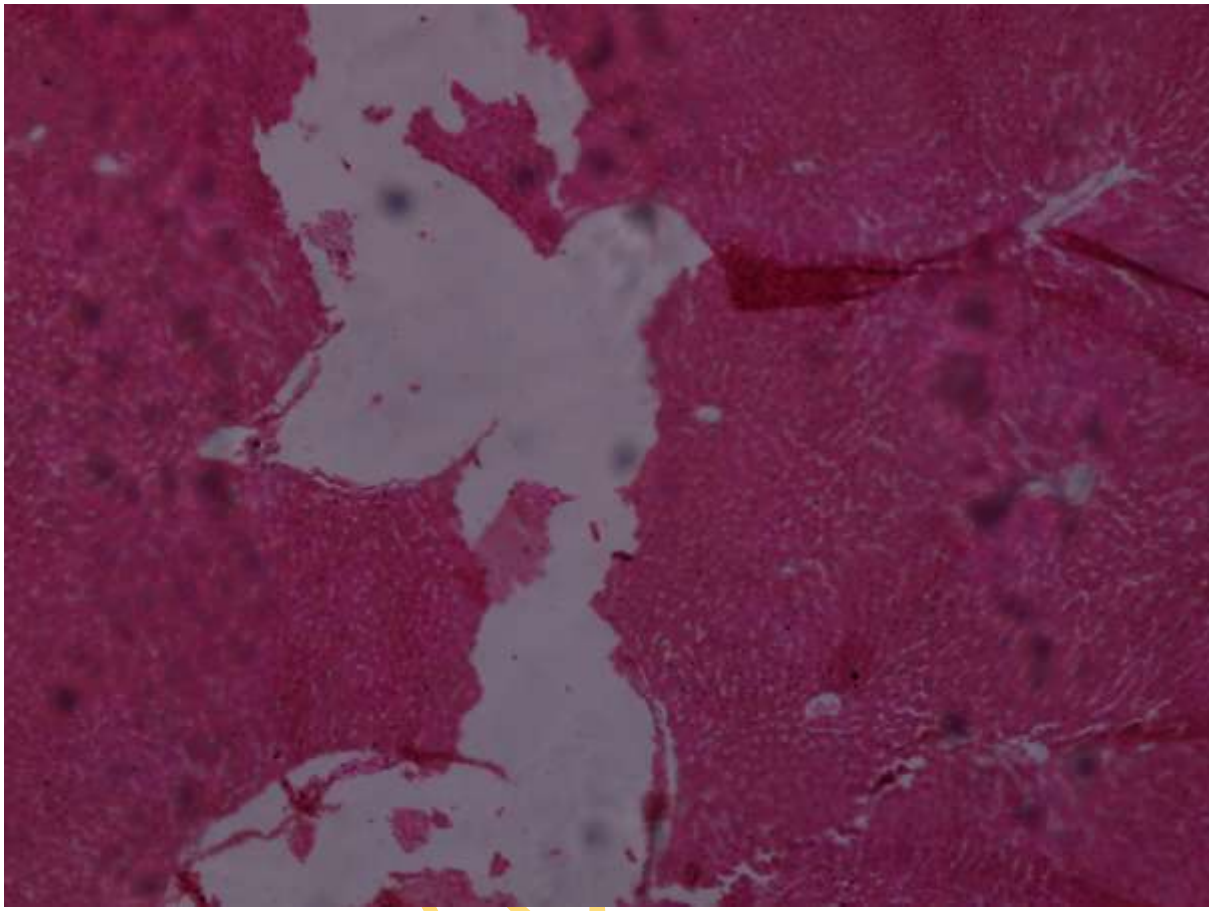

Plate 4.3. Transverse section of liver of rat in group 2 after treatment with As_2O_3 showing disorganization and degeneration of hepatocytes. HE staining (X 700).

There were visible pathological lesions.

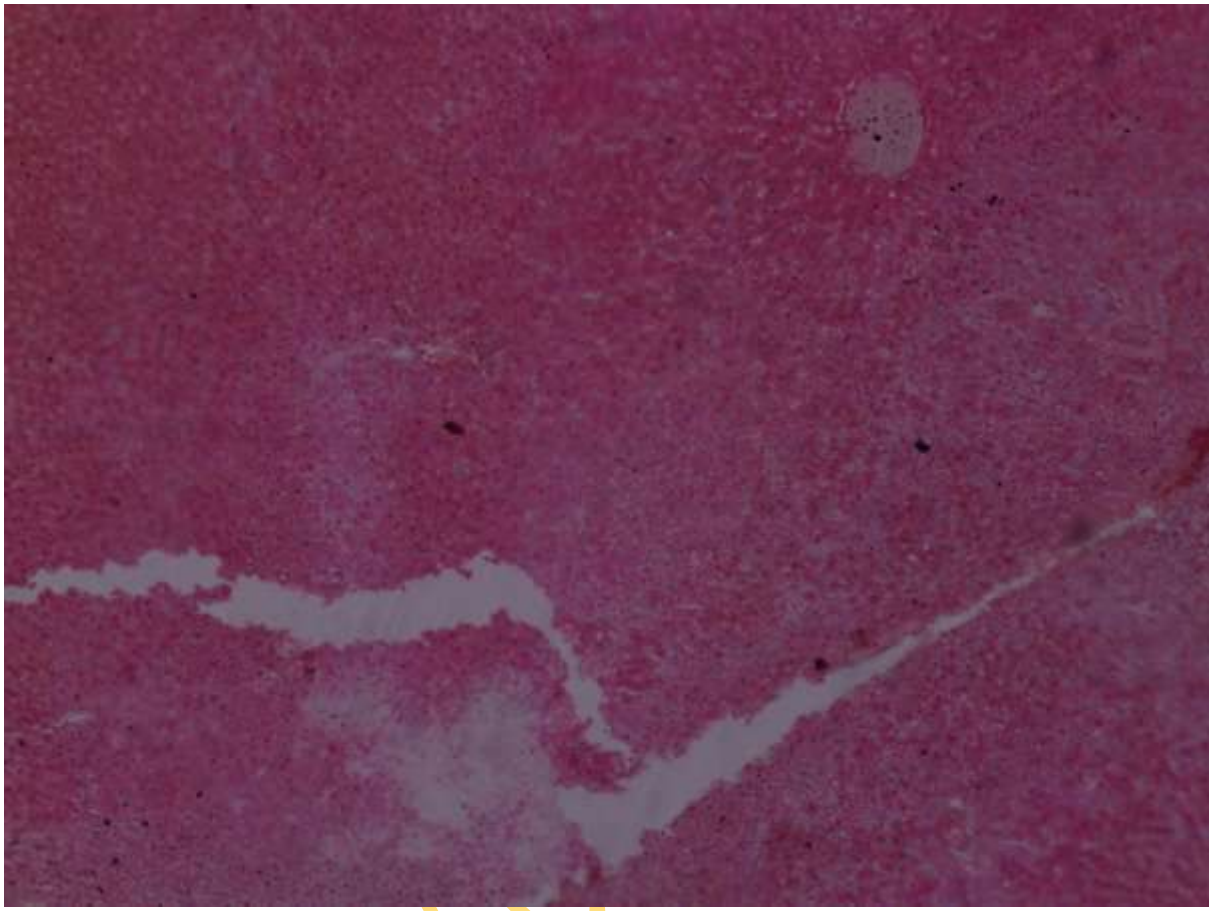

Plate 4.4 Transverse section of liver of *Allium sativum* combined with arsenic trioxide (Group 6) rat with no visible pathological lesion. HE staining (X 800).

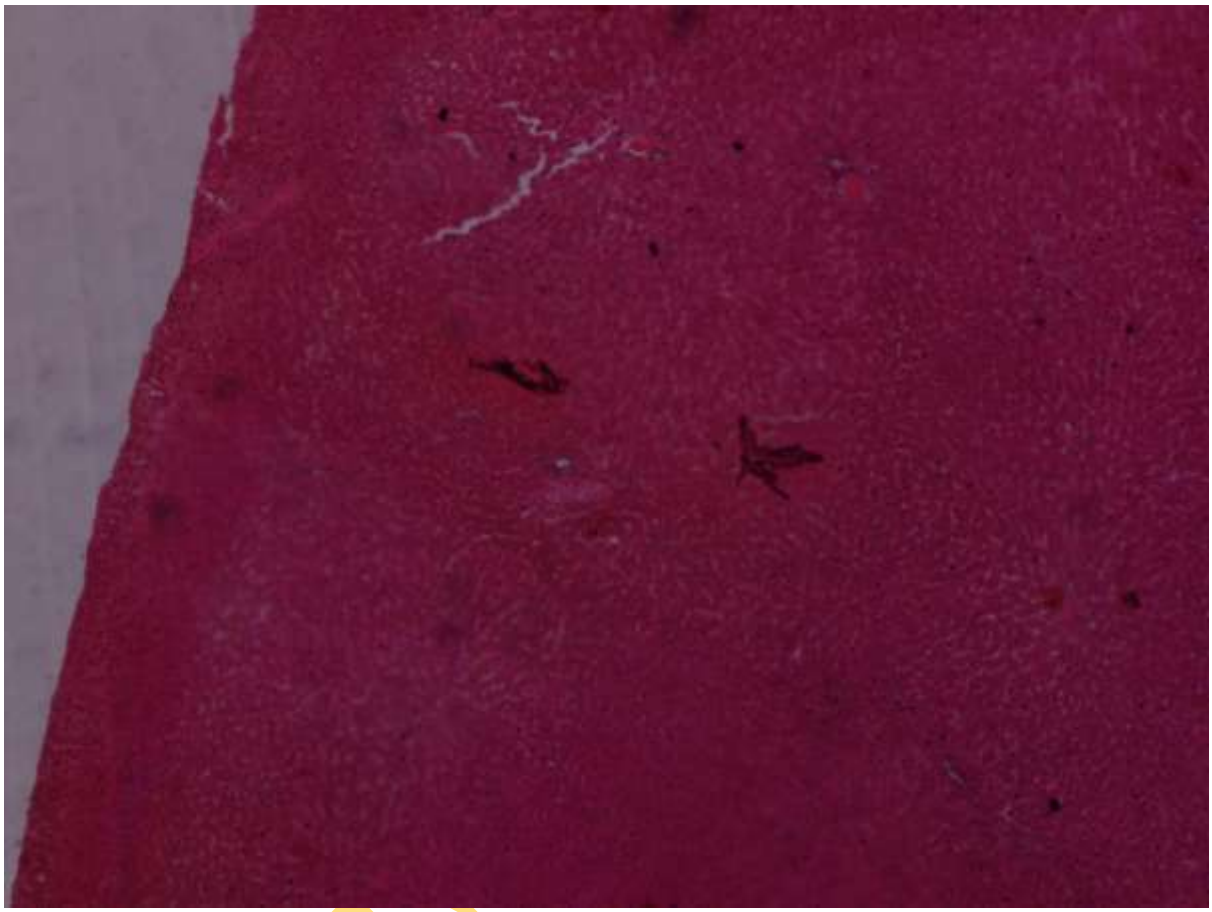

Plate 4.5 Kidney of rat in Group 7 administered both *Allium cepa* and *Allium sativum* showing no visible pathological lesion.

CHAPTER FIVE

5.0 DISCUSSION

The protective effects of Onions (*Allium cepa*) and garlic (*Allium sativum*) on arsenic toxicity were investigated in male albino rats. No mortality was recorded in the group administered arsenic trioxide only (Group 2). This was similarly observed in a study of Sodium arsenite (Na_3As) administered orally to rats carried out by Morakinyo, 2010. The detoxification pathway of arsenic in the presence of bioactive components of *Allium cepa* and *Allium sativum* such as allicin and quercetin, which are sulphur – containing compounds could be responsible for this survival recorded in the rats. (Amagase and Milner 1993, Numagami *et al*., 1996). Additional active constituents of these plants which include steroidal glycosides, lectins (Kaku *et al*., 1992), prostaglandins, fructan, pectin, essential oil, adenosine, vitamins B-1, B-2, B-6, C and E, biotin, nicotinic acid, fatty acids, glycolipids, phospholipids, anthocyanins, flavonoids, phenolics and essential amino acids (Fenwick and Hanley, 1985) could also be responsible. Morbidity and mortality depends on some variables such as concentration of the poison, route of administration, period of exposure and form of the poison.

Several studies have shown that both the acute and chronic arsenic exposure resulted in various physical changes such as reduction in body weight, fur removal, slimy nasal discharge, change in eye colour and sluggish movement (WHO, 2009). From Table 4.1, it was observed that there was a significant difference in the body weight change in the group that was administered arsenic trioxide only than when compared with the control.

The toxicological effects of arsenic on the vital organs of the rats such as, testes, kidney, liver, brain, respiratory tract, to mention few, could be responsible for this (IARC, 2004).This reduction in body weight is in agreement with a previous finding. The decrease in body weight is as a result of the animals using the sulphur-containing amino acids present in their body to detoxify arsenic. Also, in Table 4.2, there was a drastic weight gain observed in the group administered both *Allium cepa* and *Allium sativum* together which could be due to the combined effects of the bioactive components present in both plants (Fenwick and Hanley, 1985). In this

same table, nearly constant weight change was observed in the group administered *Allium cepa* only. This is consistent with the findings of Kumari and Augusti, 2002 that the bioactive components of *Allium cepa* such as quercetin, folic acid and lecithin which are very rich in antioxidant properties are responsible for the maintenance of body weight. Along with its sulphur-containing compounds, the flavonoid quercetin contained in *Allium cepa* helps to provide these antibacterial benefits (Chun *et al*., 2007). The level of feed and water intake of all the experimental groups were also shown in Table 4.1. The feed intake of the group administered *Allium cepa* and *Allium sativum* were significantly reduced in comparison with the control. Also, there is a significant reduction in water intake of the groups administered *Allium cepa*, *Allium sativum*, *Allium cepa* + arsenic and *Allium cepa* + *Allium sativum*. Group 7 that was administered both onions and garlic showed the highest increase in body weight. Both garlic and onion possess strong antioxidant and flavour properties because of their high phenolic and sulfur compounds, respectively (Griffiths *et al*., 2002).

There was no significant difference in the Packed Cell Volume in all the treatment groups thereby indicating that arsenic trioxide, *Allium cepa* and *Allium sativum* do not have any effect on the packed cell volume of the rats as shown in Table 4.3. Also, the haemoglobin, red blood cell, white blood cell, eosinophil, monocytes and platelets showed no significant difference in comparison with the control. All these are considered an integral part of a person's complete blood count results (Purves *et al*., 2004).

The level of white blood cells is used as an index of immune function because they are involved in the cellular and humoural defence of the organism against foreign material (Jimoh *et al*., 2008). This study revealed that the level of immunity of the rats was not affected by the treatments given to them. This could be attributed to the effects of antioxidant phytochemicals such as flavonoids which protect white blood cell destruction. Actually, there was an increase in white blood cell count of the group where *Allium sativum* was used to detoxify the effects of arsenic trioxide without any significant difference. These data therefore support the earlier reports by Sumiyoshi (1984) that garlic extracts stimulate immune functions. This observation

may partly explain the role of garlic in activating the natural killer cells, the function of T-lymphocytes and the level of interleukin – 2 (Tang *et al*, 1997).

In this study, the red blood cell count was not affected by the various treatments administered. The red blood cells contain haemoglobin and it is the haemoglobin which permits them to transport oxygen throughout the body.

There was a significant increase in neutrophil level of the rats administered Allium cepa only in comparison with the control (Table 4.3). This effect could be attributed to the nutritional components of Allium cepa which include dietary fibre, tryptophan, molybdenum, chromium, vitamin C, manganese, copper, folates, potassium, phosphorous and calories (Fig 2.4). Neutrophils are the first type of immune cell to respond to and arrive at the site of infection, often within an hour. During the beginning (acute) phase of inflammation, particularly as a result of bacterial infection, environmental exposure (Jacobs, *et al*, 2010) and some cancers (Waugh and Wilson, 2008), neutrophils are one of the first-responders of inflammatory cells to migrate towards the site of inflammation. This study therefore indicated an increase in neutrophil production in the body system due to the fact that neutrophils are one of the first- responders of inflammatory cells to migrate towards the site of inflammation.

Lymphocytes produce chemical substances known as lymphokines that are essential in helping the B cells destroy foreign substances (Janeway *et al*, 2001). Decrease in lymphocyte level is frequently noted in the initial stages of invasion of foreign materials indicating diseases that affect the immune system. An increase in the number of lymphocytes is usually noted in prolonged illnesses. Table 4.3 of this study showed a significant increase in lymphocyte count when compared with the control.

The results of the present study revealed no significant difference in the total protein levels in liver after treatment administration as shown in table 4.4. This could be related to the inhibition of protein synthesis by accumulation of free amino acids in liver and alteration in production of numerous sulfhydryl-containing proteins (Roy and Saha, 2002).

Table 4.4 also showed a significant decrease in albumin level in the group administered both *Allium cepa* and *Allium sativum* together at the same time indicating the presence of impaired liver function. However, in this same table, the albumin level of the groups in which *Allium cepa* and *Allium sativum* were used to detoxify the effects of arsenic poisoning fell within the normal range thereby indicating the antioxidant effects of both plants.

This study also revealed a significantly high level of globulin in the group administered *Allium sativum* and arsenic trioxide together in comparison with the control (table4.4). The increase in the globulin level implied increased antibody activities which could have been supplied by the sulphur-containing components of *Allium Sativum* (Amagase and Milner 1993, Numagami*et al*. 1996).

The organ involved in metabolism, secretion and excretion is the liver. This is due to its strategic location in the body and continuous exposure to environmental pollutants, xenobiotics and chemotherapeutic agents. Aspartate transaminase (AST) and alanine transaminase (ALT) are enzymes secreted by the liver into the blood as a result of liver inflammation. In this study, there was no significant difference of AST and ALT in the groups where *Allium cepa* and Allium sativum were used to detoxify arsenic trioxide (table 4.4) when compared with the control. This could be attributed to the effects ofboth plants' antioxidant activities which is attributed to biologically active lipophilic sulphur-bearing compounds such as allicin, S-allyl-cysteine (SAC), diallyl-di-sulphde (DADS), and diallyl-sulphide (DAS), a study done by Fenwick and Hanley, 1985.

In the group in which *Allium sativum* was used to detoxify arsenic trioxide, there was a significant increase in the level of Blood Urea Nitrogen (BUN) when compared with the control. One of the causes of decreased concentration of BUN could below protein diet and can be modulated by dietary protein intake (Martin *et al*., 2005). The result of this study presents a non-significant difference in creatinine level for all the groups.

In this study, histopathological examination of the liver, kidney, testes, brain and spleen of the control group showed no visible pathological lesion as indicated in table 4.5 and plate 4.2. Likewise, this same effect was observed in tables 4.9 and 4.10 where arsenic activities were detoxified by the bioactive components of *Allium*

cepa and *Allium sativum* as shown in Plate 4.5. However, there were disorganization and degeneration of hepatocytes, cytoplasmic degeneration, necrotic degeneration around renal tubules observed in the liver and kidney of the group 2 rats as shown in Table 4.6 and Plate 4.3. Necrotic degeneration of seminiferous tubules was observed in the testes of the experimental rats in this same group. Enlarged sinusoids and aggregation of inflammatory cells were also observed in the spleen. It is evident that the liver damage due to necrosis, apoptosis, histological manifestations, and oxidative stress would interfere with several hepatic functions (Jing *et al*., 1999, Shashi, 2003 and Guo *et al*., 2003). Liu *et al*, 2002 also observed that ingestion of arsenic contaminated drinking water caused infiltration of inflammatory cells in the periportal area in liver biopsy samples. All these findings have made this study supportive of the previous related studies.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSIONS

From this study, it can be concluded that onions (*Allium cepa*) and garlic (*Allium sativum*) had ameliorating effects on morphological, haematological, biochemical and histopathological indices of arsenic toxicity. *Allium cepa* and *Allium sativum* can provide protection without being appreciable harmful themselves. Also, the toxicity of arsenic can be greatly reduced by the combination of both plants.

The outcome of this study showed that it is consistent with previous findings, establishing the toxic effects of arsenic and the effects of *Allium cepa* and *Allium sativum* in abating arsenic toxicity.

This study also showed the detoxifying effects of *Allium cepa* and *Allium sativum* when consumed at the administered concentration to combat arsenic poisoning that may be acquired by man to detoxify arsenic through different exposures such as inhalation, oral ingestion and dermal absorption.

6.2 RECOMMENDATIONS

1. Further studies on the detoxifying effects of onions and garlic when combined together in meals against other heavy metals (especially the heavy metals having wider range of exposure) is recommended for research.
2. Studies such as activity guided fractionation of molecules involved in the ameliorative properties of the plants (onions and garlic) on arsenic toxicity are recommended.
3. A definitive understanding of the mechanism of action of arsenic will allay any uncertainties associated with the risk assessment for this chemical.
4. There should be mandatory education and continuous training of all age groups exposed to arsenic poisoning in the community either directly or indirectly especially schools and colleges, road transport workers, etc. This will reduce the exposure rate of the populace to arsenic poisoning.

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