Effects of *Allium sativum* Extract in Arsenic Induced Toxicity in *Swiss albino* mice

By

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A thesis submitted to the Department of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

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Brac University
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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

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Approval

The project titled "Effects of *Allium sativum* Extract in Arsenic Induced Toxicity in *Swiss albino* mice" submitted by Rusafa Antashin Adhara (14346006) of Summer'14, has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on 8th August 2019.

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Ethics Statement

Animal tests had performed in this study and ethical permission had taken from the Department of Pharmacy, Jahangirnagar University.

Abstract

Arsenic (As) is metal toxic element to the body and it is available in environment. Arsenicosis, elevated level of As in body, can be treated by chelation therapy. In chelation therapy, a chelate is used to form a complex chemical structure with the metal ion. As, *Allium sativum*is a natural chelate and this study focuses on the *in vivo* evaluation of the chelating effect of *Allium sativum*(methanolic extract) of different doses (150, 300, 500 mg/kg) on the depletion of arsenic from the cellular level, using *Swiss albino* mice. Body weight, hematological and biochemical parameters like, WBC, RBC, NEU, ALP, CRE, cellular structure of skin, brain, kidney, liver were observed and significant amount of changes were found. *Allium sativa*, popularly known as the garlic, is an influential antioxidant that, have the potential to detox the body via its chelating characteristics by absorbing toxic substances or metal sections.

Keywords: Arsenicosis, *Allium sativum*, toxic, biochemical parameters, chelation, antioxidant, garlic, haematological parameters, detox.

Dedication

I dedicate this work to my parents and brother for their unconditional love and support.

Acknowledgement

I would like to express my special thanks to the almighty for enabling me to carry out this research project in good health and frame-of –mind.

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Table of Contents

List of Tables

List of Figures

List of Acronyms

WHO- World Health Organization

UNICEF- United Nations Children's Fund

WBC- White Blood Cell

RBC- Red Blood Cell

NEU- Neutrophil

LYM- Lymphocyte

MON- Monocyte

EOS- Eosinophil

CRE- Creatinin

SGPT- Serum Glutamic Pyruvic Transaminase

ALP- Alkaline Phosphatase

Chapter 1

Introduction

1.1 What is Arsenic

The Arsenic (As) is the fiftieth most essential component on the earth's surface and twenty-second most prevalent component in ocean water with an atomic number of 33 on the periodic table of elements. Arsenic have only one stable isotope, which represents for 100% of its natural abundance with atomic weight 75 (Saxe et al 1964). Arsenic can be found in Earth's crust and it has been largely allocated to water, air, food and soil(National Institute of Environmental Health Sciences, 2019).

A metallic element which typical of a wide spectrum of toxic compounds, in nature arsenic in the most part with oxygen, chlorine and sulfur is measured at small degrees. This is called arsenic compounds inorganic. Arsenic incorporates carbon and hydrogen in crops and livestock.

The organic arsenic is known as this. In general, organic arsenic is much less unsafe than inorganic arsenic (Shiel, 2019). Arsenic has for generations held up our fascination, partly because its toxic characteristics have been long recognized. It has often been considered a "practical" way of killing an individual, as arsenic after death was so hard to be aware of.Today, murder with arsenic is less hard to identify, but it has become a fresh forensic task to identify sources of arsenic in the environment (Saxe, 1994)

Figure 1 Arsenic Metal (Arsenopyrite, 2019)

1.2 Therapeutic Use of Arsenic

In the 19th century, the solution of Fowler's 1% AsO_3preparing was commonly used. In 1958 Martindale's handbook on British Pharmaceutical and Therapeutic Products, indicated the solution Fowler had to offer: leukemia, skin conditions, stomatitis and gingivitis of babies, and Vincent's angina (Ratnaike, 2003). Arsenic trioxide is now broadly used to result in remission in sufferers with acute promyelocytic leukaemia, primarily Based on their mechanism as an apoptosis inducer (Chen et al., 1997). Arsenic induces apoptosis by means of releasing an apoptosis-inducing factor (AIF) from the mitochondrial inter membrane area from the place it translocates to the cell nucleus. They are used for psoriasis, syphilis, asthma, rheumatism, hemorrhoids, cough and pruritus and are also prescribed as a tonic for fitness, an analgesic, anti-inflammatory agent and as a cure for some malignant tumors(Antillon et al., 1998). In India, herbal drug treatments containing arsenic are used in some homoeopathic preparations and hematological malignancies and in Korea, arsenic is prescribed in herbal remedy for hemorrhoids (Kew et al., 1993; Mathews et al., 2011;Mitchell-Heggs, Conway, & Cassar, 1990).

1.3 Arsenic Poisoning

The metalloid substance Arsenic (As), is found throughout the environment and an upraised level of arsenic in body (termed as “arsenicosis”) is more likely to react with the cells by displacing certain elements from the cell and create abnormal changes in cell’s functions. For an example, arsenite, a form of As can emulate and replace phosphates from the cells. Phosphates have major role in generating energy within the cell and cell signaling. However, this potential is an aid in some cancer therapy, but systemic exposure of arsenic has serious consequences on physical fitness which are notable by skin eruptions, swelled eyelids and limbs, vomiting, diarrhea, muscle cramps (Puddock, 2018).

From many sources including food, water and air, human beings are exposed and the predominant way of exposure is As contaminated consuming water. Populations that consume As-contaminated soilwater excrete larger than those that consume water alone as in urine, implying that people are subjected to As from sources other than potable water. Diet exposure was thus involved in the human body as a significant supply of arsenic. The most important food components and toxic to humans are inorganic As (III) and As(V). Cooking techniques and ingredients used to cook foods may influence the retention of as in the baked food and consequently the consumption of products (M. A. Rahman, Rahman, & Hasegawa, 2018).

1.4 Arsenicosis: Worldwide Situation

The Agency for Environmental Protection reduced the authorized arsenic amount in drinking water in 2001. In the US the rate has decreased from 50% per billion (ppb) to 10% (ppb). 79,9 million and 42,7 million individuals are respectively subjected to levels of soil water arsenic above and beyond the maximum permissible limit of 50 μg / L in 42 counties of southern Bangladesh and in nine counties adjacent to western Bengal (Ratnaike, 2003). The

guideline for water specimens in many areas of the globe, e.g. U.S., China, Bangladesh, Nepal, Vietnam, Taiwan, Mexico, Argentina, Poland, Italy, Finland, Spain, Canada, Hungary, New Zealand, Japan and India, established in levels over 10 µg / L. In contrast, regions in central and southern America are places where arsenic from potable sources contaminated with drinking water. Around 70 million people worldwide exposed to arsenic by drinking water with a concentration of more than 50 µg / L. The Bengal Delta population is estimated to be at highest risk for over 150 million people at concentrations of arsenic exceeding ten 50 µg / L (James, 2017).

Arsenic sections were first found in soil in Bangladesh in 1993. According to the UNICEF, roughly 8.6 million tube-wells were screened in Bangladesh in 2008, while the water was tested at 4,75 millions of tube wells (55%), of whom 1,4 million (16%) were labeled as reds, showing that the arsenic was highly dangerous for use. Recent results indicate that approximately 20 million individuals use arsenic-contaminated tubes in Bangladesh above the permitted level.The usual situation indicates that the epidemic of arsenic toxicity and arsenic-related illnesses threatens Bangladesh most. Arsenic is a common social stigma here as individuals see it as a contagious disease or a curse. The human person is a disease. The focus on arsenic poisoning has now accelerated considerably and relies on remarkable enjoyment. This problem is being addressed by many international organizations, such as WHO, UNICEF and domestic NGOs. In the near future, we can hope that Bangladesh can overcome arsenic terror by continuing to apply awareness (Uddin & Huda, 2011).

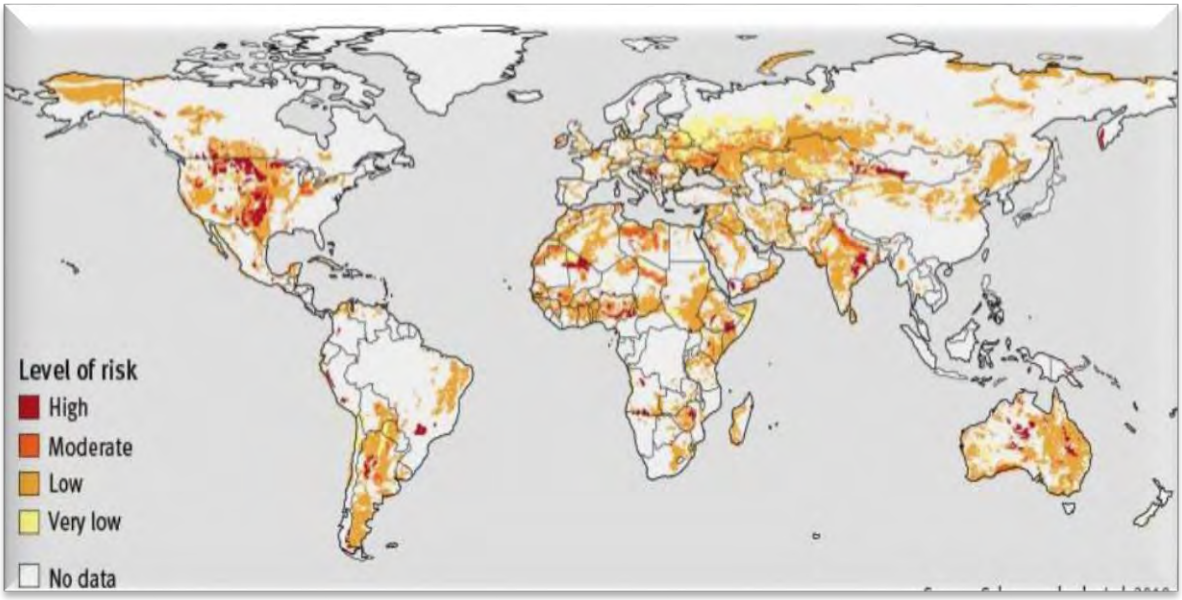

Figure 2Projected hazards for drinking water arsenic corrosion Depending on hydrogeological circumstances(UN University, 2019)

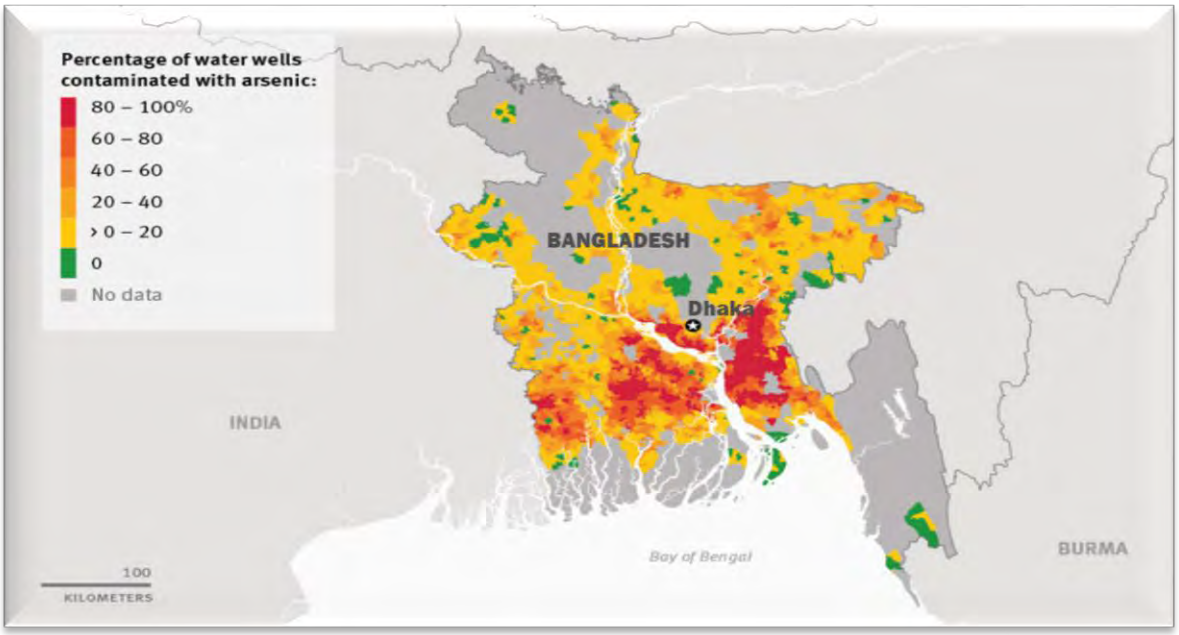

Figure 3Recent Situation of Arsenic Contamination in Bangladesh(David & April, 2019)

1.5 Stages of Arsenic Depletion

1.5.1 Acute Poisoning

Health problems of acute poisoning usually occur within thirty minutes of ingestion, but with the food, arsenic notification may be delayed. At first, an individual with a metallic taste could also feel or notice a mild garlic odor linked to a dry mouth and concern for swallowing. Additionally, muscle pain, weak point with flushing skin, may be early clinical indications and symptoms for acute arsenic poisoning. Unintended assurance of severe nausea and vomiting, colic abdominal pain, or abundant diarrhea with rice-water stools.Capillary harm results in widespread vasodilatation, plasma transudation and vasagenic shock. In the bowel lumen, fluid is being transudated, the vesico-mucosal formation and the lightening of tissue pieces are caused by the effects of arsenic on the supply, not by direct corrosive actions. The individual may also be prompt to muscle cramps, arms and feet numbness, physical rash and excessive sedation.The pores and skin turn bloodless and clammy in severe empoisonment, and a certain amount of circulatory crumple occurs usually with renal injury and reduced urine production. Drowsiness and confusion may also occur alongside the enhancement of paranoid delusion, hallucination and delirium-related psychosis. Finally, convulsions, coma, and death can occur, generally as a result of shock.

Multisystem organ injury may also happen after the gastrointestinal stage. If the loss of irreversible circulatory insufficiency no longer occurs in the first twenty-four hours, the result can be additionally liver or renal failure for a number of days. Active cardiomyopathy, sub cardiac bleeding and electrocardiographic changes consist of cardiac manifestations. cardiac manifestations. Extended QT intervals and non-specific ST segment shifts are the most common modifications to an electrocardiogram (Saha,Dikshit, Bandyopadhyay, Saha, 1999).

1.5.2 Chronic poisoning

Chronic arsenic intoxication is in nature much more insidious. The skin, lung, liver and blood systems are the most prominent continuous manifestations. The dermal changes are typical but non-specific. The skin is patchy and the "rainrops on a dusty route" has been poetically described. A diffuse finger and sole desquamation is elevated. The progression of multicenter basal cellular and squamous cell carcinomas is long-term cutaneous havoc. Some found that both monocentric and multicentric, but Basally mobile carcinoma had been found to be no longer present in pores and skin from 222 malignancies in arsenicosis in particular, telephone carcinoma, and Bowen's infection. The unusual skin lesion of Bowen is linked with every human papilloma and arsenic virus (HPV). Bowen syndrome has the illness. Arsenic and HPV are both responsible for the majority of epithelial tissue cancers,79 and arsenic can be thought to cause most people to develop an arsenic-like virus, such as HPV. This is because arsenic encourages epithelial tissue cancer in human humans, but not in rodents that generally do not lift the papilloma virus.Brittle nail, patchy alopecia, and facial edema are reported in the literature in arsenical skin diseases. Anemia and leukopenia with constant exposure to arsenic are almost common. There is also frequent occurrence of thrombocytopenia. The anaemia is usually normal chromium and normal, and at least partially precipitated by hemolysis. Stopping the metabolism of folate and synthesized DNA may also lead to megaloblastic modifications. Anemia, leucopenia and arsenic cytopenia are a frequent association of malnutrition to anemia and leucopenia in underdeveloped countries like India and Bangladesh (Saha et al., 1999).

1.6 Arsenic Toxicity Mechanism

Arsenic is present as pentavalent (As5 +, arsenate) and trivalent (As3 +, arsenate) forms in the environment and in comparison with arsenate arsenitearsenite is deemed more toxic (Domingo, 1995). Arsenic in liver, kidney, heart, and lungs is stored during absorption. The

decline in arsenic in muscle groups and neuronal tissues is determined (Kaur, Singh, & Goel, 2011).Arsenic build-up in these tissues is linked to many diseases, such as cancer, diabetes, hepatotoxicity, neurotoxicity and heart dysfunction. The metabolism of arsenic is critical for its toxicity and exercises its toxicity through the inhibition of around 200 cellular energy enzymes and DNA synthesis and repair, and so on (Ratnaike, 2003). This is metabolized via discount and methylation responses, catalyzed with one-carbon arsenic metabolism by using S-adenosyl methionine (SAM) to be methane donor, and requiring decreased glutathione (GSH) in reductase response, by Glutathione-S-transferase omegas-1 (GSTO1) and arsenic(III) methyltransferase (AS3MT). GSTO1 decreases methylarsenic [MA(V)] and arsenic [As(V)] to meteorological [MA(III)] and arsenic [As(III)] respectively and, by means of AS3MT to methylarsenic [MA(V)] and dimethyllarsinate [DMA(V)], these toxic trivalent arsenics formed by a discount point are detoxified by means of AS3MT and less toxic pentavalent Arsenicals.(Lindberg et al., 2007).Chronic ingestion of arsenite through

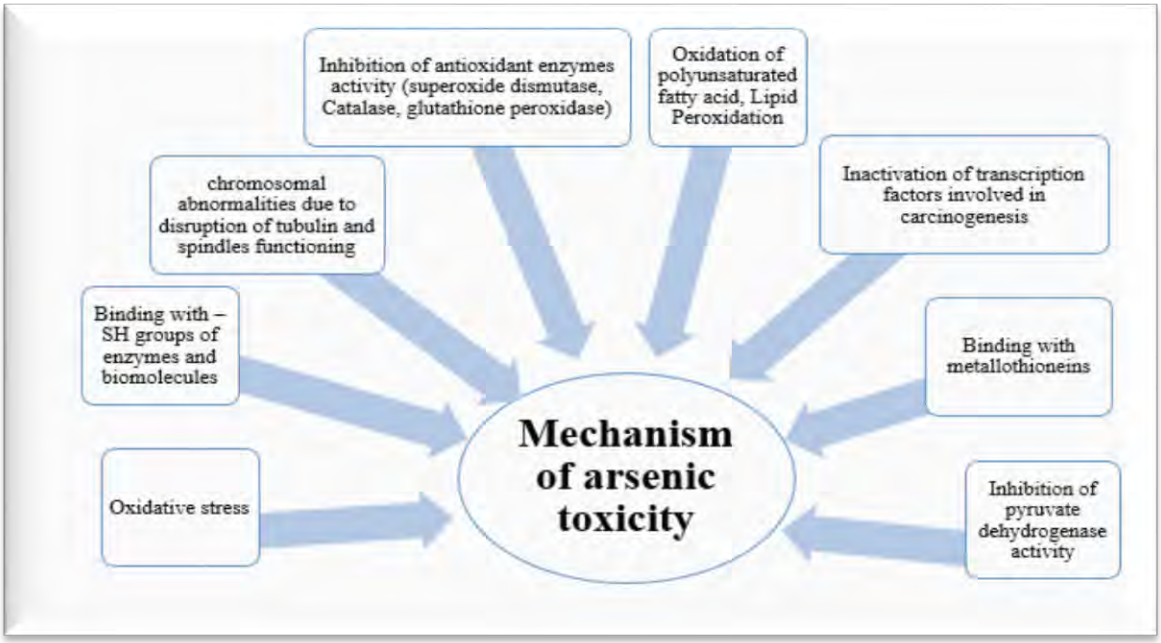

Figure 4 Mechanism of Arsenic Toxicity(Saha et al., 1999)

contaminated water results in accumulation of arsenite and MA(III) in essential organs and tissues.Atherosclerosis, hypertension, ischemic heart diseases, diabetes, hepatotoxicity nephrotoxicity, and most cancers of the skin, bladder, and lungs are the major outcomes of arsenic exposure in body (International commission for protection against environmental mutagens and carcinogens, 1979, Kaur et al., 2011, Liu, 2000,Gurr et al., 2003).

1.7 Natural Chelate: *Allium sativum*

1.7.1 *Allium sativum*

Allium sativum., commonly known as garlic, is an *Alliacean* species in the family. Garlic is an aromatic agent that is used as a bulb root for *Allium sativum* and bumps away with food in all parts of the world.

Figure 5 Allium sativum (Local name-Garlic)

1.7.2 Medicinal aspect of *Allium sativum*

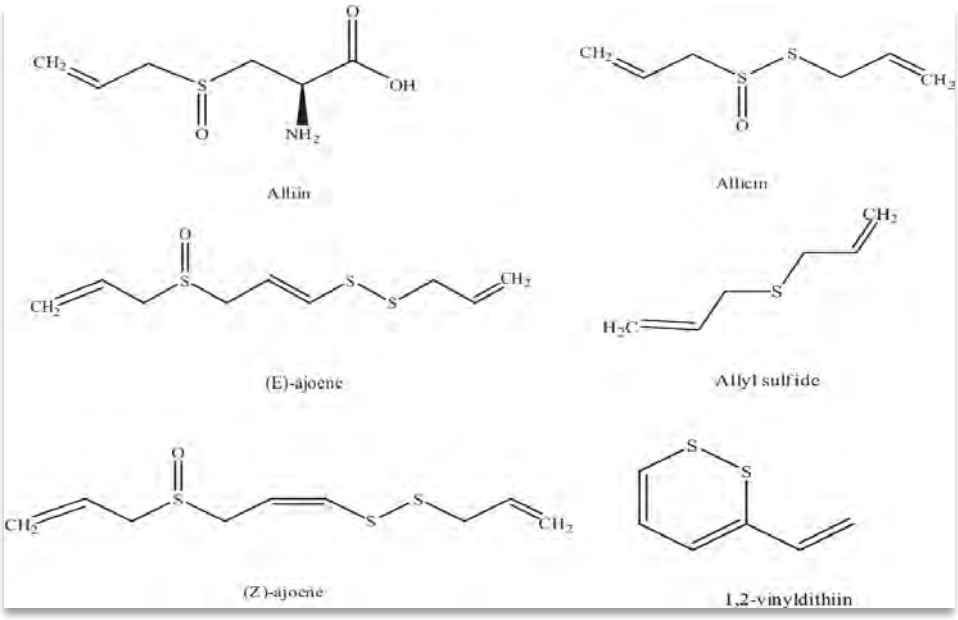

Figure 6 Chemical Constituents of Allium sativum (Martins, Petropoulos, & Ferreira, 2016)

Diallylsulfide, primarily sulfur with hazardous garlic compound, decreased oral pouch in the stomach, inhibiting colon, liver, esophagus, and tumor development in experimental designs.diallylsulfideAllicin inhibits the growth of murine and human cancer cells. Appoptotic body formation, nuclear condensation and a common DNA ladder in cancer cells were triggered by allicin. In addition, activation by means of allicin was precipitated for the caspases-3,-8 and-9 and cleavage polymerase (ADP-ribose) (Silva, 2015).

Allium sativum has an impact on cadmium, methyl mercury and phenyl mercury to prevent heavy- metal toxicity, heavy- metal accumulation in the liver, kidneys, bones and testing, histopathological injuries and inhibition of heavy-metal serum alkaline phosphatases (Cha CW,1987).

1.7.3 Rational of the Study

As some rural areas of Bangladesh still have some arsenic containing water tube wells and people of those areas are at high risk of having high amount of arsenic within their body, which can later on create life-threatening complications.

For treating arsenic toxicity, chelation therapies are conducted to treat arsenicosis and in this study, it is aimed to observe that if there is any effect of natural chelates more specifically natural extracts, on the reduction of arsenic toxicity from the cellular level of body. Chelation therapy is a non-evasive scheme in which heavy metals are removed from the body. *Allium sativum* has chelating characteristics that demonstrate important variations in cell damage concentrations. *Allium sativum*is anatural chelating agent and availability of this plant is high to the people. Therefore, people can easily afford the treatment of arsenic toxicity.

1.7.4 Aim of the Study

The aim of this study is the *in vivo* evaluation of the chelation effect of *Allium sativum* on the depletion of arsenic from the cellular level, using animal model.

1.7.5 Objectives of the Study

The objectives of this study by using natural extract of *Allium sativum*are -

- To develop arsenicosis within mice model
- To investigate the hematological and histopathological changes
- To observe changes in the organs like, kidney, liver, thoracic artery, brain of mice model
- To identify whether the selected plant has any effect on the depletion of arsenic
- To observe if further complication arises or not

Chapter 2

Literature Review

In ancient and contemporaneous documents, *Allium sativum,* has been used in the development of various circumstances and disease as a natural medicinal medicine. There is evidence that garlic is used in Middle East and East Asia to treat bronchitis, hypertension, tuberculosis, liver disease, intestinal worms, rheumatism, diabetes and fewer. Garlic is a medicinal plant of the highest quality that is effective against bacterial, viral, fungal and parasites owing to its preventive impact on cardiovascular disorders, decrease of blood sugar and LDL cholesterol (Korean Medical Science, 1987;Sadi, Ouldali, & Aoues, 2015;Vale & Bradberry, 2001; Yun, Kim, Kwon, Kang, & Om, 2011;Bajpai, Kim, & Kang, 2017).

Garlic, furthermore, was recommended as an addicting agent for heavy steel poisonings, as hypolipidemic, hypoglycemic, anticoagulant, antimicrobial, anticancerous, antitumor, hepatoprotective, anti-atherosclerotic, antimo-modulator, anti-diabeticand antioxidant. Several studies have verified that garlic is effective in the exercise of antigenotoxic, anticlastogenic results, with slow reporting of atherosclerosis, with several beneficial impacts on health reputations composed of disslipeaemia, anti-Thrombotic and vasodilator. A number of chosen viruses made from herpes virus kinds 1 and 2, parainfluenza virus type 3, etc. have also been recorded as virucidal and act as a full-size vasodilator. The radical capacity to scavenge garlic is another significant property. The BaP-induced neoplasms of the mouse forestomach have been found to suppress diallylsulfide, allyl methyl and diallyltrisulfide. These elements improve the phase of forestomach transfer by glutathione and thus accelerate the detoxification of the carcinogen (Silva, 2015)

Chapter 3

Methodology

3.1 Collection of Plants

For the investigation *Alliumsativum* was chosen. Although some previous study was conducted on its chelation activity, it was chosen for investigation decided to be chosen to compare with the previous study and for its availability. *Allium sativum*was collected from local market from Dhaka, Bangladesh.

3.1.1 Chemical Investigation of *Allium sativum*

Name of the Plant: *Allium sativum*

Family: Amaryllidaceae

Plant part: Cloves

3.2 Extraction Procedure

The extraction process can be divided into 2 steps:

1. Plant material preparation and drying
2. Extraction

3.2.1 Plant Material Preparation and Drying

The bulbs were separated and washed thoroughly with clean water so that any dirt or dust can be removed. Thereafter, the washed plant parts were shed dried for some days until they were properly dried and ready for size reduction.

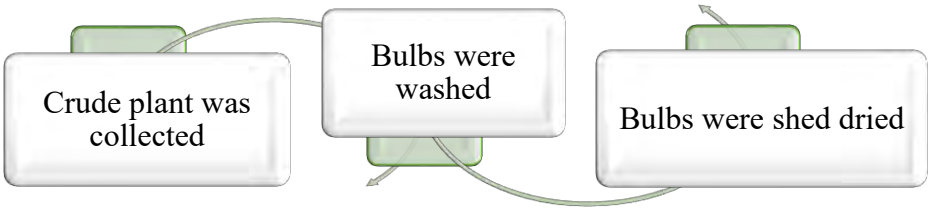

Figure 7 Plant material preparation and drying

3.2.2 Extraction Process

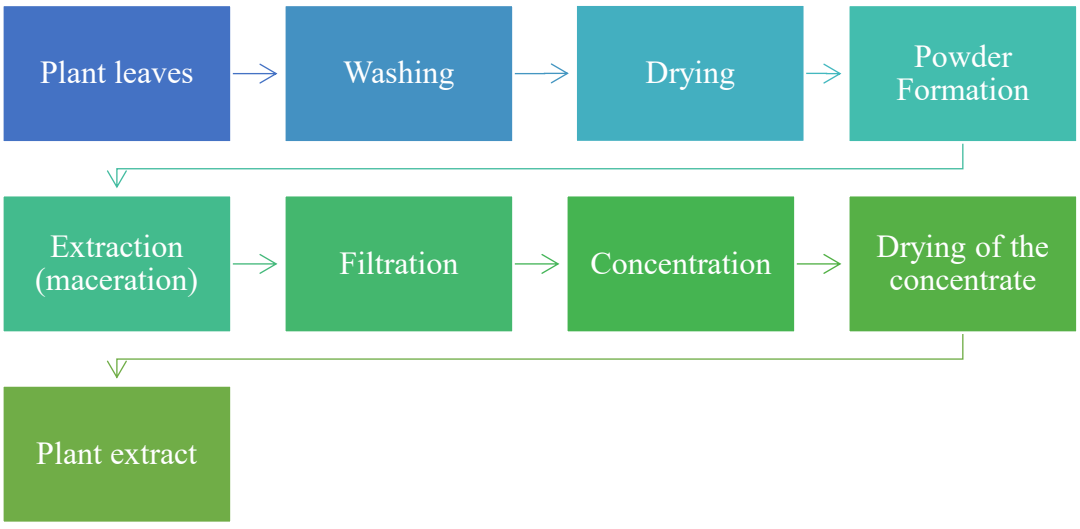

Figure 8 Step by step process for extraction of medicinal plant

3.2.3 Size Reduction and Weighing

The Bulbs were taken and grounded in a high capacity grinding machine separately to turn them in to coarse powder. The powder was then kept safely in an air tight glass container which ensures no risk of contamination. Then the container was labeled with necessary information for and then it was left in a cool and dark place awaiting the necessity of extraction procedure. The total amount of the powder of cloves was determined by weighing them and the amount was recorded.

3.2.4 Soaking

To make extract of the bulb powder of *Allium sativum*, methanol was used. The amount of methanol was calculated depending on the weight of the leaf powder. The powder was kept into methanol solution almost for 20 days in the room temperature (22-25 °C). Regular stirring have been done to make the powders properly soaked into the solvent.

3.2.5 Filtration

After soaking, cotton filtration was employed to get the filtrate of the cloves.

3.2.6 Concentration

Filtrate had been transferred to rotary evaporator to concentrate the filtrate and removal of methanol. It took more than two hours to get the proper concentration of extract.

3.2.7 Drying of Concentrate

Laminar Air Flow (LAF) was used to evaporate solvent to stay away from any chance of microbial augmentation at the time of drying the extract. Right following the concentration process the petri-dishes were positioned below Laminar Air flow so as to disperse the solvent from the extract. When the extract is successfully dried, the petri-dishes were safely enclosed with aluminum foil and then chilled them for more investigation.

3.2.8 Yield of Extract

Weight of plant extract was 120.6 gm after drying.

$$\%\ \text{of Yieldvalue} = \frac{\text{Extract weight}}{\text{Powderweight}} \times 100$$

$$= \frac{120.6}{856.45\ gm} \times 100$$

$$= 14.08\ \%$$

3.3 Phyto-chemical Screening

With a view to access the qualitative chemical composition for instance, alkaloids, reducing sugar (carbohydrates), sapponiside, flavonoids, glycosides, protein, sterol, triterpene Phytochemical screening was done on the crude extort of *Allium sativum.*

The following qualitative tests were performed:

3.3.1 Analyzing the Presence of Alkaloid

In order to analyze alkaloid compound in the sample extract, 2 tests were conducted - Mayer'stest and Wagner's test. For the test, methanolic extract of 2 gm of *A. sativum* was dissolved in 5 mL of 1 % hydrochloric acid separately. After that it was boiled in a water bath and then filtration was done. The filtrate was used to perform the following tests.

Mayer's test:

Mayer's reagent (10 mL) was arranged by dissolving 0.1358 g of mercuric (2) chloride and 0.5 g of Potassium Iodide in 10 mL distilled water.To1 mLoffiltrate,3 to 4 drops of Mayer's reagent are added by the side of the test tube. A white or creamy precipitate would indicate the test as positive. (Susmitha, 2013)

Wagner's test:

By dissolving 0.2 g of iodide crystals and 0.6g of potassium iodide in10 mL distilled water, Wagner's reagent (10 mL) were prepared. To 1 mL of filtrate, 3 to 4 drops of Wagner's reagent were added by the side of the test tube. A reddish–brown precipitate would confirm the test as positive. (Neelapu, et al, 2010)

3.3.2 Analyzing the Presence of Glycosides

Two tests were done to evaluate the presence of glycosides in the plant extract: Borntrager's test and Modified version of Borntrager's test.

Borntrager's test:

The sample drug was boiled with 5 ml of 10% sulphuric acid for 2 minute. It was filtered while hot, then cooled and the filtrate was shaken with equal volume of chloroform. The

chloroform layer was allowed to separate completely from the lower layer, which was pipetted out and transferred out to a clean test tube. Then half of its volume of aqueous ammonia (10%) was added and shaken gently and the layers were allowed to separate. The lower ammonia layer will show red pink color due to presence of free anthraquinone (Dhani, 2018).

Modified Borntrager's test:

5ml of dilute HCl and 5 ml of 5% solution of ferric chloride were added in a small portion of sample. Then the mixture was boiled for few minutes and then subsequently cooled and filtered part is shaken with chloroform. The organic layer was separated and shaken with 3/5 mL of dilute ammonia solution. A pink-red color in the upper aqueous layer indicates the presence of free anthraquinone.(Dhani, 2018).

3.3.3 Analyzing the presence of carbohydrates

In order to evaluate the presence of carbohydrate in the sample extract, three tests were conducted: Fehling's test, Benedict's test and Barfoed's test.

Fehling's Test:

In a test tube, 2 ml of the sample solution and equal volumes of Fehling A & Fehling B were placed and it was kept in boiling water bath for few minutes. When the contents of the test tube came to boiling, it was observed if any change in color or precipitate formation were seen.

Benedict's Test:

In the test tube, with 2 ml of Benedict's reagent, 5-6 drops of the sample solution were added and it was mixed well. The test tube was kept in a boiling water bath for five minutes and observed any change in color or precipitate formation. (Qualitative Analysis , 2015)

Barfoed's Test:

2 ml of the test solution add about 2-3 ml of Barfoed's reagent were mixed well and the test tube was boiled for one minute in the water bath. When the contents of the test tube came to boiling, it was observed if any change in color or precipitate formation were seen. Since Barfoed's reagent is slightly acidic, this test is specific for monosaccharide (Qualitative Analysis, 2015).

3.3.4 Analyzing the Presence of Saponins

In order to be assured we have conducted Foam test.

Foam Test:

A small portion of sample was diluted with distilled water.The tube was shaken for 15 s and allowed to stand for 15 min. A height of persistent foam greater than 1 cm would indicate the presence of saponins.(Reuters, 2014).

3.3.5 Analyzing the Presence of Flavonoids

To evaluate flavonoids in the sample, Shinoda Test was done.

To the extract solution, few drops of Sodium hydroxide solution was added. Formation of an intense yellow color would turn to colorless on addition of few drops of dilute acetic acid would indicate the presence of flavonoids.(Nirmala, et al, 2015)

3.3.6 Analyzing the Presence of Proteins

To detect the presence of protein in the sample, Biuret Test was done.

Biuret Test

A small quantity of the dispersion was taken in a test tube and 2 ml of NaOH was added into it. Four to five drops of 1 % CuSO4 solution were also added. Formation of bluish solution will indicate the presence of protein (Biuret test : Principle, 2018).

3.3.7 Analyzing the Presence of Sterols

Salkwaski test was done to observe the presence of strolls in the compound.

Salkwaski Test:

Extract was treated with chloroform and concentrated sulphuric acid was added from the sides of the test tube. Formation of red colored lower layer indicates the presence of steroids (Neelapu et al., 2011)

3.3.8 Analyzing the Presence of Triterpenes/Triterpenoids

In order to analyze triterpenes in the sample extract, two tests were conducted - Salkowski test and Lieberman Burchardt test.

Salkwaski Test:

Extract was treated with chloroform Concentrated sulphuric acid was added from the sides of the test tube and formation of yellow colored lower layer indicates the presence of Triterpenoids(Neelapu et al., 2011)

Lieberman Burchardt Test:

Extract was treated with few drops of acetic anhydride. Then it was boiled and cooled down. Concentrated sulphuric acid was added from the sides of the test tube formation of deep red colored layer indicates the presence of Triterpenoids (Neelapu et al., 2011).

3.4 Animal grouping

Experiment on animal model had been done in Department of Pharmacy, Jahangir Nagar University. *Swiss albino* mice species was selected for the experiment. Thirty-six (36) healthy female mice were taken and divided into 6 groups. The experiment was conducted for eight weeks. One group was treated with arsenic (0.0075 mg/mL) only; one group was treated with extract 500 mg/kg, three groups were treated with arsenic and extract 150 mg/kg, 300 mg/kg and 500 mg/kg and another group was for distill water which was control.

Figure 9 Swiss albino mice (The Jackson Laboratory, 2019)

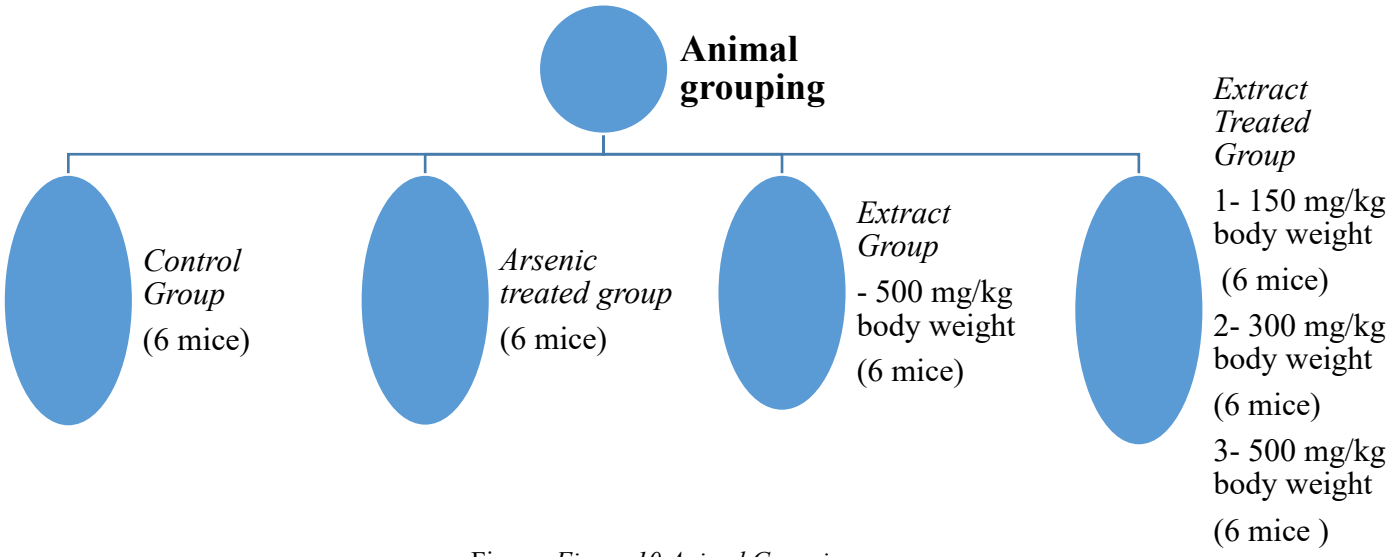

Figure: *Figure 10 Animal Grouping*

3.5 Doses of Chemical product and Organic extract

Table 1 Doses of Chemical product and Organic extract

To create arsenicosis	To treat arsenicosis
Sodium arsenite - 0.0075 mg/mL	Plant Extract- • 150 mg/kg body weight • 300 mg/kg body weight • 500 mg/kg body weight

Table 2 Animal Observation Period

Number of weeks	
1st week, 2nd week	▪ Starting of inducing (orally) Na arsenite to create arsenicosis in to four animal groups: - Positive control group/ Arsenic treated group - Extract treated group 1 - Extract treated group 2 - Extract treated group 3 ▪ Negative Control group had only distilled water. ▪ Extract group had plant extract at 500mg/kg body weight dose.

3rd week, 4th week, 5th week, 6th week, 7th week, 8th week	▪ Extract treated groups (1, 2, 3) had extracts in different doses along with Na arsenite dose. ▪ Positive control group/Arsenic treated continued with Na arsenite dose. ▪ Negative Control group had only distilled water. ▪ Extract group continued with plant extract at 500mg/kg body weight dose.

3.6 Materials and reagent

- ✓ Plant extract: *Allium sativum*(Methanol extract)
- ✓ Distilled water;
- ✓ Normal saline;
- ✓ Syringe;
- ✓ Cotton and antiseptic agent;
- ✓ Arsenicosis inducing agent (Sodium arsenite);
- ✓ Formalin;
- ✓ Ketamine;
- ✓ Test tubes;
- ✓ Appendrop tubes;
- ✓ Scissors;
- ✓ Glass slides;
- ✓ Petri-dish;

3.7 Experimentation

After administration of extract doses according to previous researches, eight weeks later animals were sacrificed and the dissection took place.

3.7.1 Collection of blood samples and serum evaluation

Blood was sample collected by syringe from heart and later those were centrifuged for analyzing different hematological parameters like, White Blood Cell Count (WBC), Red Blood Cell Count (RBC), Neutrophil count (NEU), Lymphocyte Count (LYMPH), Monocyte Count (MON) and Eosinophil Count (EOS).

Plasma and serum specimens were obtained and used for determination of Creatinine (CRE), Serum Glutamic Pyruvic Transaminase (SGPT), Alkaline Phosphatase (ALP) levels.

3.7.2 Histopathological study

Organs like; livers, kidneys, skin and brains were removed and kept in 10% buffered formalin solution, passed through ascending series of saline baths for further histopathological study.

3.8 Statistical analysis

Data is expressed as Means ± Standard Error Mean (S.E.M.). Data comparisons were carried out using one-way analysis of variance followed by independent samples T-Test to compare means between groups and to observe the significance level. Significant level has been considered <0.05 (p-value).

Chapter 4

Results

4.1 Phytochemical Screening of Methanolic Extract of *Allium sativum*

Table 3 Screening of Methanol Extract of Allium sativum

Class of compounds	Name Of The Test	Presence – (+) Absence – (-)
Carbohydrates	Fehling's Test	-
	Benedict's Test	+
	Barfoed's Test	-
Alkaloids	Mayer's Test	+
	Wagner's Test	+
Glycosides	Bortrager's Test	-
	Modified Bortrager's Test	-
Saponins	Foam Test	+
Protein	Biurete Test	+
Sterol	Salkwaski Test	-
Triterpenes	Salkwaski Test	+
	Lieberman Burchardt Test	+
Flavonoids	Shinoda Test	+

4.1.1 Phytochemical Screening of Methanolic Extract of *Allium sativum* (images)

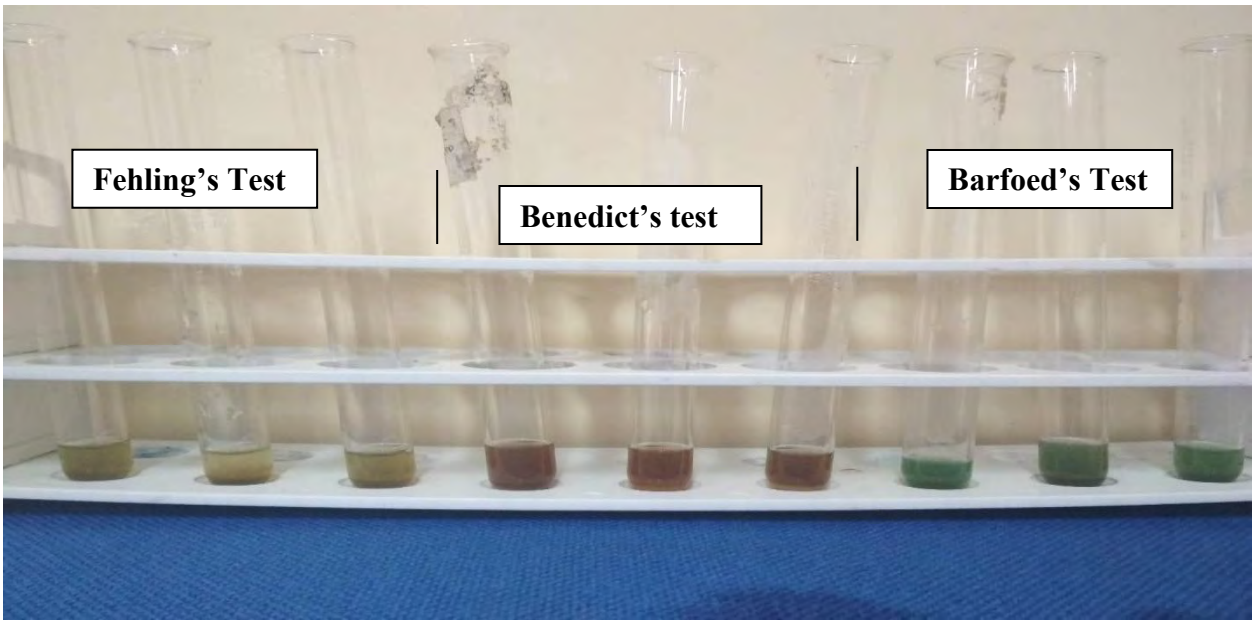

Figure 11 Screening of Carbohydrates

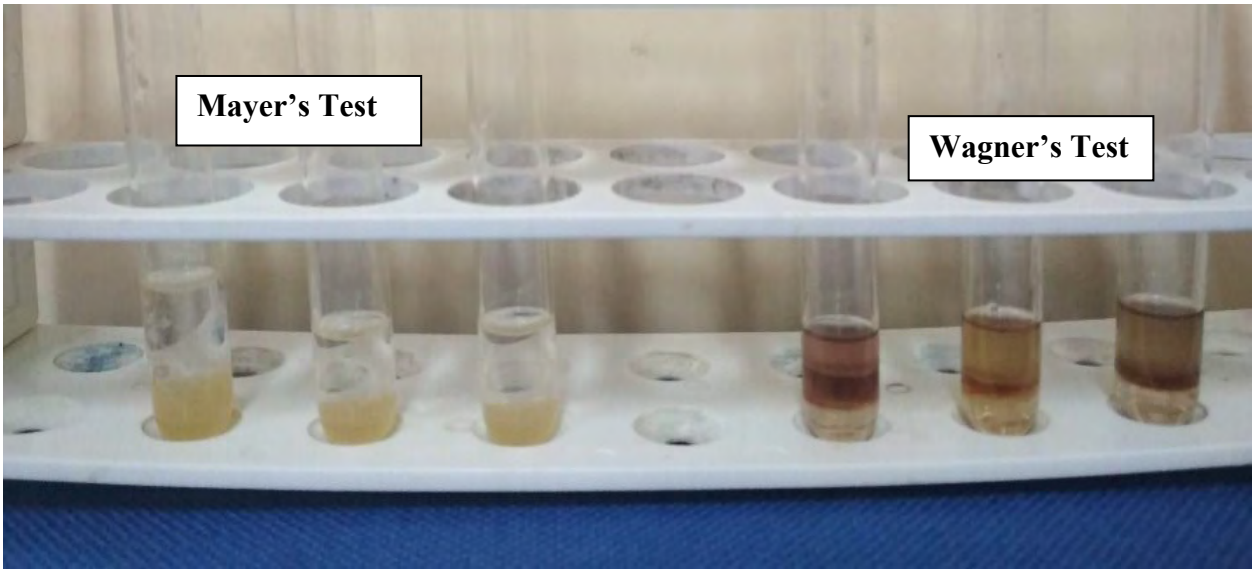

Figure 12 Screening of Alkaloids

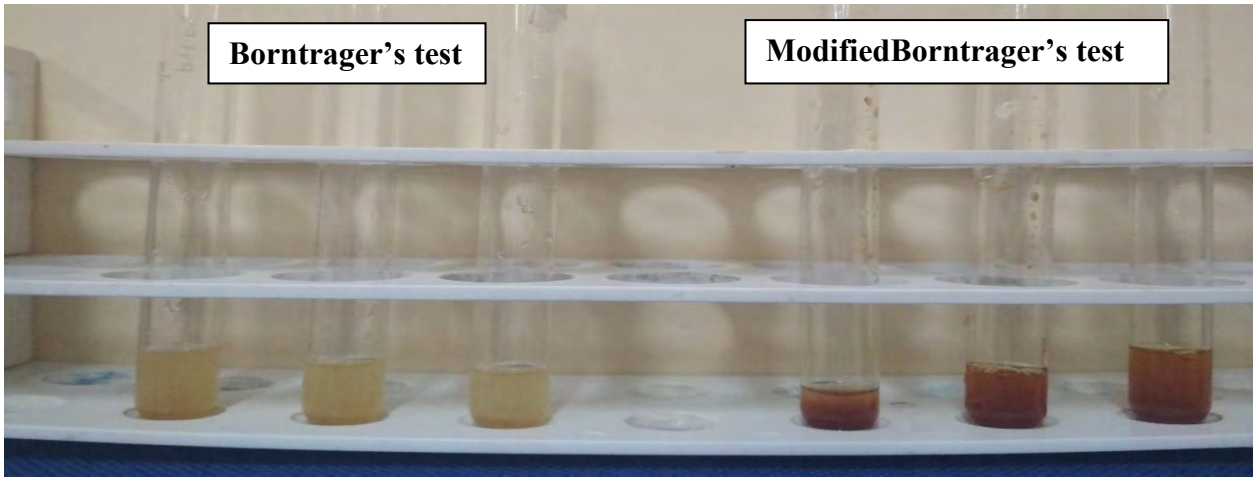

Figure 13 Screening of Glycoside

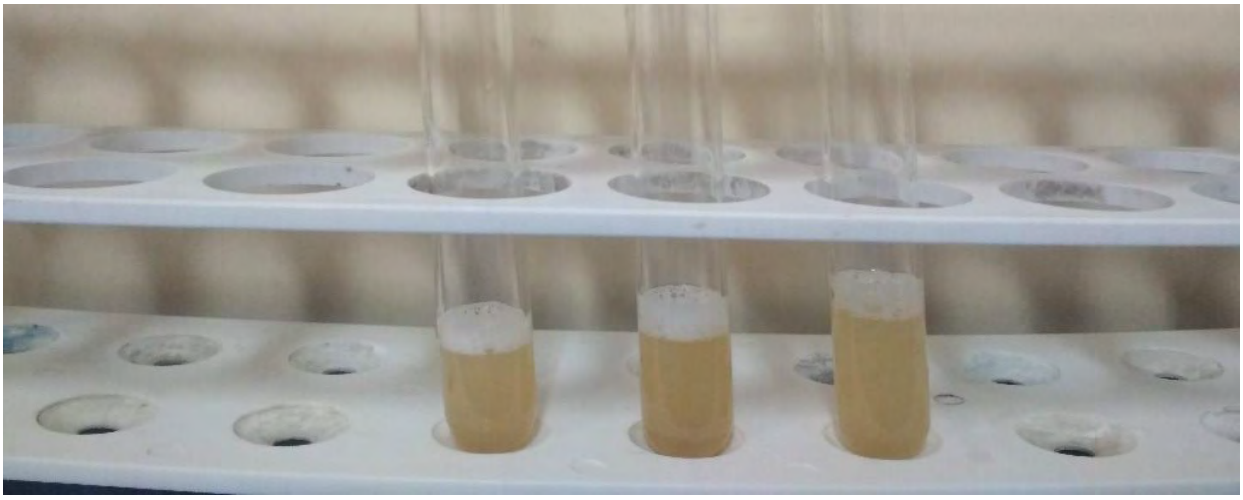

Figure 14 Screening of Saponiside

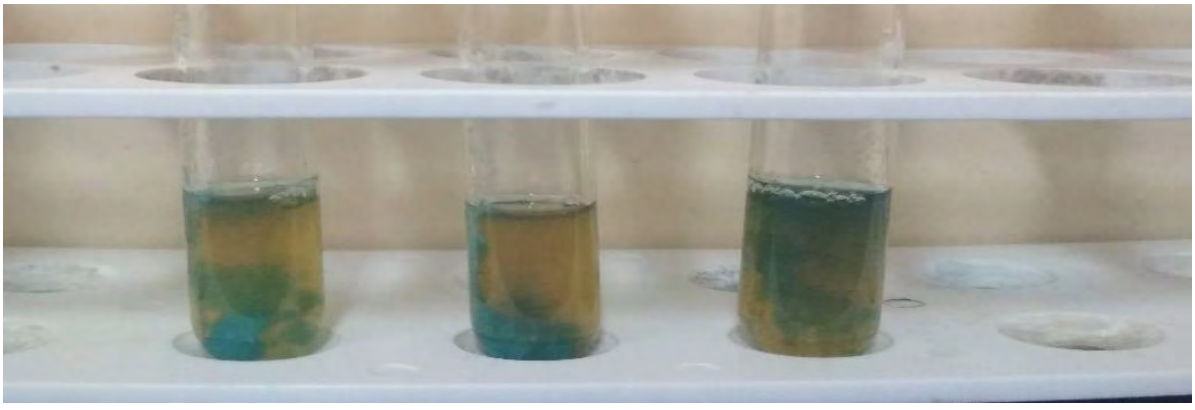

Figure 15 Screening of Protein

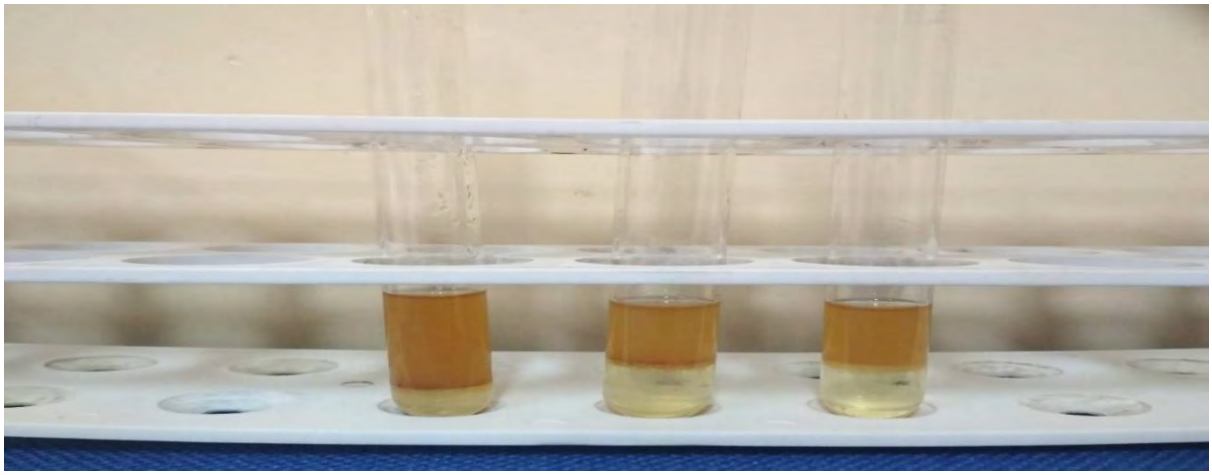

Figure 16 Screening of Flavonoid

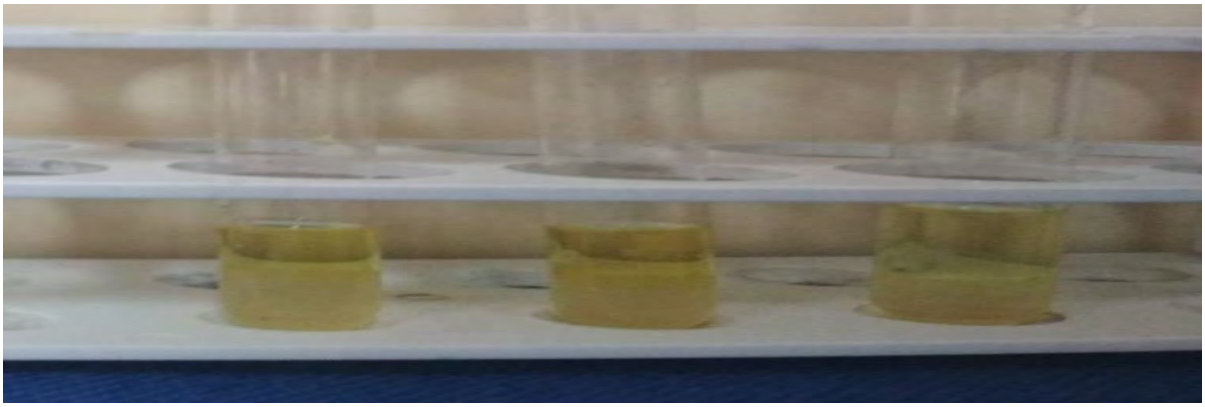

Figure 17 Screening of Sterols

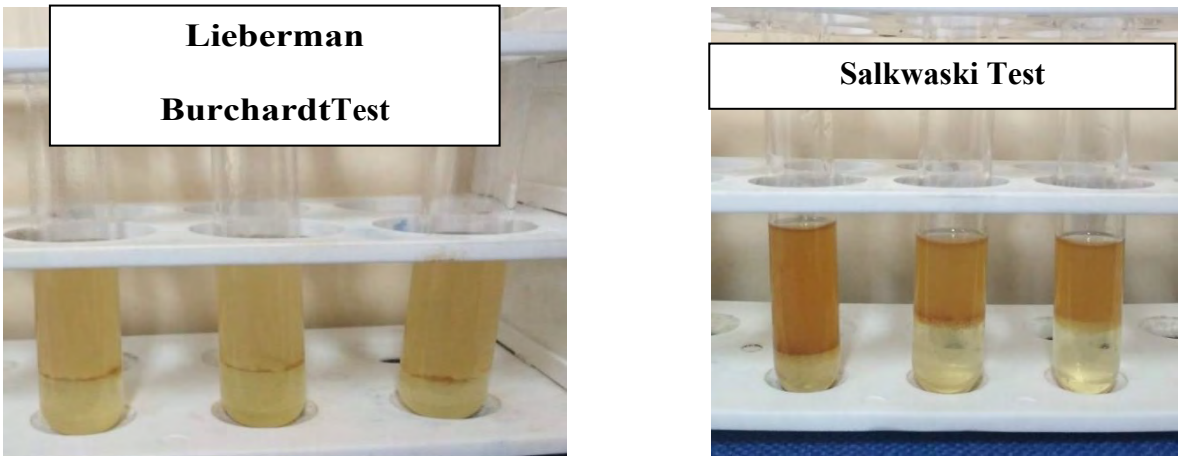

Figure 18 Screening of Terpinoids

4.2 Hematological and Biochemical Parameters

In order to evaluate the changes in blood and body enzymes of *Swiss albino* mice after eight weeks due to the continuous administration of Garlic Extract (GE) on three different doses (150, 300, 500 mg/kg), Na arsenite (Arsenic treated) as well as GE 500 mg/kg solely, few hematology and biochemical tests were conducted such. As it was mentioned, the parameters are the White Blood Cell Count (WBC), Red Blood Cell Count (RBC), Neutrophil count (NEU), Lymphocyte Count (LYM), Monocyte Count (MON) and Eosinophil Count (EOS), Creatinine (CRE), Serum Glutamic Pyruvic Transaminase (SGPT), Alkaline Phosphatase (ALP) levels.

Below the test results and their significance towards change are shown using different tables.

*Table 4 Effects arsenic and different doses of GE**

Parameters	**Arsenic Treated**	**Arsenic + GE 150 mg/kg**	**Arsenic + GE 300 mg/kg**	**Arsenic + GE 500 mg/kg**
WBC (x 10^9 cells/ liter)	6.30±0.316	4.5± 0.184***	5.66± 0.57	5.68± 0.43
RBC (cells/µL)	5.26± 0.06	5.20± 0.15	5.21± 0.06	5.36± 0.07
NEU (cells/µL)	59.33± 1.45	50.33± 2.48	53.83± 2.85	54.16± 2.42
LYM (x 10^9 cells/ liter)	33.16± 1.13	42.16± 2.61**	38.33± 2.40	40.00± 2.25**
MON (cells/ liter)	3.83± 0.65	4.16± 0.40	4.33± 0.42	3.00± 0.00

Parameters	Arsenic Treated	Arsenic + GE 150 mg/kg	Arsenic + GE 300 mg/kg	Arsenic + GE 500 mg/kg
EOS (cells/ liter)	3.16± 0.30	3.33± 0.33	3.50± .42	3.00± 0.36
CRE (mg/dL)	0.75± 0.71	0.80± 0.05	1.06± 0.88**	0.78± 09
ALP (U/L)	1.34± 0.02	1.59± 0.63**	1.61± 0.06**	1.50± 0.07**
SGPT (U/L)	51.66±2.18	56.16 ± 3.19	62.50± 3.34	55.16± 5.83

* Values are given as Mean ± Standard Error Mean (SEM) for group of six animals each

** p< 0.05, significance level when compared to arsenic exposed *Swiss albino* and extract treated *Swiss albino*

*** p< 0.001, significance level when compared to arsenic exposed *Swiss albino* and extract treated *Swiss albino*

Here, some significant changes are observed in case of WBC, LYM, and ALP when arsenicosis induced mice are treated with 150 mg/kg *Allium sativum* extract (GE). In case of treatment with 300 mg/kg, ALP and CRE levels have significant difference, whereas 500 mg/kg dose of extract has significant effect on LYM and ALP levels.

*Table 5 Effects of control and arsenic treatment**

Parameters	Control	Arsenic Treatment
WBC (x 10^9 cells/ liter)	7.83± 0.542	6.30± 0.316
RBC (cells/µL)	5.36± 0.098	5.26± 0.06
NEU (cells/µL)	54±3.08	59.33± 1.45
LYM (x 10^9 cells/ liter)	39.50± 3.53	33.16± 1.13
MON (cells/ liter)	11.66± 8.27	3.83± 0.65
EOS (cells/ liter)	2.66± 0.33	3.16± 0.30
ALP (U/L)	1.61± 0.09	1.34± 0.02**
SGPT (U/L)	56.83± 2.98	51.66± 2.18
CRE (mg/dL)	0.86± 0.08	0.75± 0.07

*values are given as mean ± standard error mean (SEM) for group of six animals each.

**$p < 0.05$, significance level when compared to control and arsenic treated *Swiss albino* mice

If comparison between control group which only had distilled water with arsenic treated group which was exposed to As in the dose of 0.0075 mg/mL, only the levels of ALP have significantly changed whereas other hematological and biochemical parameters did not show any significance.

Table 6 Effects GE 500 mg/kg and three dose of GE for As treatment

Parameters	GE 500 mg/kg	Arsenic + GE 150mg/kg	Arsenic + GE 300mg/kg	Arsenic + GE 500mg/kg
WBC (x 10^9 cells/ liter)	6.43± 0.56	4.50± 0.56**	5.66± 0.57	5.68± 0.43
RBC (cells/µL)	5.33± 0.12	5.20± 0.15	5.21± 0.06	5.36± 0.07
NEU (cells/µL)	53.83± 3.23	50.33± 2.48	53.83± 2.85	54.16± 2.42
LYM (x 10^9 cells/ liter)	39.50± 3.41	42.16± 2.61	38.33± 2.40	40.00±2.25
MON (cells/ liter)	3.33± 0.49	4.16± 0.40	4.33± 0.42	3.00±0.00
EOS (cells/ liter)	3.33± 0.33	3.33± 0.33	3.50± 0.42	3.00± 0.36
ALP (U/L)	1.63± 0.11	1.59± 6.39	1.61± 0.11	1.50± 0.07
ALT (U/L)	62.83± 5.56	56.16± 3.91	62.50± 3.34	55.16± 5.83
CRE (mg/dL)	0.83± 0.06	0.80± 0.05	1.06± 0.88**	0.78± 0.09

*values are given as mean ± standard error mean (SEM) for group of six animals each.

**p< 0.05, significance level when compared to control and arsenic treated *Swiss albino* mice

When it comes down to the comparison of the three different doses of Garlic extracts (GE) which are 150 mg/kg, 300 mg/kg and 500 mg/kg induced into As exposed mice to treat arsenicosis with the maximum dose (500 mg/kg) solely (absence of As), only the levels of WBC and CRE show significant change. On the other hand, other parameters are not that much moved.

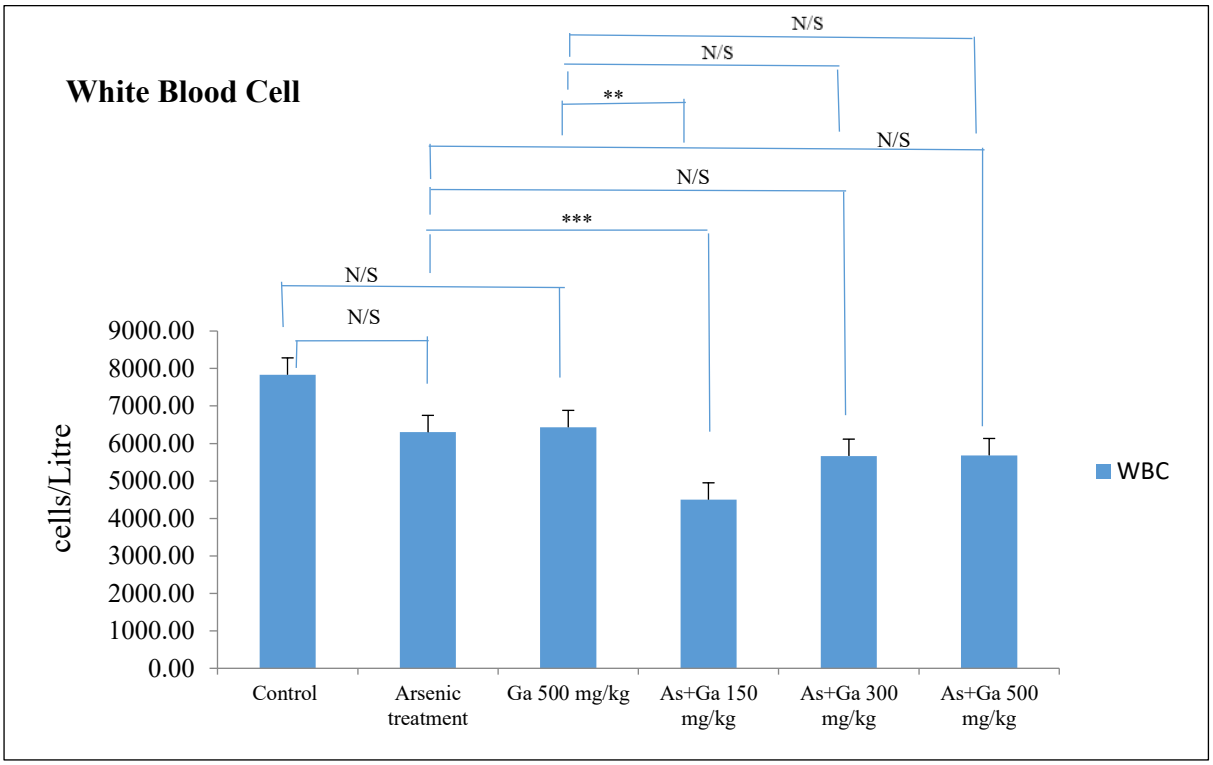

Figure 19 White Blood Cell (WBC) count

In Figure 20, White Blood Cell (WBC) count of control, arsenic treatment, garlic extract (GE) 500 mg/kg, arsenic and GE 150 mg/kg, arsenic and GE 300 mg/kg and arsenic and GE 500 mg/kg are shown.

Values are given as Mean ± Standard Error Mean (SEM) for group of six mice each. Significant differences:

Arsenic Treated group versus Garlic Extract 150 mg/kg : ***p <0.001;

Garlic Extract 500 mg/kg versus Arsenic and Garlic Extract 150 mg/kg : **p <0.01.

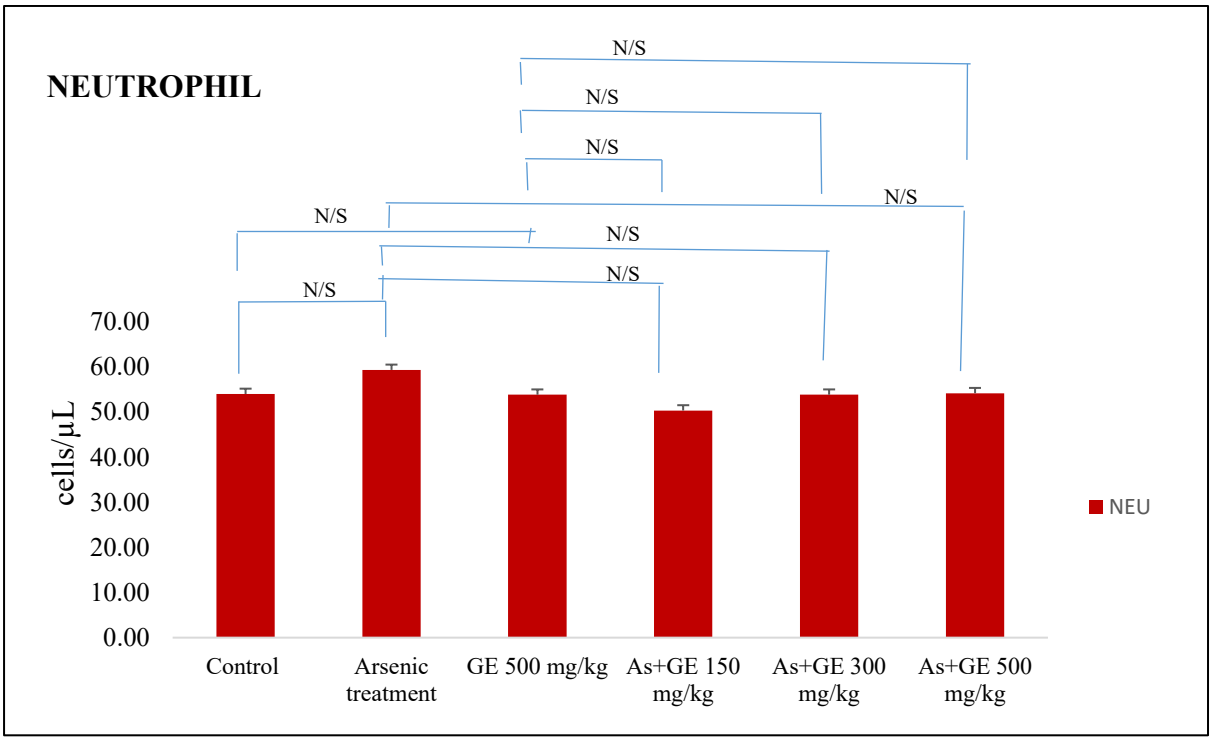

Figure 20Neutrophil count

In Figure 21, Neutrophil (NEU) count of control, arsenic treated, GE 500 mg/kg, arsenic and GE 150 mg/kg, arsenic and GE 300 mg/kg and arsenic and GE 500 mg/kg are shown.

Values are given as Mean ± Standard Error Mean (SEM) for group of six animal each.

No significant difference has seen.

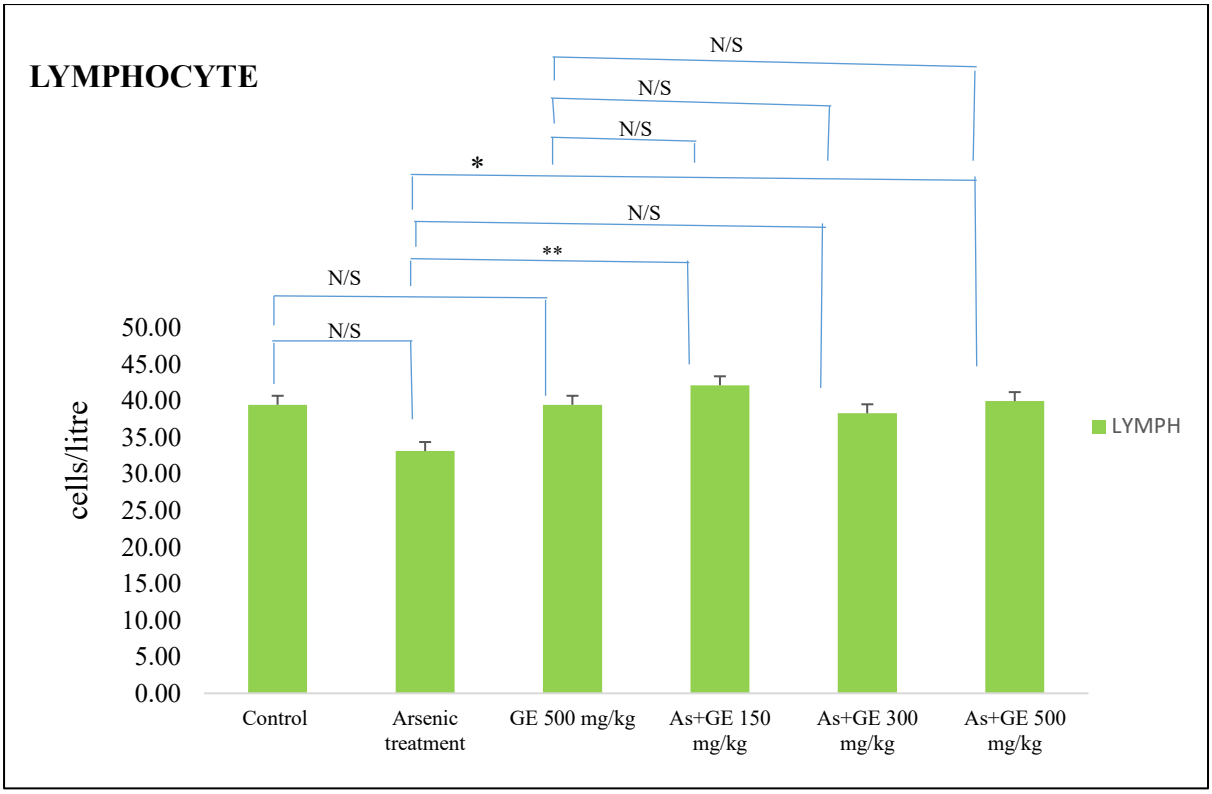

Figure 21Lymphocyte count

In Figure 22, Lymphocyte (LYMPH) count of control, arsenic treated, GE 500 mg/kg, arsenic and GE 150 mg/kg, arsenic and GE 300 mg/kg and arsenic and GE 500 mg/kg are shown.

Values are given as Mean ± Standard Error Mean (SEM) for group of six mice each. Significant differences:

Arsenic Treated group versus Garlic Extract 150 mg/kg : **p <0.01;

Arsenic Treated group versus Garlic Extract 500 mg/kg :*p <0.05.

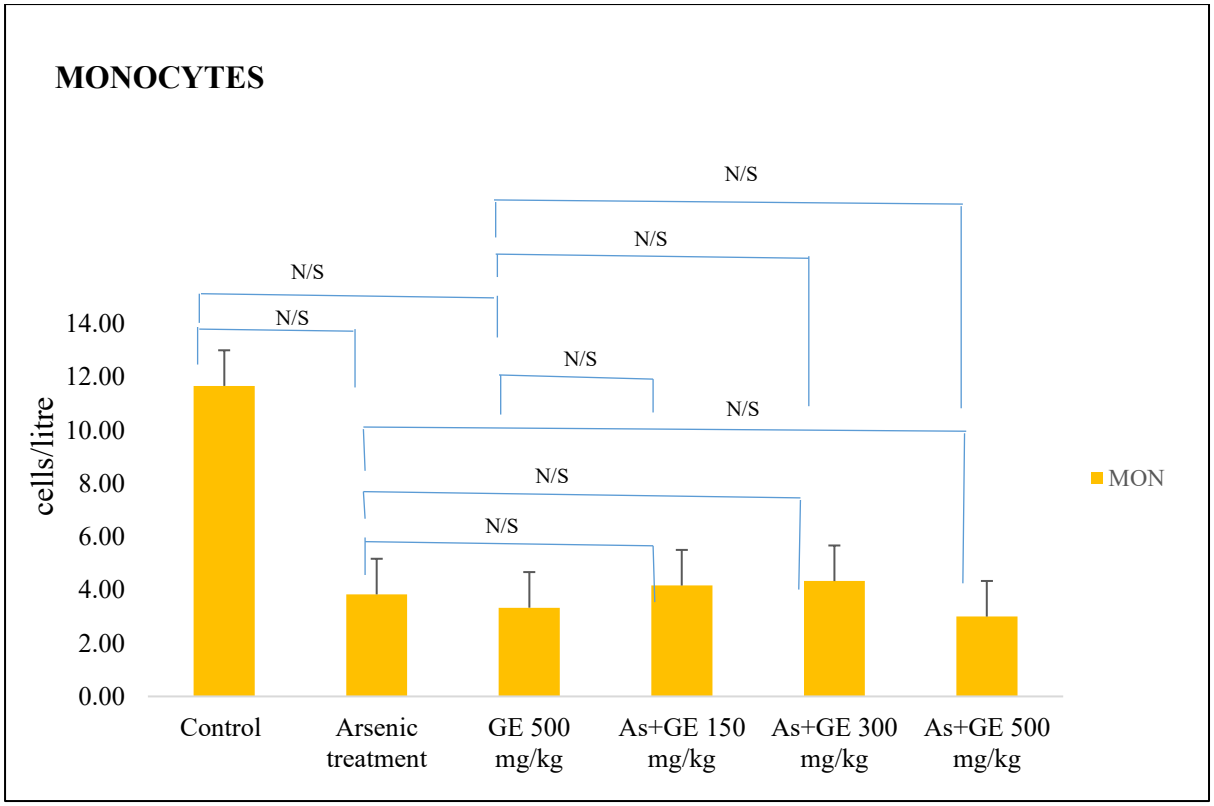

Figure 22Monocyte count

In Figure 23, Monocyte (MON) count of control, arsenic treated, GE 500 mg/kg, arsenic and GE 150 mg/kg, arsenic and GE 300 mg/kg and arsenic and GE 500 mg/kg are shown.

Values are given as Mean ± Standard Error Mean (SEM) for group of six mice each.

No significant difference has seen.

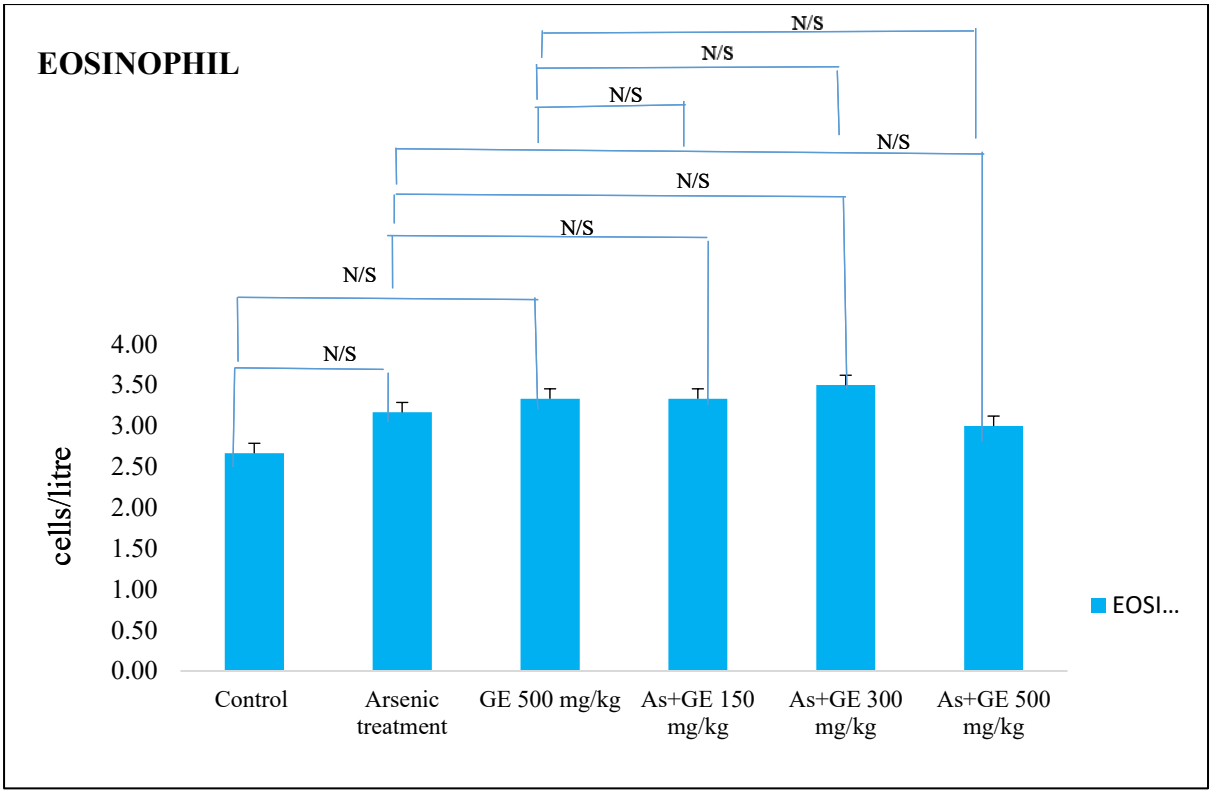

Figure 23Eosinophil count

In Figure 24, Eosinophil (EOS) count of control, arsenic treated, GE 500 mg/kg, arsenic and GE 150 mg/kg, arsenic and GE 300 mg/kg and arsenic and GE 500 mg/kg are shown.

Values are given as Mean ± Standard Error Mean (SEM) for group of six mice each.

No significant difference has seen.

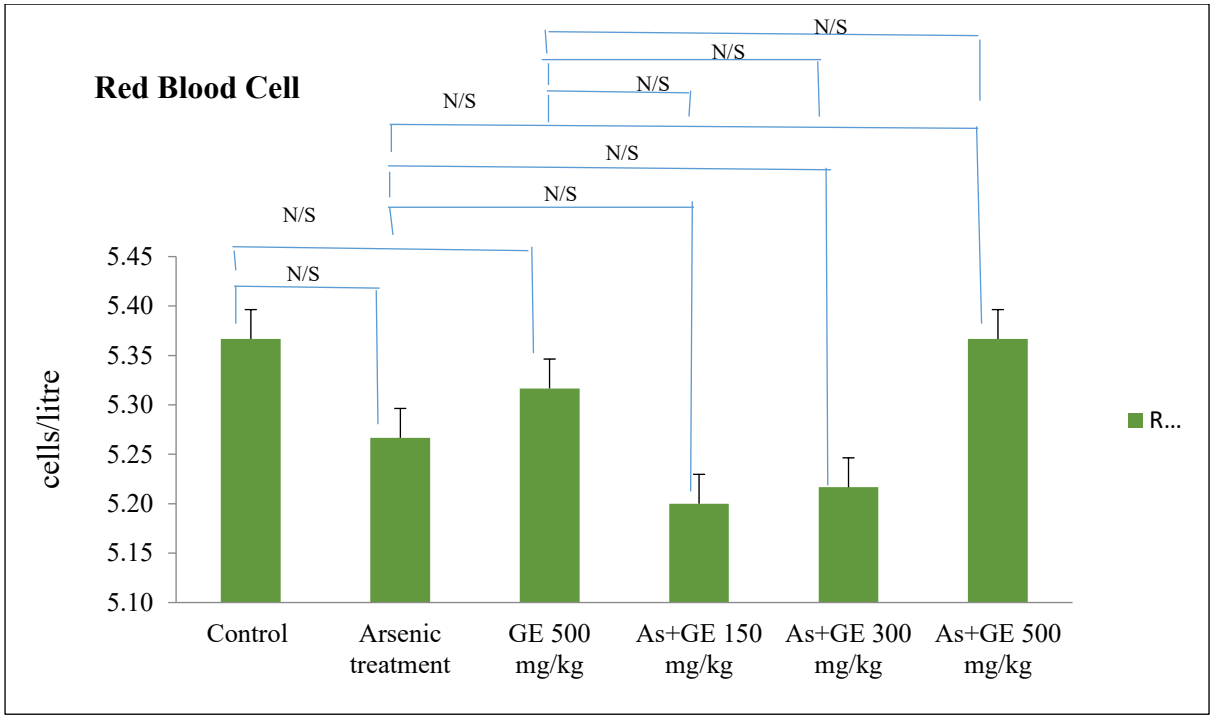

Figure 24Red Blood Cell count

In Figure 25, Red Blood Cell (RBC) count of control, arsenic treated, GE 500 mg/kg, arsenic and GE 150 mg/kg, arsenic and GE 300 mg/kg and arsenic and GE 500 mg/kg are shown.

Values are given as Mean ± Standard Error Mean (SEM) for group of six mice each.

No significant difference has seen.

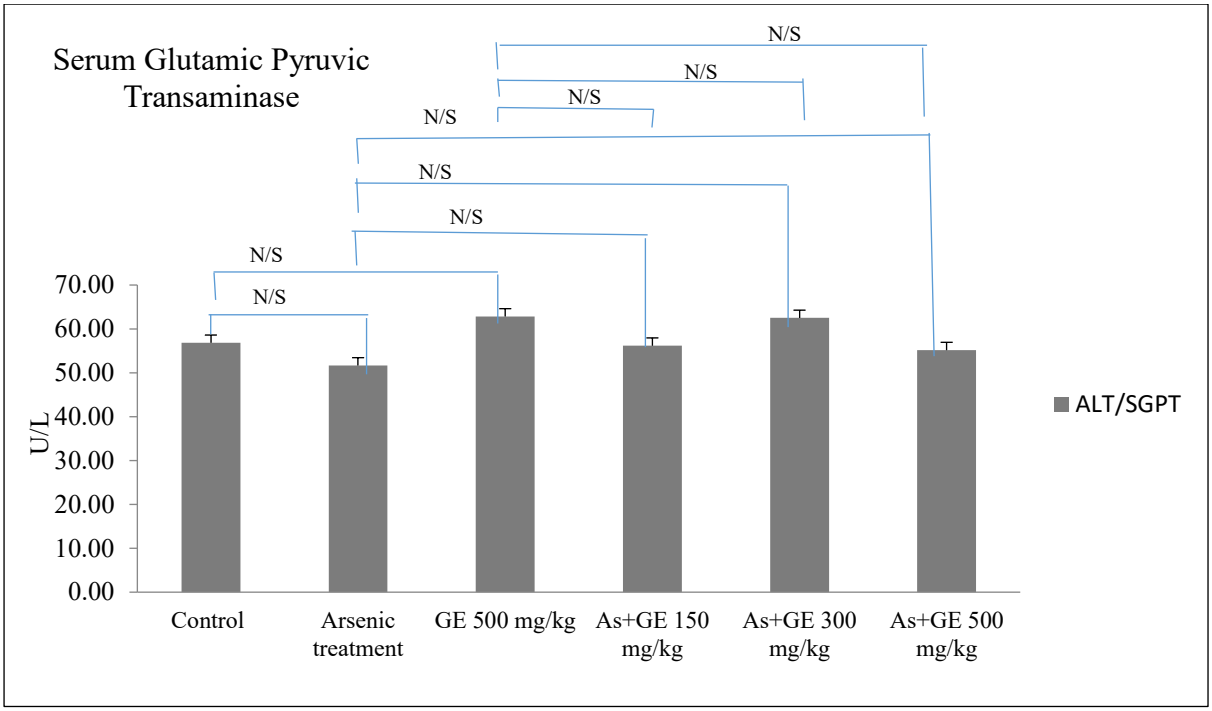

Figure 25Serum Glutamic Pyruvic Transaminase Level

In Figure 26, Serum Glutamic Pyruvic Transaminase (SGPT)of control, arsenic treated, GE 500 mg/kg, arsenic and GE 150 mg/kg, arsenic and GE 300 mg/kg and arsenic and GE 500 mg/kg are shown.

Values are given as Mean ± Standard Error Mean (SEM) for group of six mice each.

No significant difference has seen.

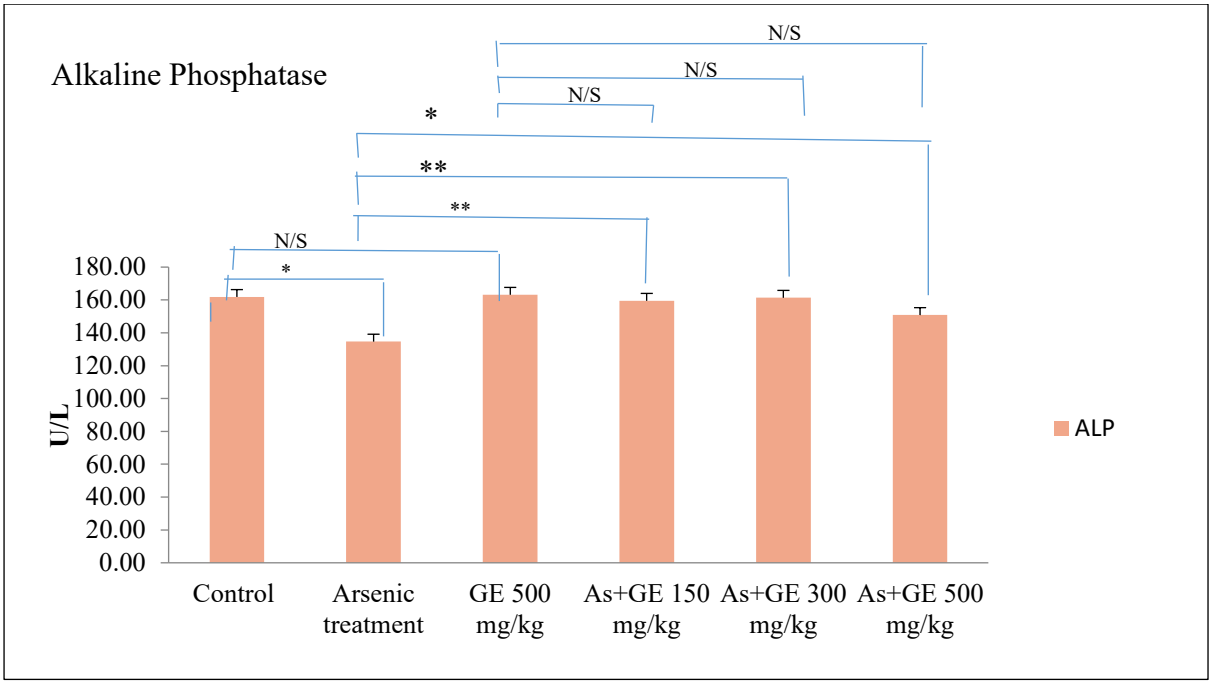

Figure 26Alkaline Phosphatase Level

In Figure 27, Alkaline Phosphatase (ALP) level of control, arsenic treated, GE 500 mg/kg, arsenic, GE 150 mg/kg, arsenic, GE 300 mg/kg, arsenic, and GE 500 mg/kg are shown.

Values are given as Mean ± Standard Error Mean (SEM) for group of six mice each. Significant differences:

Arsenic Treated group versus GE 150 mg/kg : **p <0.01;

Arsenic Treated group versus GE 300 mg/kg : **p <0.01;

Arsenic Treated group versus GE 500 mg/kg :*p <0.05.

Arsenic Treated group versus Control: *p <0.05.

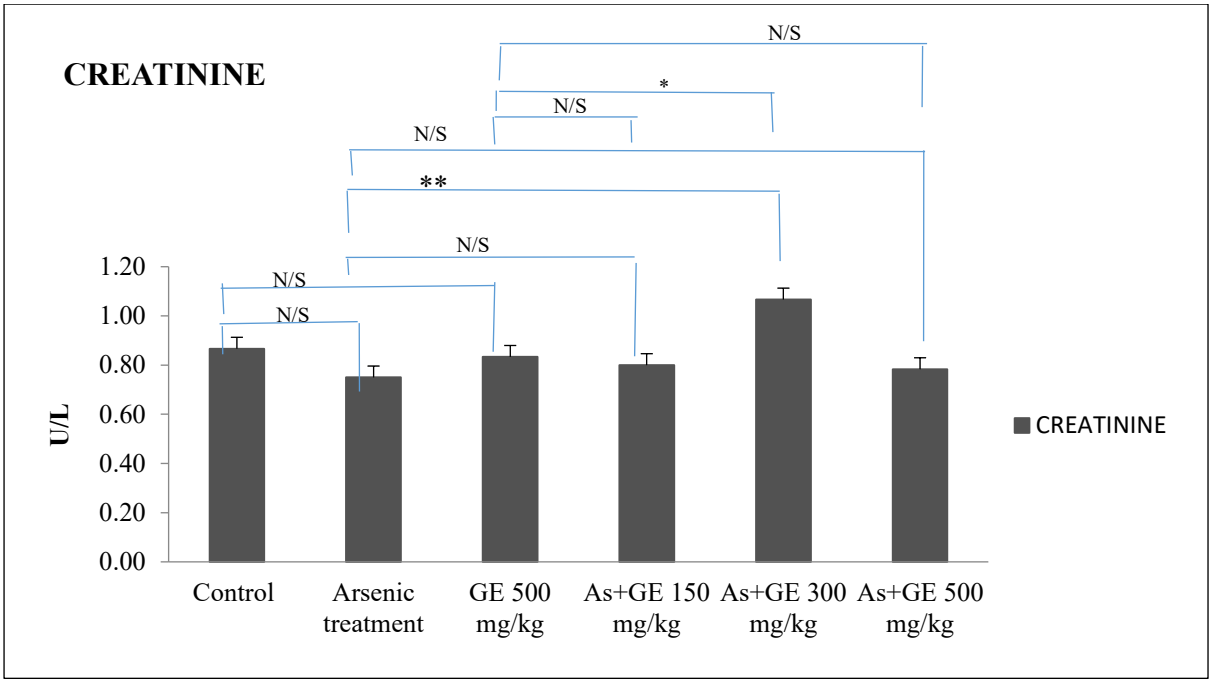

Figure 27Creatinine Level

In Figure 28, Creatinine (CRE) level of control, arsenic treated, GE 500 mg/kg, arsenic, GE 150 mg/kg, arsenic, GE 300 mg/kg, arsenic, and GE 500 mg/kg are shown.

Values are given as Mean ± Standard Error Mean (SEM) for group of six mice each. Significant differences:

Arsenic Treated group versus GE 300 mg/kg :**p <0.001;

Garlic Extract 500 mg/kg versus Arsenic and GE 300 mg/kg : *p <0.01.

Table 7 Average Data of Different Hematological And Biochemical Tests

Test Parameters	WBC ($\times 10^9$ cells/L)	NEU (cells/ µL)	LYM (cells/L)	MON (cells/L)	EOS (cells/L)	RBC (cells/ µL)	SGPT (U/L)	ALP (U/L)	CRE (U/L)
Control	7833.33	54.00	39.50	11.67	2.67	5.37	56.83	161.83	0.87
Arsenic Treatment	6300.00	59.33	33.17	3.83	3.17	5.27	51.67	134.67	0.75
GE 500 mg/kg	6433.33	53.83	39.50	3.33	3.33	5.32	62.83	163.17	0.83
As+GE 150 mg/kg	4500.00	50.33	42.17	4.17	3.33	5.20	56.17	159.50	0.80
As+GE 300 mg/kg	5666.67	53.83	38.33	4.33	3.50	5.22	62.50	161.33	1.07
As+GE 500 mg/kg	5683.33	54.17	40.00	3.00	3.00	5.37	55.17	150.83	0.78

4.3 Effects of Treatment on Body Weight

The variations in the body weights of *Swiss albino* mice subjected to different treatments are shown in Figure 29. In the present investigation, it is seen that the weights of animals have been increased throughout the study progressively. Although, the group which was induced As and GE 500 mg/kg shows the highest data comparing to other groups. On the contrary, As treated group was much influenced by the dose of Na arsenite which was 0.0075 mg/mL as this group did not gain weight drastically as the Treatment Group 3 has gained. Again, Treatment Group 3 had both As and GE 500 mg/kg.

Treatment Group 2 and 3 (As+GE 150 mg/kg and As+GE 300 mg/kg) have less effect on weight gaining compared to Treatment Group 1 and almost showing close Average data comparing with As treated group.

Adding to that, the control group is showing a significant data in case of body weight compared to other five groups.

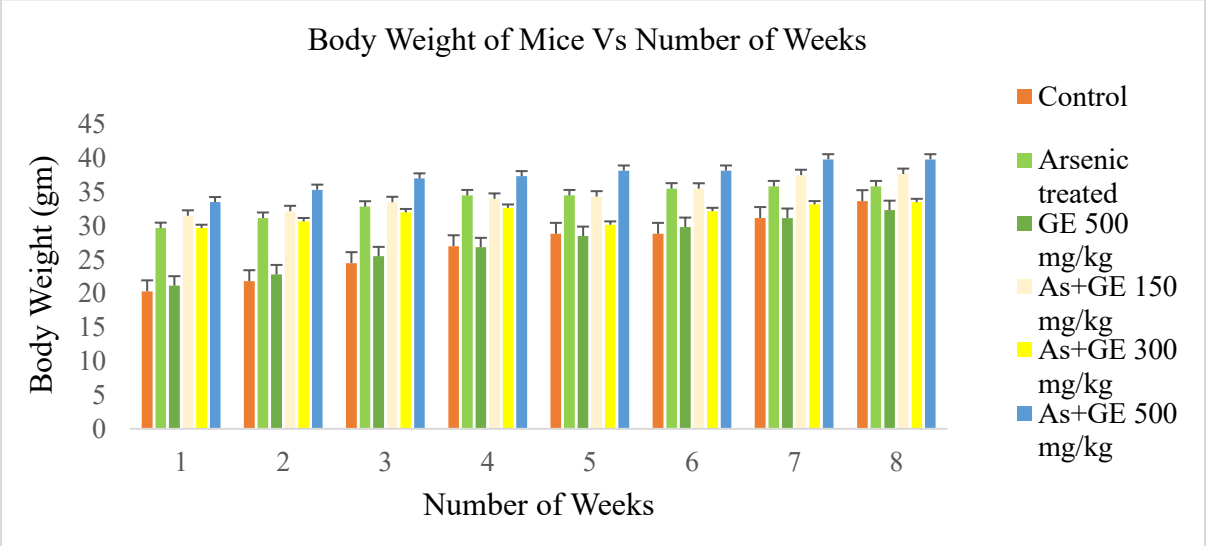

Figure 28 Effects of Different Treatments on Body Weight of Swiss albino

4.4 Histopathological Analysis

In order to evaluate the changes of tissue of *Swiss albino* mice due to induction of Arsenic, histopathology of liver, kidney, brain and skin were performed.

4.4.1 Histopathology of Liver Tissue

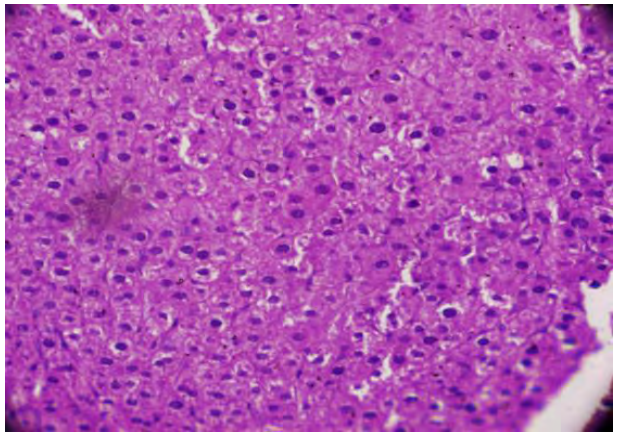

Figure 29Microphotograph of liver sections taken from mice of the control group. H and E staining (× 400).

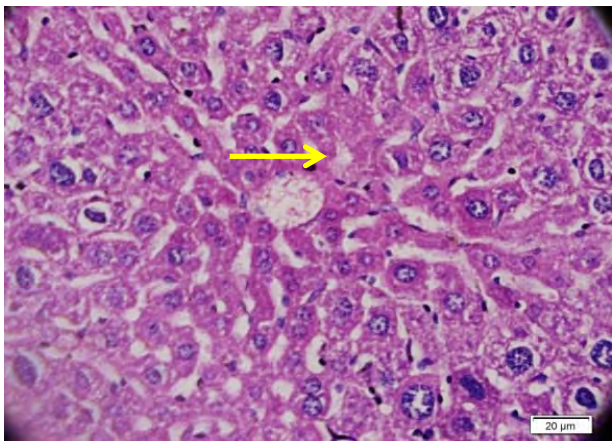

Figure 30 Microphotograph of liver sections taken from mice of the Arsenic treated group. H and E staining (× 400).

In Figure 32, section of mice liver structure from Arsenic treated group revealing slight sinusoidal dilation, fatty change edema and infiltration of inflammatory cell.

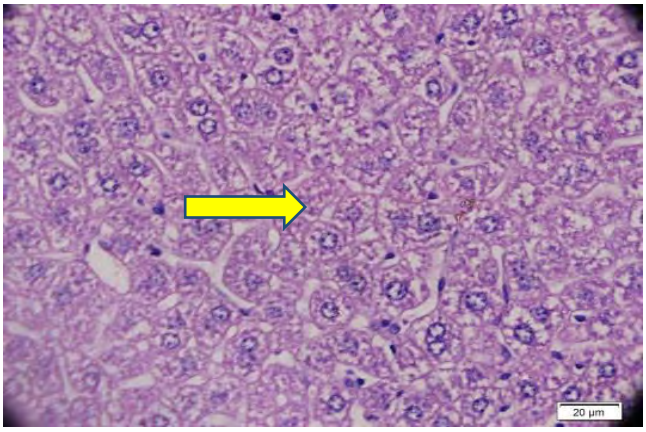

Figure 31Figure 4.4.3: Microphotograph of liver sections taken from mice of the As and GE 150 mg/kg extract treated group.

H and E staining (× 400).

In Figure 33, microphotograph of liver sections taken from mice of GE 150 mg/kg extract treated group showed slight signs of amelioration of Arsenic evoked liver injury which was evident from the presence of normal hepatic pods and the absence of necrosis and reduced fatty changes

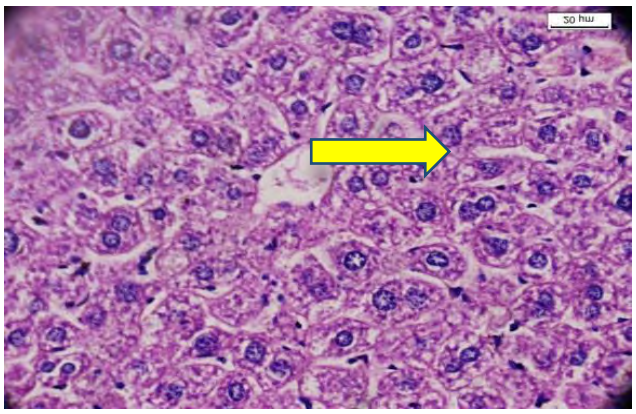

Figure 32Microphotograph of liver sections taken from mice of the As and GE 300 mg/kgtreated group.

H and E staining (× 400).

In Figure 34, GE 300 mg/kg treated group showed reduction of edema but three are still fatty deposit indicating slight improvement.

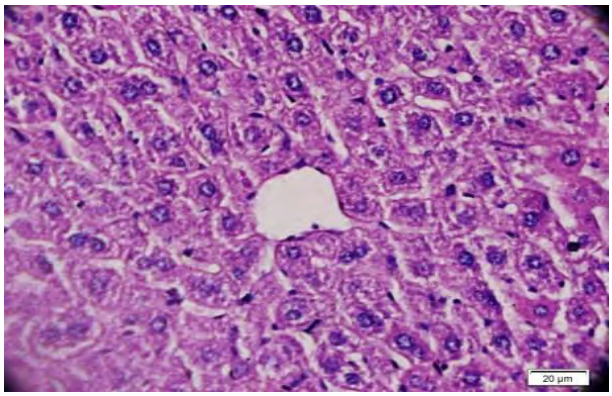

Figure 33Microphotograph of liver sections taken from mice of the As and GE 500 mg/kg treated group. H and E staining (× 400).

In Figure 35, Garlic 500 mg/kg extract treated rat liver showed regular hepatic lobules and no sign edema indicating significant improvement.

4.4.2 Histopathology of Kidney Tissue

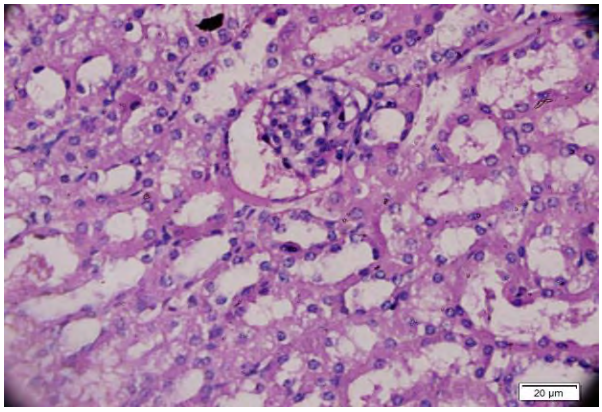

Figure 4.4.6:*Figure 34Microphotograph of Kidney sections taken from mice of the control group* . H and E staining (× 400).

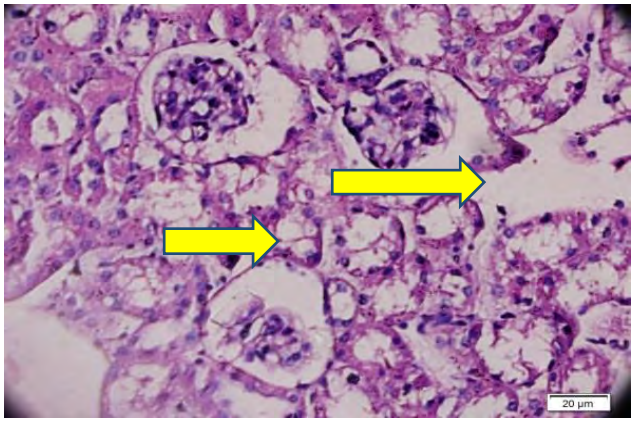

Figure 35Microphotograph of Kidney sections taken from mice of the Arsenic treated group. H and E staining (× 400).

In Figure 36, Photomicrograph of arsenic treated kidney showing fat bodies, desquamation of epithelium, tubular degeneration, intra-tubular degeneration, and congestion.

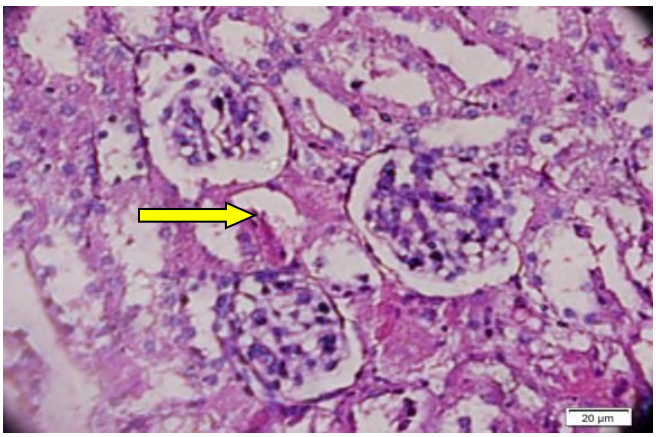

Figure 36Microphotograph of Kidney sections taken from mice of the As and 150 mg/kg extract treated group. H and E staining (× 400).

In Figure 37, which is GE 150 mg/kg treated group, there are desquamation of epithelium, tubular degeneration, intra-tubular degeneration, and congestion.

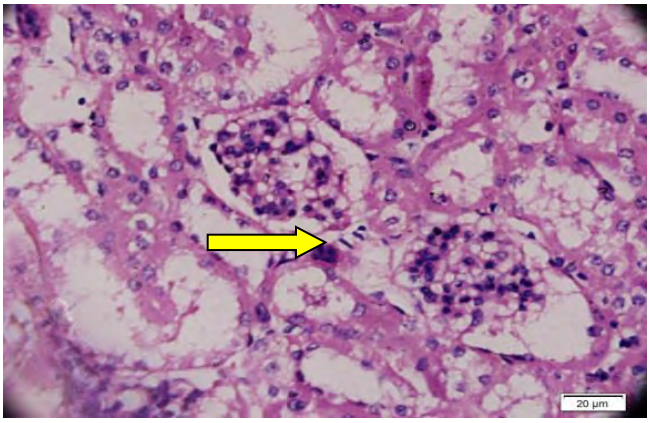

Figure 37Microphotograph of Kidney sections taken from mice of the As and GE 300 mg/kg treated group. H and E staining (× 400).

In Figure 4.4.9, GE 300 mg/kg treated group showed slight improvement restoration of epithelium, tubular degeneration, intra-tubular degeneration, and congestion.

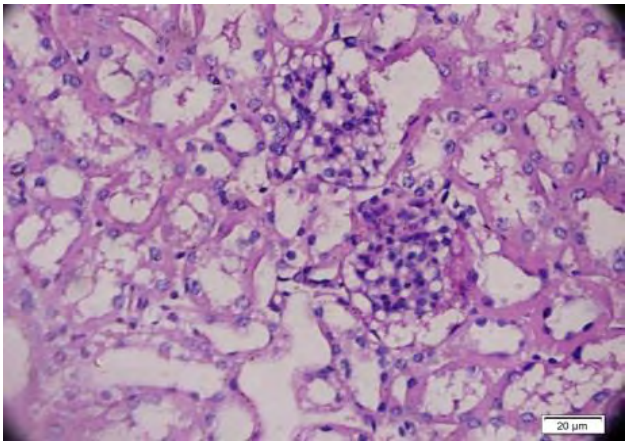

Figure 38Microphotograph of Kidney sections taken from mice of the As and GE 500 mg/kg treated group. H and E staining (× 400).

In Figure 40, GE 500 mg/kg treated group, there are restoration of epithelium, reduce tubular degeneration, but there are sign congestion.

4.4.3 Histopathology of Brain Tissue

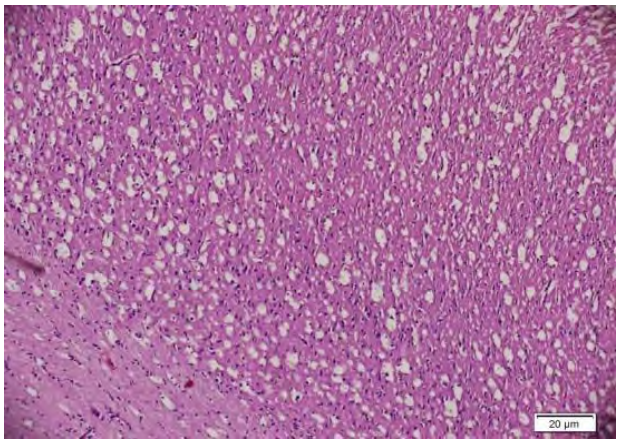

Figure 39Microphotograph of brain sections taken from mice of the control group.

H and E staining (× 400).

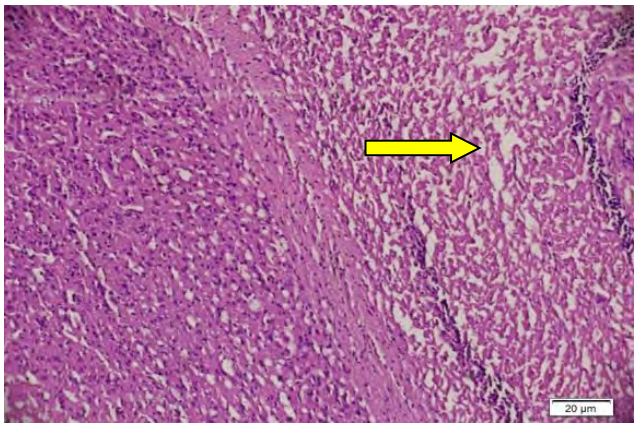

Figure 40Microphotograph of brain sections taken from mice of the Arsenic treated group.

H and E staining (× 400).

In Figure 42, Microphotograph of brain sections taken from rats of the arsenic treated group showing Intra-cellular space, edematous change.

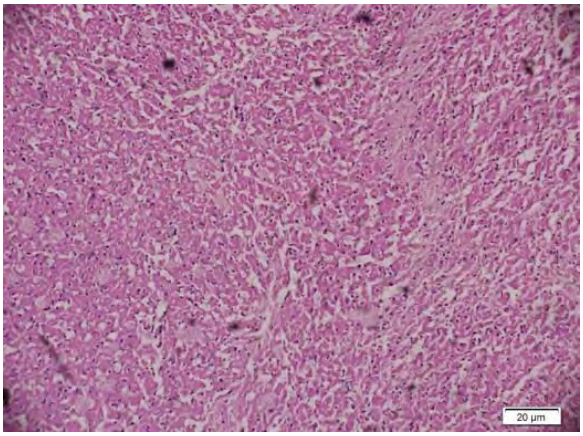

Figure 41Microphotograph of Brain sections taken from mice of the As and GE 150 mg/kg treated group. H and E staining (× 400).

In Figure 43,Microphotograph of brain sections taken from mice of GE 150 mg/kg treated group showed signs of amelioration of arsenic-evoked injury which was evident from the reduction of intracellular space and edematous change.

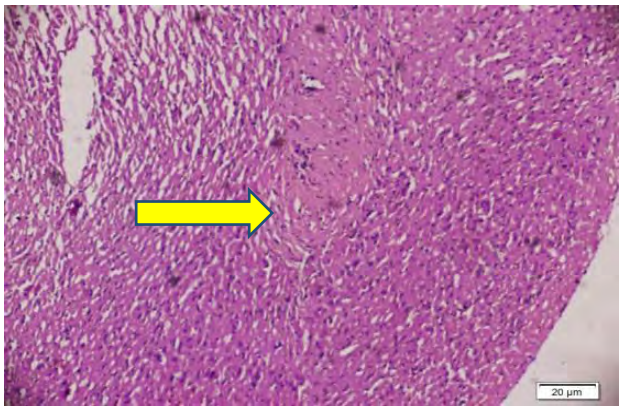

Figure 42Microphotograph of Brain sections taken from mice of the As and GE 300 mg/kg treated group. H and E staining (× 400).

In Figure 44, Microphotograph of brain sections taken from mice of GE 300mg/kg treated group showed signs of amelioration of arsenic-evoked injury which was evident from the reduction of intracellular space and edematous change.

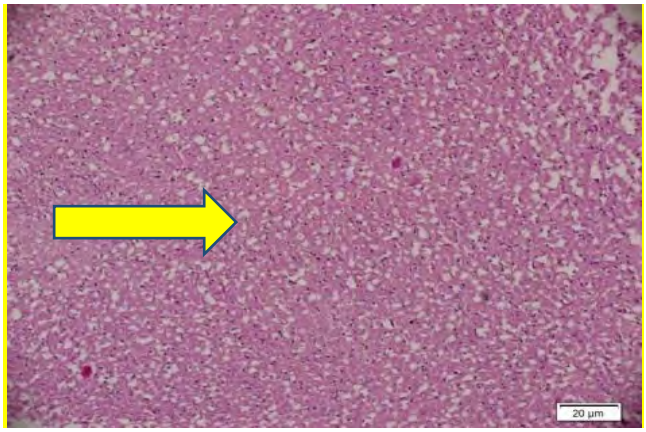

Figure 43Microphotograph of Brain sections taken from mice of the As and GE 500 mg/kg treated group H and E staining (× 400).

In Figure 45, Microphotograph of brain sections taken from mice of Garlic 500 mg/kg extract treated group showed almost normal brain structure.

4.4.4 Histopathology of Skin Tissue

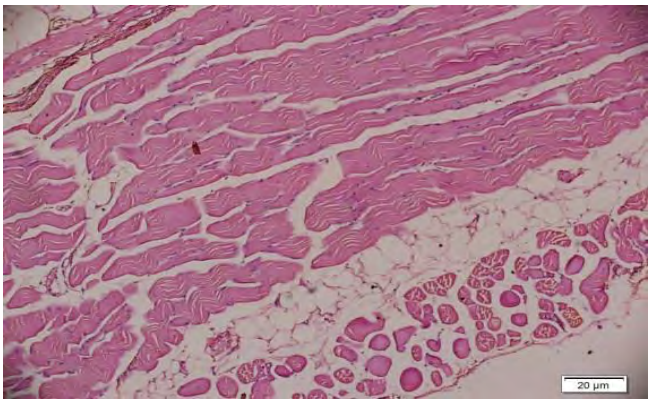

Figure 44Microphotograph of Skin sections taken from mice of the control group H and E staining (× 400).

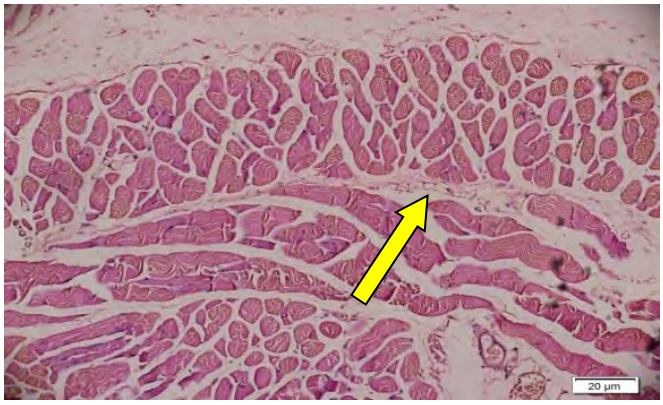

Figure 45Microphotograph of Skin sections taken from mice of the Arsenic treated group. H and E staining (× 400).

In Figure 47,Microphotograph of skin sections taken from mice of the arsenic treated group showing disruption of skeletal muscle and absence of the fat cells of the hypodermis.

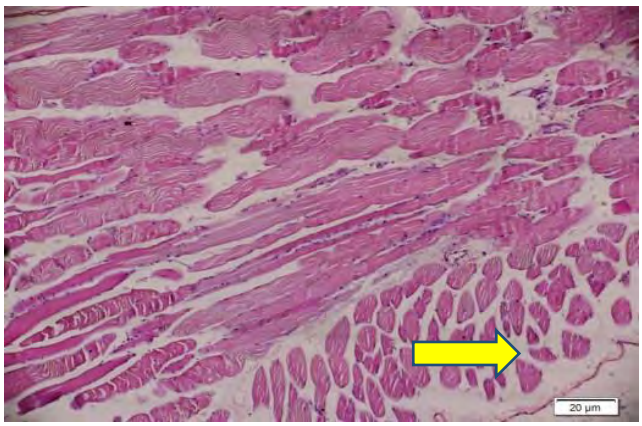

Figure 46Microphotograph of Skin sections taken from mice of the As and GE 150 mg/kg treated group. H and E staining (× 400).

In Figure 48,Microphotograph of skin sections taken from mice of the GE 150 mg/kg treated group also shows disruption of epidermis and skeletal muscle and no deposition fat cells of the hypodermis.

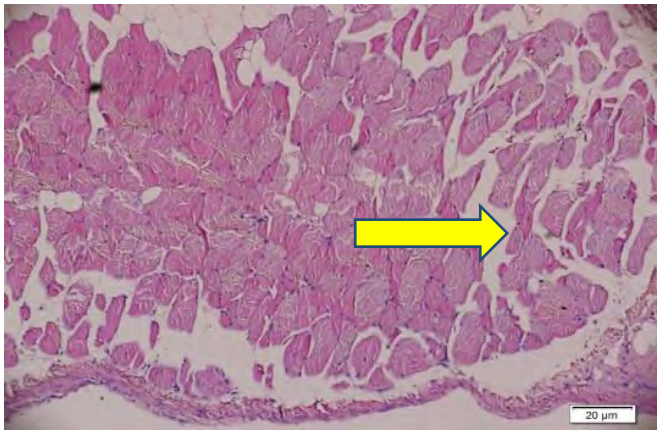

Figure 47Microphotograph of Skin sections taken from mice of the As and GE 300 mg/kg treated group. H and E staining (× 400).

In Figure 49,Microphotograph of skin sections taken from mice of the GE 300 mg/kg treated group also shows disruption of epidermis and skeletal muscle and no deposition fat cells of the hypodermis.

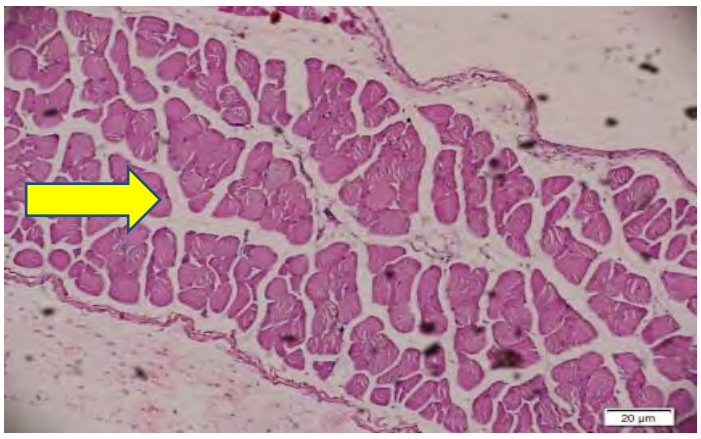

Figure 48 Microphotograph of Skin sections taken from mice of the As and GE 500 mg/kg treated group. H and E staining (× 400).

In Figure 50,Microphotograph of skin sections taken from mice of the garlic 500 mg/kg extract treated group shows moderate restoration of disrupt epidermis and skeletal muscle and no deposition fat cells of the hypodermis.

Chapter 5

Discussion

5.1 Phytochemical screening

According to Nazir et al., *Allium sativum* contains saponin, alkaloids, carbohydrates, Glycosides, protein, flavonoids, reducing sugar, sterols (Nazir & Chauhan, 2019). In this study, the presence of carbohydrate was detected only in the Benedict's test and other two test (Fehling's and Barfoed's) did not show the desired color for the presence of carbohydrates. Alkaloids, saponin, protein, triterpenes and flavonoids were present as their respective tests showed the colors that are needed to be sure. However, glycoside tests gave negative result as well as sterol test (Table 3).

5.2 Effects on Hematological, Biochemical, Physiological (Body Weight), Histopathological Parameters

There are established facts about the presence of arsenic in environment and this heavy metal is able to create some serious consequences regarding physiological, biochemical and histological changes within body. Human body and other animals can be introduced to As from contaminated water, soil and food. Hence, the evaluation of toxic potential of arsenic is crucial for the risk assessment of living creators ordinarily unprotected to this metal. In this study, the hematological, biochemical, physiological, histopathological influence of arsenic were observed using *Swiss albino* mice. Statistically, different scientific studies that were conducted indicated that the degree of toxic manifestation of As can be altered due to the administration of treatment procedure.

5.2.1 Effect on Body Weight

Decrease in body weight is an established indicator of decaying of normal health status. It has been mentioned that arsenic ought to set off toxicological effects and biochemical dysfunctions representing serious risks to fitness (Messarah et al., 2012). In the present study, the body weights of Arsenic treated group did not enhance much compared to the Extract treated groups (Figure 30), whereas, the Extract treated groups showed progressive increase of body weight throughout the whole time period of eight weeks. The lowered body weight found in found in As-treated rats in assessment to manipulate shows loss of weight dueto immoderate break down of tissue proteins (Chatterjea and Shinde, 2002.). One of the most major strategies used to treat As-induced toxicity is the administration of agents with effective antioxidant properties. *Allium sativum* is a natural remedy containing allin, as its foremost amino acid. Allinincludes sulfur substitutes and in the presence of alliinase is transformed to allicin, which then produces different compounds such as: vinyldithiines, ajaenes, and poly sulfides(Mehrandish, Rahimian, & Shahriary, 2019). Adding to that, these are some powerful compounds that have strong antioxidant properties(Chung, 2006).

5.2.2 Effect on Hematological and Biochemical Parameters

If the hematological and biochemical studies are focused in this experiment, some major alteration can be seen between Arsenic treated group and Extract treated groups.

Lymphocyte:

The highest level of LYM was found in case of As+GE 150 mg/kg and the lowest was found in Arsenic treatment which are 42.17 cells/litre and 33.17 cells/liter. (Table 7)

Significant changes in Lymphocyte (LYM) count are highlighted in As group and As+150 mg/kg ; As group and As+500 mg/kg individually (Figure 22). In As group, the level of LYM decreased, whereas in As+150 mg/kg and As+500 mg/kg group, the LYM level were close to

the Control group. Inhibition of stimulation and proliferation of Lymphocyte can be altered due to exposure of arsenic in blood (Gonsebatt et al, 2002) This consequence confirm the truth that persistent arsenic introduction can affect the proliferation of total blood lymphocytes.

White Blood Cell:

From the extract treated groups, As+GE 500 mg/kg group had the highest level which is 5683.33×10^9 cells/litre and is close to the control group 7833.33×10^9 cells/liter. (Table 7)

The White Blood Cell (WBC) count showed a change in Arsenic Treated group versus Garlic Extract 150 mg/kg group (Figure 20). Adding to that, comparing with control, As group has decreased level of WBC which was also reported by Farzand and et al. in their study (Ferzand, Gadahi, Saleha, & Ali, 2008). This may be due to apoptotic effect of arsenic on plasma cells as also studied by Rousselot et al. (Rousselot et al., 2004). There, a decreased data is seen in case of Garlic Extract 150 mg/kg group compared to As treated group, it can be suggest that more WBC induced their phagocytic activity to fight against As exposure.

Alanine Phosphatase

The average levels of ALP of different groups are very close, however As treated group showed 134.67 U/L which is the lowest. (Table 7)

Although, the ALP levels of Arsenic treated group comparing with the three doses of extract treatment have significantly altered (Figure 27). Alkaline Phosphatase (ALP) test is a liver function test. The altered levels indicate that As exposure has created an imbalance between pre-oxidation and antioxidation homeostasis in liver system which result in hepatic

degenerative and this fact has also been included by Mohammod et al. in their study (Mohammod, 2015).

Creatinine

The highest CRE level was showed by As+GE 300 mg/kg (1.07 U/L) and the lowest was shown by As treated group which was 0.75 U/L (Table 7).

The Creatinine level in case of As treated group and Dose 300 mg/kg signifies that As has immense effect in organs like kidney (figure 28). Kidney is one of the most vulnerable organs which can easily be damaged due to perfusion and increased concentration of renal tubular wastes. Serum creatinine level is more potent indicator than other renal indicators to show the primary stages of kidney disease. (Messarah et al., 2013).

Neutrophil, Monocyte, Eosinophil, Red Blood Cell, Serum Glutamate Aminotranferase

In many research articles, it have been found that As exposure and treatment of Arsenicosis have elevated result in terms of Neophyte, Monocyte, Eosinophil, Red Blood Cell, Serum Glutamate Pyruvate Transaminase levels (Milton et al., 2005; Rahman et al., 2003; Watanabe et al., 2003).

However, in this study, no significant changes have been seen in those cases, and it is being assumed that this scenario may have arisen because of the time duration of this research study. A Instead of eight weeks, if the study was carried out a bit longer it may had bring better results in those test parameters (figure 21, 23, 24, 25, 26,).

On the other hand, the average levels showed drastic changes between the groups. (Table 7)

5.2.3 Histopathological Study

Kidney

As it is said earlier, increased oxidative stress in tissue due to arsenic exposure is seen,so the major cause for arsenic-induced toxicity in mice is due to oxidative stress (Parrish, Xing Hui Zheng, Turney, Younis, & Gandolfi, 1999).In the kidney during its urinary elimination, As compounds affect the function of proximal convoluted tubules. We have observed the degenerative changes in kidney tissue in arsenic treated mice (Chapter 4.4.2).

Liver

Liver is identified as a target organ of arsenic exposure due to its unique metabolic functions and this organ is related to the gastrointestinal tract. The sections of liver showed moderate degeneration and necrosis in hepatic parenchyma with mild to moderate fatty change in As and treatment exposure whereas control did not reveal any lesions of pathological significance (Chapter 4.4.1). In previous studies, arsenic mediated, hepatocytic degeneration was characterized by vacuolar degeneration followed by hepatic necrosis observed in case of rat (Gora, Baxla, Kerketta, Patnaik, & Roy, 2014).

Brain

It has also been observed in many studies that inorganic arsenicals have been reported to be accumulated in brain astrocytes and also can disturb the mitosis of granule cells and interfere with the normal development of mice cerebellum (Koehler, Luther, Meyer, Schwerdtle, & Dringen, 2014; Ding et al., 2013). In this report, we have found edema, intracellular space, edematous change in arsenic exposed mice brain tissue and also the recovery from the damage due to administration of *Allium sativum* extract in different doses (Chapter 4.4.3).

Skin

Various types of skin diseases as well as skin cancer can be exposed in human body due to the chronic levels of As in blood(Hunt, Srivastava, Elmets, & Athar, 2014). In this study, the skin layers of the different mice group also have been analyzed and it was highlighted that disruption of skeletal muscle and absence of the fat cells of the hypodermis too place due to exposure of As. Until the maximum dose which is 500 mg/kg as treatment, other two doses that include 150 mg/kg and 300 mg/kg did not show significant recovery from the damage. Garlic 500 mg/kg extract treated group shows moderate restoration of disrupt epidermis and skeletal muscle however, no deposition fat cells of the hypodermis (Chapter 4.4.4).

Chapter 6

Conclusion

By focusing on the above study, there is a legit data on the fact that *Allium sativa*, locally called garlic is a significant anti-oxidant agent which can detox the body by absorbing toxin or metal segments by its chelating property. So it would be beneficial for an individual's health condition if a certain amount of garlic is taken as a dietary supplement as it helps in reducing metal toxicity risk from the body.

Chapter 7

Future Works

The extended work that need to be done-

- As mice was used in the animal study, only a few amount of blood and serum could be collected and only some hematological and biochemical tests were done. In future, other animals like, rabbit, guinea pigs etc. could be used so that other related hematological and biochemical tests can be performed.
- Same Plant extracts of different concentrations should be a part of study.
- In future, comparison between a standard drug for As treatment and garlic extract of different concentrations could be a major deal.

References

Antillon, F., Behm, F. G., Raimondi, S. C., Kaste, S. C., Sandlund, J. T., & Pappo, A. S. (1998). Pediatric primary diffuse large cell lymphoma of bone with t(3;22)(q27;q11). *Journal of Pediatric Hematology/Oncology*, *20*(6), 552–555. https://doi.org/10.1097/00043426-199811000-00008

Bajpai, V. K., Kim, J. E., & Kang, S. C. (2017). Protective effect of heat-treated cucumber (Cucumis sativus L.) juice against lead-induced detoxification in rat model. *Indian Journal of Pharmaceutical Education and Research*. https://doi.org/10.5530/ijper.51.1.9

Bases, S., Azetidinone, I. T. S., & Derivatives, T. (2015). *International Journal of Innovative Pharmaceutical Sciences and Research ACTIVITY OF NOVEL 2-SUBSTITUTED BENZIMIDAZOLE*. *3*(574), 574–583.

Biuret test : Principle , requirements , Procedure and Result Biuret test : Principle , requirements , Procedure and Result Principle : (2018). 1–6.

Chen, Z., Shen, Z.-X., Chen, G.-Q., Ni, J.-H., Li, X.-S., Xiong, S.-M., … Wang, Z.-Y. (1997). Use of arsenic trioxide (As2O3) in the treatment of acute promyelocytic leukemia (AFL): II. Clinical efficacy and pharmacokinetics in relapsed patients. *Blood*, *89*(9), 3354–3360.

Chung, L. Y. (2006). The Antioxidant Properties of Garlic Compounds: Allyl Cysteine, Alliin, Allicin, and Allyl Disulfide. *Journal of Medicinal Food*, *9*(2), 205–213. https://doi.org/10.1089/jmf.2006.9.205

David, B. Y., & April, F. (2019). *Report Charges ‘ Nepotism and Neglect ’ on Bangladesh Arsenic Poisoning*. 1–7.

Ding, X., Su, Q., Jiang, M., Xie, H., Cong, J., & Wang, L. (2013). Arsenic affects on cerebellar development of mice. *Toxicology Mechanisms and Methods*, *23*(9), 672–677. https://doi.org/10.3109/15376516.2013.840347

Domingo, J. L. (1995). Reproductive Toxicology Review BY CHELATING AGENTS OF METAL-INDUCED DEVELOPMENTAL TOXICITY. *Reproductive Toxicology*, *9*(2), 105–113.

Ferzand, R., Gadahi, J. A., Saleha, S., & Ali, Q. (2008). Histological and haematological disturbance caused by arsenic toxicity in mice model. *Pakistan Journal of Biological Sciences*, *11*(11), 1405–1413. https://doi.org/10.3923/pjbs.2008.1405.1413

Geometry, R., & Analysis, G. (n.d.).

Gora, R., Baxla, S., Kerketta, P., Patnaik, S., & Roy, B. (2014). Hepatoprotective activity of Tephrosia purpurea against arsenic induced toxicity in rats. *Indian Journal of Pharmacology*, *46*(2), 197. https://doi.org/10.4103/0253-7613.129317

Gurr, J. R., Yih, L. H., Samikkannu, T., Bau, D. T., Lin, S. Y., & Jan, K. Y. (2003). Nitric oxide production by arsenite. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis*, *533*(1–2), 173–182. https://doi.org/10.1016/j.mrfmmm.2003.08.024

Hunt, K. M., Srivastava, R. K., Elmets, C. A., & Athar, M. (2014). The mechanistic basis of arsenicosis: Pathogenesis of skin cancer. *Cancer Letters*, *354*(2), 211–219. https://doi.org/10.1016/j.canlet.2014.08.016

International commission for protection against environmental mutagens and carcinogens: News no. 2. (1979). *Environmental Mutagenesis*, *1*(3), 297–301.

https://doi.org/10.1002/em.2860010315

James, K. A. (2017). *Chemical Characteristics*. *1*, 170–175. https://doi.org/10.1016/B978-0-12-803678-5.00025-4

Kaur, T., Singh, A., & Goel, R. (2011). Mechanisms pertaining to arsenic toxicity. *Toxicology International*, *18*(2), 87. https://doi.org/10.4103/0971-6580.84258

Kew, J., Morris, C., Aihie, A., Fysh, R., Jones, S., & Brooks, D. (1993). Arsenic and mercury intoxication due to Indian ethnic remedies. *Bmj*, *306*(6876), 506–507. https://doi.org/10.1136/bmj.306.6876.506

Knowledge, M. P. (2016). *What is arsenic poisoning ? What is arsenicosis ?* (January 2018), 1–9.

Koehler, Y., Luther, E. M., Meyer, S., Schwerdtle, T., & Dringen, R. (2014). Uptake and toxicity of arsenite and arsenate in cultured brain astrocytes. *Journal of Trace Elements in Medicine and Biology*, *28*(3), 328–337. https://doi.org/10.1016/j.jtemb.2014.04.007

Lindberg, A. L., Kumar, R., Goessler, W., Thirumaran, R., Gurzau, E., Koppova, K., … Vahter, M. (2007). Metabolism of low-dose inorganic arsenic in a central European population: Influence of sex and genetic polymorphisms. *Environmental Health Perspectives*, *115*(7), 1081–1086. https://doi.org/10.1289/ehp.10026

Liu, J. (2000). Metallothionein-I/II Null Mice Are More Sensitive than Wild-Type Mice to the Hepatotoxic and Nephrotoxic Effects of Chronic Oral or Injected Inorganic Arsenicals. *Toxicological Sciences*, *55*(2), 460–467. https://doi.org/10.1093/toxsci/55.2.460

Martins, N., Petropoulos, S., & Ferreira, I. C. F. R. (2016). Chemical composition and

bioactive compounds of garlic (Allium sativum L.) as affected by pre- and post-harvest conditions: A review. *Food Chemistry*, *211*, 41–50. https://doi.org/10.1016/j.foodchem.2016.05.029

Mathews, V., Chendamarai, E., George, B., Viswabandya, A., & Srivastava, A. (2011). Treatment of acute promyelocytic leukemia with single-agent arsenic trioxide. *Mediterranean Journal of Hematology and Infectious Diseases*, *3*(1), 1–18. https://doi.org/10.4084/MJHID.2011.056

Mehrandish, R., Rahimian, A., & Shahriary, A. (2019). Heavy metals detoxification: A review of herbal compounds for chelation therapy in heavy metals toxicity. *Journal of Herbmed Pharmacology*, *8*(2), 69–77. https://doi.org/10.15171/jhp.2019.12

Messarah, M., Klibet, F., Boumendjel, A., Abdennour, C., Bouzerna, N., Boulakoud, M. S., & El Feki, A. (2012). Hepatoprotective role and antioxidant capacity of selenium on arsenic-induced liver injury in rats. *Experimental and Toxicologic Pathology*, *64*(3), 167–174. https://doi.org/10.1016/j.etp.2010.08.002

Messarah, M., Saoudi, M., Boumendjel, A., Kadeche, L., Boulakoud, M. S., & Feki, A. El. (2013). Green tea extract alleviates arsenic-induced biochemical toxicity and lipid peroxidation in rats. *Toxicology and Industrial Health*, *29*(4), 349–359. https://doi.org/10.1177/0748233711433934

Milton, A. H., Smith, W., Rahman, B., Hasan, Z., Kulsum, U., Dear, K., … Ali, A. (2005). Chronic arsenic exposure and adverse pregnancy outcomes in Bangladesh. *Epidemiology*, *16*(1), 82–86. https://doi.org/10.1097/01.ede.0000147105.94041.e6

Mitchell-Heggs, C. A. W., Conway, M., & Cassar, J. (1990). Herbal Medicine as a Cause of Combined Lead and Arsenic Poisoning. *Human & Experimental Toxicology*, *9*(3), 195–

196. https://doi.org/10.1177/096032719000900314

Mohammod, A. S. (2015). Arsenic-induced Histological Alterations in Various Organs of Mice. *Journal of Cytology & Histology*, *06*(03). https://doi.org/10.4172/2157-7099.1000323

Nazir, I., & Chauhan, R. S. (2019). *Qualitative phytochemical analysis of Allium sativum (Garlic) and Curcuma longa (Turmeric)*. *7*(1), 545–547.

No Title. (2019). https://doi.org/10.1016/B978-0-12-812069-9.00020-0

Of, E. (n.d.). *T Extbook of T Herapeutic*.

Parrish, A. R., Xing Hui Zheng, Turney, K. D., Younis, H. S., & Gandolfi, A. J. (1999). Enhanced transcription factor DNA binding and gene expression induced by arsenite or arsenate in renal slices. *Toxicological Sciences*, *50*(1), 98–105. https://doi.org/10.1093/toxsci/50.1.98

Rahman, M. A., Rahman, I. M. M., & Hasegawa, H. (2018). Effects of Cooking on Dietary Exposure to Arsenic From Rice and Vegetables: Human Health Risks ☆. In *Reference Module in Earth Systems and Environmental Sciences* (2nd ed.). https://doi.org/10.1016/b978-0-12-409548-9.10996-0

Rahman, M. M., Mandal, B. K., Roy Chowdhury, T., Sengupta, M. K., Chowdhury, U. K., Lodh, D., … Chakraborti, D. (2003). Arsenic groundwater contamination and sufferings of people in North 24-Parganas, one of the nine arsenic affected districts of West Bengal, India. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering*, *38*(1), 25–59. https://doi.org/10.1081/ESE-120016658

Ratnaike, R. N. (2003). *Acute and chronic arsenic toxicity*. 391–397.

Required, M. (2015). *Qualitative Analysis of Carbohydrates Materials Required : Procedure :* 2–5.

Reuters. (2014). *Gulf States Seek Food Security in Europe, US After African Problems*. *7*(8), 2938–2946. Retrieved from http://gulfbusiness.com/2014/01/gulf-states-seek-food-security-in-europe-us-after-african-problems/%5Cnhttp://gulfbusiness.com/2014/01/gulf-states-seek-food-security-in-europe-us-after-african-problems/?utm_medium=email&utm_campaign=Daily Gulf Business Da

Rousselot, P., Larghero, J., Labaume, S., Poupon, J., Chopin, M., Dosquet, C., … Fermand, J. P. (2004). Arsenic trioxide is effective in the treatment of multiple myeloma in SCID mice. *European Journal of Haematology*, *72*(3), 166–171. https://doi.org/10.1046/j.0902-4441.2003.00194.x

S, H., & R, S. (2002). Looking for relevant research ? *Science Direct*, *50*(1), 25–29.

Sadi, N., Ouldali, O., & Aoues, A. E. K. (2015). L'effet des pectines extraites de datte (phoenix dactylifera) contre la neurotoxicité du plomb:étude neurocomportementale. *Annales de Cardiologie et d'Angeiologie*. https://doi.org/10.1016/S0003-3928(16)30253-0

Saha, J. C., Dikshit, A. K., Bandyopadhyay, M., & Saha, K. C. (1999). A review of arsenic poisoning and its effects on human health. *Critical Reviews in Environmental Science and Technology*, *29*(3), 281–313. https://doi.org/10.1080/10643389991259227

Scientists, O. I., Sheet, N. F., & States, U. (2019). *7/16/2019 Arsenic*. 1–6.

Scrolling, C., Click, O. R., & For, H. (2019). *Medical De nition of Diabetes mellitus*. 1–53.

Silva, J. P. M. C. S. De. (2015). Ac ce p d t. *Environmental Toxicology and Pharmacology*. https://doi.org/10.1016/j.etap.2015.09.016

Susmitha, P. V. (2013). PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF AQUEOUS EXTRACT OF THE AERIAL PARTS OF VIBURNUM PUNCTATUM BUCH. - HAM. Ex. D. DON. *International Journal of Pharmaceutical Sciences and Research*, *4*(6), 2322–2326. https://doi.org/10.13040/IJPSR.0975-8232.4(6).2322-26

Uddin, R., & Huda, N. H. (2011). Arsenic poisoning in Bangladesh. *Oman Medical Journal*, *26*(3), 207. https://doi.org/10.5001/omj.2011.51

UN University compares technologies that remove arsenic from groundwater. (2019). 8–11.

Updates, L. (2018). *EXTRACTION AND CHEMICAL INVESTIGATION OF LEAVES OF*. (November), 1–5.

Vale, J. A., & Bradberry, S. M. (2001). Lead intoxication: oral DMSA-vs-intravenous sodium calcium edetate . *Journal of Toxicology: Clinical Toxicology* .

Watanabe, C., Inaoka, T., Matsui, T., Ishigaki, K., Murayama, N., & Ohtsuka, R. (2003). Effects of arsenic on younger generations. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering*, *38*(1), 129–139. https://doi.org/10.1081/ESE-120016885

Xanthocarpum, S. (2011). *Available online through A PRELIMINARY PHYTOCHEMICAL INVESTIGATION ON THE LEAVES OF*. *2*(3), 845–850.

Yun, H., Kim, I., Kwon, S.-H., Kang, J.-S., & Om, A.-S. (2011). Protective Effect of Chlorella vulgaris against Lead-Induced Oxidative Stress in Rat Brains. *JOURNAL OF HEALTH SCIENCE*. https://doi.org/10.1248/jhs.57.245

(1987). A study on the effect of garlic to the heavy metal poisoning of rat. *Journal of Korean Medical Science*. https://doi.org/10.3346/jkms.1987.2.4.213