EFFECT OF SPIRULINA AND VITAMIN C AGAINST ARSENICOSIS IN RAT

A Thesis

By

MD. HANNAN ALI
Registration No. 1605512
Session: 2016-17
Semester: January-June, 2018

MASTER OF SCIENCE (M.S.)
IN
PHARMACOLOGY

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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DEDICATED
TO MY
BELOVED PARENTS

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ABSTRACT

The experiment was conducted with Long Evans rats in a view to observe the comparative effects of Spirulina (*Spirulina platensis*) and Vitamin C in the prevention of Arsenic Toxicity. Sixty male (Long Evans) rats (age about 35 days; average body weight at day 0 was 75 g) were randomly divided into five equal groups (n=7), namely, control group (T_0), Arsenic treated group (T_1), Arsenic plus Spirulina treated group (T_2), Arsenic plus Vitamin C treated group (T_3) and Arsenic plus Spirulina plus Vitamin C treated group (T_4). Rats of T_0 group were given normal feed and water and kept as control. Rats of T_1, T_2, T_3 and T_4 were given (Arsenic trioxide) As_2O_3 100 mg/kg body weight daily for 45 days orally. In addition to As_2O_3 rats of group T_2 and T_4 were simultaneously fed with Spirulina @ 1 gm/kg of feed and T_3 and T_4 were simultaneously feed with Vitamin C @ 250mg /kg body weight up to 45 days respectively. Four rats from each group were sacrificed at 15 days interval and blood samples were collected. No visible toxic clinical signs were observed in any experimental rats during the study period. Highly body weight were found except arsenic (T_1) treated group of rats and administration of spirulina tended to bring the body weight towards the normal. The values of SC (Serum creatinine) increased significantly ($P<0.01$) in all the treated groups of rats (T_1, T_2, T_3 and T_4) compared to the control (T_0) group, but Spirulina combined with Vitamin C produced significant values comparable to the control group. Though the values of SGOT (Serum glutamate oxaloacetate transaminase) showed little significance ($P<0.05$) but SGPT (Serum glutamate pyruvate transaminase) showed highly significant value ($P<0.01$) at 45^{th} day of blood collection among different treated groups. Spirulina combined with Vitamin C appeared most effective in managing arsenic treatment. Spirulina with Vitamin C increased the values of TEC, TLC and Hb (Total erythrocyte count, Total leukocyte count and Hemoglobin) against arsenic toxicity in rats and showed highly significance compared to control group of differences treatment. In conclusion, the combination of Spirulina and vitamin C were found more effective to decreases the level of arsenic and prevention of chronic arsenicosis in rat than sole use of Spirulina or Vitamin C.

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LIST OF ABBREVIATIONS

B. wt.	:	Body weight
Conc.	:	Concentration
Cu. mm	:	Cubic millimeter
d.w.	:	Drinking water
et al.	:	Associates
Fig.	:	Figure
gm	:	Gram
Hb	:	Hemoglobin
i.e.	:	That is
J.	:	Journal
Kg	:	Kilogram
Lit	:	Liter
Ltd.	:	Limited
mg	:	Milligram
mm^3	:	cubic millimeter
No.	:	Number
SE	:	Standard Error
SM	:	Sample Mean
TEC	:	Total Erythrocyte Count
Vol.	:	Volume
µg	:	Microgram
%	:	Percent
&	:	And
@	:	At the rate of
<	:	Less than
>	:	Greater than
0^0C	:	Degree centigrade
TEC	:	Total Erythrocyte Count
TLC	:	Total Leukocyte Count
SGOT	:	Serum Glutamate Oxaloacetate Transaminase
SGPT	:	Serum Glutamate Pyruvate Transaminase

CHAPTER I

INTRODUCTION

As a natural element, Arsenic (As) is found in earth's crust. It is frequently not only found in the biosphere but also occurs naturally in both organic and inorganic forms like in water, food, soil, dust, wood and other materials. Arsenic exists in nature in three allotropic forms, α (yellow), β (black) and γ (grey) of the metallic state and in a number of ionic forms. The most common oxidation numbers of arsenic are +5, +3 and −3, in which the element is able to form inorganic and organic compounds both in the environment and within the human body (Orloff *et al.,* 2009).

Arsenic trioxide (As_2O_3) is the most prevalent inorganic arsenical found in air, while a variety of inorganic arsenates (AsO_4^{3-}) or arsenites (AsO_2^-) occur in water, soil or food (Magalhaes, 2002; Chou *et al.,* 2007). In consequence of its widespread use in the microelectronics industry, gallium arsenide (GaAs) is an inorganic arsenic compound which may also impact adversely on human health. The largest source of arsenic and other metals is usually food, of which the main dietary forms are seafood, rice, mushrooms and poultry (Smedley and Kinniburgh, 2002; Jones, 2007; Petroczi and Naughton, 2009; Naughton, 2009; Nepusz *et al.,* 2009).

In drinking water the main source of arsenic is arsenic rich rocks through which the water has filtered. Arsenic poisoning occurs naturally in both organic and inorganic forms in water, food, soil, dust, wood and other materials. Arsenic is a well-known human carcinogen and has many other toxic effects. To produce a state of the art review on arsenic in drinking water the World Health Organization (WHO) has worked with other UN system organizations (WHO, 1999). The safety limit of arsenic accepted by Bangladesh Government is 0.05 mg/liter for drinking water (WHO, 1999). The World Health Organization limits for drinking water 0.01 mg/liter and far foodstuffs is 2 mg/liter on a fresh weight basis (Robinson *et al.,* 2003).

In different developing countries now arsenic creates a serious public health issue (Rahman, 2006), where the drinking water contaminated with inorganic form of arsenic. At present Chronic arsenic toxicity is a global health issue (Yoshida *et al.,* 2004).

In Bangladesh and surrounding regions it is also a major health problem (Guha Mazumder *et al.,* 2001; Kalia, 2005; Khalequzzaman *et al.,* 2005). Chronic Arsenic

poisoning can cause serious health problems including cancers, hyperkeratosis, restrictive lung disease, and ischaemic heart disease (Mandal and Suzuki 2002; Rossman, 2003) and increases the risk for As-induced diseases such as noncancerous skin lesions, bronchitis, hepatomegaly, neuropathy, peripheral vascular diseases (e.g., gangrene), cardiovascular disease, skin cancer, lung cancer, and bladder cancer (Smith *et al.* 1998; Guha Mazumder, 2003). Arsenic Changes in liver enzyme activities, thiobarbituric acid reactive substances (TBARS) level, antioxidants and reduced glutathione (GSH) contents. Arsenic exposure enhanced an oxidative stress by disturbing the tissue antioxidant defense system, but the Se co-administration protected liver tissues against As intoxication probably owing to its antioxidant properties (Messarah *et al.,* 2012). Methanolic extract of *Glycosmis pentaphylla* (GP) against sodium arsenite ($NaAsO_2$) induced toxicosis extraction of *G.pentaphylla* and leaves may have ameliorative effect in arsenicosis in rats at dose dependent manner (De *et al.,* 2015).

About half of the total populations (more than 50 millions) of Bangladesh are consuming arsenic (As) through drinking and cooking. Among them, more than 40,000 people have already developed the signs and symptoms of chronic arsenic toxicity (Mudur 2000; Misbahuddin, 2003). Nearly 61, out of 64 districts of the country`s tube wells contain dangerous levels of inorganic As, tube wells, which are serving as main sources of waterfor drinking and cooking purposes. The general populations are exposed to As through drinking water, dust, fumes and dietary sources. An organic arsenical compound Roxarsone (3-nitro-4- hydroxyphenylarsonic acid) is used widely in poultry production to control coccidial intestinal parasites. It is excreted unchanged in the manure and introduced into the environment when litter is applied to farmland as fertilizer. Although, the toxicity of roxarsone is less than that of inorganic As, roxarsone can be degraded biotically and abiotically, to produce more toxic inorganic forms of As, such as arsenite and arsenate (Bednar *et al.,* 2003). Spirulina was found to be beneficial in goats of chronic arsenic poisoning (Halim, 2007) and Spirulina extract plus zinc was found to be beneficial in patients of chronic arsenic poisoning (Misbahuddin *et al.,* 2006). The potential of dietary antioxidants to reduce the arsenic burden in human by increasing its metabolism has drawn increasing attention in recent years (McCall, 1999; Dey, 2002). Some investigators suggested that the effectiveness of retinol, β-carotene (Chung *et al.,* 2006), zinc (Valko *et al.,* 2005), ascorbic acid (Saha, 2003), selenium (Spallholz *et al.,*

2004), tocopherol (Ramanathan *et al.,* 2003), á-lipoic acid (Tabassum, 2006) spinach (Umar, 2007) for the treatment of chronic arsenic toxicity.

Spirulina, a microscopic blue-green algae. The cyano-bacterium Spirulina is a filamentous blue-green alga belonging to the *Oscillatoriaceae* family that is generally found in tropical and subtropical regions in warm alkaline water (Samir *et al.,* 2016). It has a property of reducing heavy metals and nephrotoxic substance from the body. It is characterized by high nutritional value where it contains high protein content (60–70% by dry weight), plenty of vitamins, amino acids, gamma-linoleic acid, and minerals (Hoseini *et al.,* 2013). The consumption of Spirulina as a diet supplement has health benefits in preventing or managing hypercholesterolemia (Ferreira-Hermosillo *et al.,* 2010), hyperglycerolemia (Deng *et al.,* 2010), obesity, inflammation (Coskun *et al.,* 2011), cancer (Ismail *et al.,* 2009), and cardiovascular disease (Khan *et al.,* 2005). In addition, Spirulina has antidiabetic effect (Karkos *et al.,* 2011). The use of Spirulina is the most beneficial in treatment of different diseases and disease conditions of man and animal. It reduced mercury and other toxic metal accumulation in the tissue (Johson *et al.,* 1986). It is not only a whole food, but it seems to be an ideal therapeutic supplement. Spirulina alone or in combination with other vitamin and/or mineral was found to be effective in the removal of arsenic from arsenic-loaded tissues in various species including man (Fariduddin *et al.,* 2001; Misbahuddin *et al.,* 2006; Awal, 2007), in the treatment of chronic arsenic poisoning (Khan *et al.,* 2001), Antioxidants can reduce arsenic toxicity through chelating it and scavenging free radicals (Rana *et al.,* 2007). Spirulina and thankuni significantly lowered the arsenic burden of rats whereas spirulina is more effective than thankuni against arsenic burden in rats (Hasan *et al.,* 2015). The protective actions of *S. platensis* against arsenic are believed to originate from its free radical scavenging, antioxidant activities, maintenance of antioxidant enzymes, and a decrease in the production of inflammatory mediators that are implicated in the pathogenesis of arsenic-induced testicular injury. Therefore, S. platens is represents a potential agent to prevent testicular injury and dysfunction induced by arsenic exposure. Millions of people in Bangladesh, India, Taiwan, and Chile are consuming high concentration of arsenic through drinking water, and thousands of them have already developed chronic arsenic poisoning. (Misbahuddin *et al.,* 2006).

It is clearly evidenced that spirulina could play consistent role in the treatment of arsenicosis patients. The overall response revealed that 60% patients showed

considerable improvement with spirulina treatment (Rahman *et al.,* 2006). Some of the pharmacological properties of spirulina (*Spirulina platensis*) may be linked to its antioxidant potential, which mitigates oxidative stresses (Ghosh *et al.,* 2014).

For numerous intrinsic processes Vitamin C is essential. The most well-known and understood process is that of healing. Vitamin C is water soluble vitamin and also act as the effective antioxidant. It is white crystalline solid related to hexoses. Because of its relation to other sugars like L-isomers it is called L-ascorbic acid. It also acts as a strong reducing agent. The formation of intracellular material of subcutaneous tissue, cartilage and teeth are the Major functions of Vit. C (Ramanath, 1998). In collagen synthesis thereby wound healing it also plays important role (Balakumar1995). Ascorbic acid and n-aceltyl-l-cysteine as well as other antioxidants have different reactivates for specific oxidative species, such as hydroxyl radicals, singlet oxygen, hydrogen peroxide, peroxyl radicals, or superoxide anion (Childs *et al.,* 2001; Ramanathan *et al.,* 2002). The synergism between ascorbic acid and arsenic trioxide may, therefore, be responsible for enhancing ATO cytotoxicity. The reduction of arsenic induced higher blood glucose level by folic acid and vitamin C demonstrates that folic acid and vitamin C has significant effect in preventing arsenic induced disease (Ali *et al.* 2008). Folic acid supplementation total blood arsenic levels were reduced by 13.6 and by 2.5% in the placebo group (Gamble *et al.,* 2007).

In Bangladesh, elaborate data is available for arsenic only on tube-well water; however, data on the specific treatment in prevention of arsenic toxicity in both human and animals is very limited. Therefore, data on the effective prevention of arsenicosis with Spirulina and vitamin-C and their comparative efficacy will be the expected new findings especially for Bangladesh as well as for the world. So considering all the above facts, this work has been undertaken with the following objectives:

1. To evaluate the efficacy of spirulina and vitamin C against arsenicosis rats.
2. To know the arsenic effects of spirulina and vitamin C on body weight in arsenic fed rats.
3. To determine the effects of arsenic, spirulina and vitamin C on hematological parameters in arsenicosis rats.
4. To determine the effects of arsenic, spirulina and vitamin C on biochemical parameters in arsenic fed rats.

CHAPTER II

REVIEW OF LITERATURE

The main objective of this chapter is to get up-to-date information regarding the research works on arsenic and its prevention and treatment. Important information related to the present study is represented below:

2.1 Properties and metabolism of arsenic

Arsenic is a naturally occurring element in the environment, toxic to biological systems and is released into the environment by natural and anthropogenic activities such as rock weathering and ore mining respectively. Arsenic can be found to a small extent in the elemental form. Common organic arsenic compounds are arsanilic acid, methylarsonic acid, dimethylarsinic acid (cacodylic acid), and arsenobetaine (AB). This latter compound is considered to be the most predominant organo-arsenical in marine animals. Other organo-arsenicals include arsenocholine, dimethyloxyarsyl-ethanol, trimethylarsonium lactate, arsenic containing sugars and phospholipids have also been found in fish (Friberg *et al.,* 1986; Lau *et al.,* 1987).

Messarah *et al.* 2012. Changes in liver enzyme activities, thiobarbituric acid reactive substances (TBARS) level, antioxidants and reduced glutathione (GSH) contents were determined after 3 weeks experimental period. To conclude, results suggest that Ar exposure enhanced an oxidative stress by disturbing the tissue antioxidant defense system, but the Se co-administration protected liver tissues against As intoxication probably owing to its antioxidant properties.

Methylation of inorganic arsenic might be considered a detoxification mechanism, as the end metabolites, monomethylarsonic acid (MMA) and dimethyl arsinic acid (DMA), are less reactive with tissue constituents, less toxic, and more readily excreted in the urine than inorganic arsenic, especially the trivalent forms (Vhater *et al.,* 2001). Metabolism of inorganic arsenic to MMA and DMA, and this methylation facilitated urinary arsenic excretion that had a lower risk of adverse arsenic-related health outcomes (Heck *et al.,* 2007).

It was found that more than 75% of single dose of inorganic arsenite (5.0 mg As/kg body weight, BW) accumulated in rat red blood cells (RBCs) in the form of dimethylarsinous

acid (DMA^{III}), whereas less than 0.8% of the dose accumulated in hamster RBCs, mostly in the form of monomethylarsonous acid (Naranmandura *et al.,* 2007).

2.2 Sources and routes of exposure to arsenic

Arsenic trioxide (As_2O_3) is the most prevalent inorganic arsenical found in air, while a variety of inorganic arsenates (AsO_4^{3-}) or arsenites (AsO_2^-) occur in water, soil or food (Magalhaes, 2002; Chou *et al.,* 2007). Arsenic exists in nature in three allotropic forms, α (yellow), β (black), γ (grey), of the metallic state and in a number of ionic forms. The most common oxidation numbers of arsenic are +5, +3 and −3, in which the element is able to form both inorganic and organic compounds both in the environment and within the human body (Orloff *et al.,* 2009). De *et al.,* 2015 carried an experiment to evaluate the effects of methanolic extract of *Glycosmis pentaphylla* (GP) against sodium arsenite ($NaAsO_2$) induced toxicosis in adult albino rats. They showed that extraction of *G. pentaphylla* leaves may have ameliorative effect in arsenicosis in rats at dose dependent manner.

In consequence of its widespread use in the microelectronics industry, gallium arsenide (GaAs) is an inorganic arsenic compound which may also impact adversely on human health.

The largest source of arsenic and other metals is usually food, of which the main dietary forms are seafood, rice, mushrooms and poultry (Smedley and Kinniburgh, 2002; Jones, 2007; Petroczi and Naughton, 2009; Nepusz *et al.,* 2009). While there is more arsenic *per se* in seafood, this is mostly in an organic form called arsenobetaine which is much less harmful than others. Mostly, arsenic poisoning occurs through industrial exposure, from contaminated wine or moonshine, or by malicious administration.

Very recently, it has been reported that traditional Chinese herbal products, deliberately fortified with arsenic for therapeutic purposes, may represent a serious health hazard (Martena *et al.,* 2010). Mass spectrometry has revealed that, of 292 tested samples sold in Dutch market, 20% significantly exceeded safety levels not only of arsenic but also of lead and mercury. It has been concluded that traditional herbal preparations of Chinese and Tibetian medicine require strict control by local authorities.

Color pigments that are used in the cosmetic industry in the production of eye-shadows frequently contain toxic elements, including arsenic (Sainio *et al.,* 2000). The skin of the

eyelids is very delicate and the application of eye-shadows may produce eczemas. In addition, arsenic particles can be water soluble and therefore may undergo percutaneous absorption through the wet skin. When it enters the circulatory system via percutaneous absorption, at high concentrations, arsenic may represent a potential risk of carcinogenesis. Based on the available toxicology data, it has been recommended that cosmetic products should contain less than 5 ppm of metal impurities.

As contained in water, soil or food, ingested arsenic may quickly enter the human body. When air containing arsenic dusts is breathed in, the majority of the dust particles settle onto the lining of the lungs (Chen *et al.,* 2006). Very little internal exposure to arsenic occurs via the material passing through the skin into the body, and so there is little risk of arsenic poisoning posed by this route.

The majority of arsenic enters the body in the trivalent inorganic form As(III) via a simple diffusion mechanism (Cohen *et al.,* 2006). Only a small amount of pentavalent inorganic arsenic can cross cell membranes via an energy-dependent transport system, after which it is immediately reduced to trivalent arsenic.

Both organic and inorganic forms of arsenic leave the body in urine and thus most inorganic arsenic will be expelled after several days, although some will remain for a number of months or even longer (Aposhian *et al.,* 2000). The majority of organic arsenic is expelled more rapidly and usually within several days.

Groundwater contamination by arsenic and other metals has impacted severely on the health of the populations of various regions in the world. Some of the most profound examples of contamination by arsenic occur in Bangladesh and West Bengal, in India, where it has been discovered that almost 43 million people have been drinking water that is laden with arsenic (Chowdhury *et al.,* 2000). To place this in perspective, the WHO recommended limit for arsenic in water is 10 $\mu g\ l^{-1}$ (WHO factsheet no. 210, May 2001), while concentrations in the range 50–3200 $\mu g\ l^{-1}$ have been measured (Bhattacharya *et al.,* 2003).

Rahman *et al.* (2005) reported that out of a total of 336 hand-pumped tube-wells in Rajapur, 91% (307/336) contained As at concentrations of >10 µg/L, and 63% (213/336) contained As at >50 µg/L in Rajapur, India. Arsenic concentrations in drinking water varied markedly among locations, from <1 to about 200 µg/L over a 10-year period in

San Antonio de Los Cobres, Northern Argentina fluctuated within 140 and 220 µg/L, with no trend of decreasing concentration (Concha *et al.,* 2006).

Certain prokaryotes use arsenic oxyanions for energy generation, either by oxidizing arsenite or by respiring arsenate and these microbes are phylogenetically diverse and occur in wide range of habitats. In aquifers, this microbial reaction may mobilize arsenic from the solid to the aqueous phase, resulted in contaminated drinking water (Oremland and Stolz, 2003).

Almost 57 million people at 50 µg As/L (Bangladesh standard) and 35 million people at 10 µg As/L (WHO standard) in drinking water were affected in Bangladesh (Mahmood, 2002; Kadono *et al.,* 2005; Rahman, 2006). Department of Public Health Engineering (DPHE), British Geological Survey (BGS) in 1999 and Bangladesh Arsenic Mitigation and Water Supply Project (BAMWSP) in 2001 report that 61 out of total 64 districts of Bangladesh are reported to have the presence of dangerous levels of inorganic arsenic (>50 µg/L) in most of the tube wells, which are currently serving as water points mainly for drinking and cooking purpose. Khan *et al.* (2003) reported that 59 out of 64 districts in Bangladesh with 97% of socio-economically disadvantaged rural people have been already affected by arsenic in underground drinking water show skin lesions which lead to skin cancer when continuous arsenic exposure occur. Ground water As level exceeding the recommended value of WHO (0.01miligram/Liter; mg/L) found in 52 districts and above WHO's maximum permissible limit (0.05mg/L) in 41 districts out of total 64 districts of Bangladesh (Dhar *et al.,* 1998). Arsenic levels of 40% of the mostly studied tube wells in Bangladesh exceeded the Bangladesh standard limit (0.05 mg/L) of As contamination (DPHE-BGS, 2000).

Arsenic contamination in drinking water often exceeded limit of 50 µg As/L in Bangladesh, West Bengal, India and Nepal as well as other areas occupying much of the Ganges-Brahmaputra delta and found As produce arsenicosis in human population (Spallholz *et al.,* 2004). Eight districts of West Bengal, As in ground water has been found above the maximum permissible limit in 61 blocks and 863 village/wards of these districts by the side of the river Ganga are affected (Mandal *et al.,* 1998).

2.3 Arsenic, oxidative and nitrosative stress

Many mechanistic studies of arsenic toxicity have suggested that reactive oxygen species and reactive nitrogen species are generated during inorganic arsenic metabolism in living cells (Shi *et al.,* 2004).

Arsenic induces morphologic changes in mitochondrial integrity and a rapid decline of mitochondrial membrane potential. Mitochondrial alterations are considered to be primary sites where an uncontrolled random formation of superoxide anion radical occurs. Cascade mechanisms of free radical formation derived from the superoxide radical combined with a decrease in cellular oxidant defense by treatment with glutathione-depleting agent's results in an increased sensitivity of cells to arsenic toxicity (Valko *et al.,* 2005; Cohen *et al.,* 2006). Experimental results based on both *in vivo* and *in vitro* studies of arsenic-exposed humans and animals suggest the possible involvement of increased formation of peroxyl radicals ($ROO^{\bullet}$), superoxide anion radical ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), dimethylarsenic radical ($(CH_3)_2As^{\bullet}$), blood non protein sulfy dryls and/or oxidant-induced DNA damage (Flora *et al.,* 2007). The exact mechanism responsible for the generation of all these reactive species has yet to be fully elucidated, but some studies have proposed the formation of intermediary arsine species.

2.4 Genotoxicity of arsenic

There have been a large number of *in vitro* and *in vivo* studies made, devoted to determining the genotoxicity of inorganic arsenicals (Yamanaka *et al.,* 2004; Cohen *et al.,* 2006). *In vitro* studies on human fibroblasts, leukocytes, lymphocytes and hamster embryo cells have shown that arsenic induces chromosomal aberrations and sister chromatid exchange (Helleday *et al.,* 2000). Similar studies using human, mouse and hamster cells explored a potential enhancement of DNA damage, DNA repair enhancement or the inhibition of DNA synthesis.

Investigations of genotoxic effects of ingested arsenic in Taiwanese residents have yielded mixed results, possibly due to the different types of cells being examined and the different exposure levels experienced by the populations studied. Arsenic-related skin cancer has shown an accompanying much higher rate in p53 mutations in comparison with those found in UV-induced skin cancer (Hsu *et al.,* 1999).

Occupational exposure of arsenic among workers in a glass plant in India whose levels of blood arsenic were five times higher than in the control group was reported to lead to increased DNA damage in leukocytes (Vuyyuri *et al.,* 2006). These studies suggest that ingested arsenic may cause chromosomal effects, but the data really are too limited to extract any firm conclusions.

The genotoxicity of organic arsenic has also been thoroughly investigated (Kuroda *et al.,* 2004). DMA causes several genotoxic effects, including single strand DNA breaks, the formation of apurinic and apyrimidinic sites, an enhancement in oxidative stress as documented by oxidation of DNA bases, formation of DNA–protein crosslinks and chromosomal aberrations (Kitchin, 2001). Clastogenic effects of arsenic have been attributed to the high afinity of arsenic to sulfydryl groups of proteins. Several tests indicate that not only DMA but also roxarsone (3-nitro, 4-hydroxyphenylarsonic acid) may be able to cause mutations and DNA strand breaks. *In vitro* studies with MMA did not find significant increases in the occurrence of chromosome aberrations, mutations or unscheduled DNA synthesis. In addition, an increased number of DNA strand breaks was detected in lung and other tissues of mice and rats given oral doses of ~1500 mg kg^{-1} DMA (Okada and Yamanaka, 1994); this effect appeared to be related to the formation of some active oxygen species. Since the breaks were largely repaired within 24 h, the relevance related to any health risk is uncertain.

A study of p53 mutations in arsenic-related skin cancers from patients in Taiwan exposed to arsenic from drinking water found a high rate of p53 mutations and different types of p53 mutations compared with those seen in UV-induced skin cancers; similar results have been found in mice (Salim *et al.,* 2003).

The most extensively studied DNA lesion is the formation of 8-OH-G, one of the major products of DNA oxidation, which originates from the reaction of hydroxyl radical with guanine (Valko *et al.,* 2006). 8-OH-G is a sensitive genotoxic marker of oxidatively damaged DNA. Associations with increased urinary 8-OH-G concentrations have been seen also for arsenic exposure. As described above, arsenic is metabolized via methylation. A high concentration in urine, of the monomethylated As metabolite, methylarsonic acid (MMA), which is a susceptibility factor for As-induced toxicity, including carcinogenicity, has been correlated with similarly high urinary concentrations of 8-OH-G (Hu *et al.,* 2006). Interestingly, based on clinical trials, for a wide range of As

exposure with urinary-As concentrations up to 1200 μg l^{-1}, accepting the known pro-oxidative effects of As, the association of 8-OH-G with urinary-As was shown to be weaker than that for moderate exposure to cadmium. Thus, 8-OH-G may not be as sensitive a biomarker for As-induced oxidative stress as it is for Cd and for oxidative stress induced by other metals (Engström *et al.,* 2010).

2.5 Arsenic and human disease

Arsenic-induced genotoxicity may involve an alteration of the integrity of the cellular genetic material by oxidants or free radical species. Many recent studies have provided experimental evidence that arsenic-induced generation of free radicals and oxidative stress can cause cell damage and cell death through activation of oxidative sensitive signaling pathways (De Vizcaya-Ruiz *et al.,* 2009). Arsenic exposure has been linked with various types of cancer (Miller *et al.,* 2002), cardiovascular disease (Navas-Acien *et al.,* 2005), diabetes (Díaz-Villaseñor *et al.,* 2007), neurological disorders (Vahidnia *et al.,* 2007) and dermal effects (Cohen *et al.,* 2006). Low intake of calcium, animal protein, folate and fiber may increase susceptibility to arsenic-caused skin lesions (Mitra *et al.,* 2004). Health risks caused by the chronic exposure to As-contaminated groundwater have been considerable problem in many Asian countries which causes skin manifestations among the children and affect child growth and development, reproductive performance due to feeding of contaminated breast milk (Watanabe *et al.,* 2003). Over and above hyper pigmentation and keratosis, weakness, anemia, burning sensation of eyes, solid swelling of legs, liver fibrosis, chronic lung disease, gangrene of toes, neuropathy, and skin cancer are some of the other manifestations were found to be significantly higher in As exposed people (water As>0.05 mg/L) compared to control (Guha, 2003).

2.6 Dermal disease

Chronic exposure to arsenic leads to the development of lesions on the skin, including hyperkeratosis and hyperpigmentation, often used as diagnostic criteria for arsenicosis. (McCarty *et al.,* 2007). Dermal effects following the exposure to arsenic are hallmarks of the early stages of arsenic poisoning. Arsenic-induced cancers may appear later, sometimes taking several decades to develop symptoms (Lage *et al.,* 2006). Mashkoor *et al.,* 2013 carried out a study to know the arsenic (As) induced toxico-pathological

alterations in broiler chicks and their attenuation with vitamin E (Vit E) and selenium (Se). They resulted that arsenic treated groups showed significant decrease in serum. Histopathologically, liver exhibited congestion and cytoplasmic vacuolation. In kidneys, condensation of tubular epithelium nuclei, epithelial cell necrosis, increased urinary spaces, sloughing of tubules from basement membrane and cast deposition were observed. In conclusion As induced toxico-pathological alterations and vitamin E and selenium partially ameliorate the toxic effects in broilers chicks.

There is limited data accumulated for humans exposed to organic arsenic in air. Keratosis was observed in female workers in a chemical plant who were exposed to aersanilic acid (Chou *et al.,* 2007).

Nearly 2.9% of the study population (n=1654) in Bangladesh had clinical manifestations of skin lesions of As poisoning. Multivariate analysis identified age and economic status as significant predictors of arsenicosis controlling for education and gender (Hadi and Parveen, 2004). More than 50% of the villagers (n=561) showed some skin manifestations due to arsenicosis and interestingly, the skin manifestations were more severe in males than in females with clear dose-response relationship but not related with age (Kadono *et al.,* 2005).

Arsenical dermatitis in 3695 of 18,000 persons (20.6%) & evidence of arsenic neuropathy in 37.3% were observed in Bangladesh. The sub clinical arsenicosis was found in approximately 90% of children below 11 years of age living in the affected areas having hair and nail arsenic above the normal level (Rahman *et al.,* 2001). A total of 136 patients belonging to 3 Upazillas of Laxmipur, Barisal and Madaripur districts in Bangladesh, showing pigmentation were seen in all-136 cases, keratosis was found only in 110 cases, and skin ulcer was detected in 13 cases. Chronic cough increased with age, and the relative risk of chronic bronchitis increased with age due to ingestion of inorganic As (Milton *et al.,* 2003).

2.7 Cardiovascular effect

While serious and adverse effects on the cardiovascular system following oral exposure to arsenic are well known, there is some evidence from epidemiological studies that the cardiovascular system may also be affected by inhaled inorganic arsenic (Navas-Acien *et al.,* 2005; States *et al.,* 2009).

Among the more profound effects on the heart from long-term exposure to arsenic are altered myocardial depolarization and cardiac arrhythmias (Cullen *et al.,* 1995; Mumford *et al.,* 2007). Drinking water containing more As than the quantity accepted by the WHO (0.05 ppm) can cause multiple carcinomatous lesions on the oral mucosa as well as on the skin of human in Argentine Republic (Carrica**,** 2006). Inorganic forms of As are classified as carcinogens, with chronic exposure (10-40 picogram/day; pg/day) associated with skin, respiratory, and bladder cancer (Lasky *et al.,* 2004).

Wang *et al.,* 2003 found an increased incidence of disease in the blood vessels in Taiwanese populations living in areas with arsenic-polluted wells (>0.35 mg l^{-1}). In addition, attempts to assess the relative risks for stroke and peripheral arterial disease have been conducted. However, there are methodological limitations for the interpretation of the observed data and it would hence appear sensible to make such studies of the effect of arsenic on the cardiovascular system a research priority.

Vascular endothelium is well known to regulate the release of various mediators such as nitric oxide, angiotensin-II, endothelin-1, adhesion molecules, cytokines and other similarly acting species (Quyyumi, 1998; Balakumar and Kaur, 2009). NO has been considered to be a major mediator released from endothelium. It has vasodilatory and anti-inflammatory properties, and inhibits platelet adhesion and aggregation, smooth muscle cell proliferation and migration. Exposure of endothelial cells to sodium arsenite induces a decline in the integrity of vascular endothelium and endothelial cytotoxicity by inactivating protein kinase B/Akt and eNOS, so reducing the generation and bioavailability of NO, and increasing the oxidative stress and subsequently decreasing the endothelium-dependent vasorelaxation (Kitchin, 2001; Balakumar and Kaur, 2009). Experimental studies of the effect of arsenic on the vascular system have shown that oxidized lipids are present in all stages of atherogenesis which in turn generate several bioactive molecules (e.g. ROS, peroxides and isoprostanes), of which aldehydes are the major end products. Malondialdehyde (MDA) and 4-hydroxy-*trans*-2-nonenal (HNE) are the most abundant aldehydes generated from the oxidation of LDL and possess mutagenic and carcinogenic properties (Valko *et al.,* 2005, 2007). Protein adducts of MDA and HNE have been detected in atherosclerotic lesions of experimental animals and humans.

Evidence from a large number of studies indicates that inflammation plays a pivotal role in atherosclerotic plaque formation. Vascular cells generate chemokines and proinflammatory cytokines including monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6) and tumour necrosis factor α. This suggests that As-induced inflammation could be an important risk factor for atherosclerosis (Tsou *et al.,* 2005).

Hypertension is another disorder associated with increased arsenic exposure (Yang *et al.,* 2007). Arsenic-induced hypertension has been explained by an enhanced myosin light-chain phosphorylation and an increase in calcium-sensitization in blood vessels. Disruption of the antioxidant defence system leads to elevated systolic blood pressure

Impairment of vasomotor tone due to arsenic exposure is contributing factor in the development of cardiovascular disease (Lee *et al.,* 2003). Long-term exposure to ingest As has been documented to induce peripheral vascular disease, ischemic heart disease, and cerebral infarction in a dose-response relationship. This study further examined the biological gradient between ingested inorganic As and carotid atherosclerosis. Carotid atherosclerosis is associated with ingested inorganic As, showing a significant biological gradient (Chih-Hao *et al.,* 2002).

2.8 Cancer

Arsenic is a pernicious environmental carcinogen, and leads mainly to cancers of the skin, albeit that there is epidemiological evidence for lung, bladder, liver and kidney cancers being caused by exposure to arsenic (Rossman, 2003). It is thought that the mechanism by which these cancers originate may involve the promotion of oxidative stress by arsenic compounds, in which the antioxidant capacity of the living organism is overwhelmed by ROS (reactive oxygen species), resulting in molecular damage to proteins, lipids and most significantly DNA (Liu *et al.,* 2001).

Trivalent arsenic has been demonstrated to exhibit a greater toxicity than the corresponding pentavalent forms, in addition to a far more pronounced ability to release iron from the iron-storage protein ferritin (Salnikow and Zhitkovich, 2008).

However, the incidence of lung cancers has also been observed among workers exposed primarily to arsenate (Bulbulyan *et al.,* 1996). The latter study also reported an increased risk of stomach cancers among workers who had been exposed to the highest concentrations of arsenic.

Quantitative dose–response data obtained from copper smelters provide the most compelling evidence that arsenic is responsible for the development of lung cancer (Mazumdar *et al.,* 1989).

Histological examinations found an increase in several types of lung tumors, indicating that arsenic does not specifically increase the incidence of one particular type of lung cancer.

In addition to lung cancer, other minor types of nonrespiratory cancers associated with inhalation exposure to inorganic arsenic have been reported. Interline and coworkers (Enterline *et al.,* 1995) found a significantly increased mortality due to cancer of the large intestine and bone cancer. It should be noted, however, that the apparent increase in the risk of bone cancer was based on a very small number of observations.

Other studies have shown an increase in non-melanoma skin cancers as a result of exposure from a Slovakian coal-burning power plant (Pesch *et al.,* 2002) Human exposure to inorganic arsenic is associated with an increased risk of dermal malignancies (Pi *et al.,* 2008); however, arsenic has been found to act as a cofactor in the development of skin tumors in combination with ultraviolet (UV) irradiation or exposure to phorbol esters. This suggests that the events associated with arsenic-induced dermal carcinogenesis may be distinct from other target tissues.

Long-term arsenic exposure has been reported to cause a malignant transformation of human keratinocytes *in vitro* (Pi *et al.,* 2008). Arsenic-transformed cells were found to show a weakened Nrf2 (nuclear factor E2-related factor 2) -mediated antioxidant defense, activation coupled with apoptotic resistance, increased expression of casein kinase 2 (CK2) and elevated basal Nrf2 activity. Arsenic-induced apoptotic resistance and weakened antioxidant response may therefore be critical steps in development of dermal cancer after exposure to arsenic.

It is generally accepted that methylated organic arsenicals are significantly less toxic than the inorganic forms (Kitchin, 2001). Methylation is part of a natural process of enhanced excretion of arsenic and appears to be a detoxification mechanism for inorganic arsenic. However, the process of methylation may lead to formation of reactive and carcinogenic trivalent methylated arsenicals (MMAIII and DMAIII; see above) (Cohen *et al.,* 2001, 2002). Thus the process of methylation of inorganic arsenic may

provide a toxic pathway and both trivalent methylated arsenic (monomethylarsonous and dimethylarsinous acids) may possess harmful biological activity.

Animal studies did not show any signs of a prospective carcinogenic effect of MMA (V) (Cohen *et al.,* 2006).

DMA (V) has been documented by a number of studies to act as a cancer promoter in co-administration of other tumorigenic compounds (Kitchin, 2001; Wanibuchi *et al.,* 2004). DMA(V) has been reported to act as a skin tumour promoter in mice, accelerating the induction of 7,12-dimethylbenz(a) anthracene (DMBA)-induced skin tumors in mice (Morikawa *et al.,* 2000).

Rats fed with DMA (V) (200 ppm in water) exhibited an increased urinary concentration of DMA (III) in a dose-dependent manner (Okina *et al.,* 2004). This study has also shown that the levels of MMA (III) and DMA (III) may play a significant role in the toxicity and carcinogenicity towards the bladder induced by DMA (V).

It has been proposed that DMA (III) is an unstable metabolite and is stabilized through the formation of a DMA (III)–GSH conjugate, which is responsible for the toxic effect of DMA (III) (Styblo *et al.,* 2000). The cytotoxicity of trivalent (MMA (III), DMA (III) and also of As (III) arsenic can be suppressed by the application of antioxidants. Positive effects have been found following application of vitamin C and N-acetylcysteine, which preferentially interacts with trivalent arsenicals via its sulfydryl group (Wei *et al.,* 2005). Interestingly, melatonin and trolox did not show a protective effect against arsenic toxicity. The mechanism of genotoxicity of the DMA (III) does not involve direct interaction with DNA, but is most probably achieved indirectly via formation of ROS (Kitchin and Ahmad, 2003). ROS formation activates the transcription factors (e.g. AP-1, c-*fos* and NF-kB), and over secretion of proinflammatory and growth promoting cytokines, resulting in increased cell proliferation and ultimately carcinogenesis.

The exact molecular mechanism of carcinogenesis caused by arsenic is still under investigation by many researchers. Currently accepted molecular mechanisms of arsenic toxicity involve genetic and epigenetic changes, the role of oxidative stress, enhanced cell proliferation and modulation of gene expression. Arsenic is known to induce the hypoxia signaling pathway (Galanis *et al.,* 2009).

2.9 Gastrointestinal disturbances

2.9.1 Inorganic arsenicals

Clinical signs of gastrointestinal irritation, including nausea, vomiting, diarrhoea and abdominal pain, are observed in all cases of short-term high-dose and longer-term lower-dose exposures to inorganic arsenic (Uede and Furukawa, 2003; Vantroyen *et al.,* 2004).

2.9.2 Organic arsenicals

The gastrointestinal tract appears to be the critical target of toxicity following oral exposure to MMA. Ingestion of 80 mg kg^{-1} of organic arsenicals causes vomitting, abdominal pain, hyperactive bowel and diarrhoea (Lee *et al.,* 1995).

A dose level of 72.4 mg MMA kg^{-1} per day led to a thickened wall, oedema and haemorrhagic, necrotic, ulcerated or perforated mucosa in the large intestine and a significant increase in the incidence of squamous metaplasia of the epithelial columnar absorptive cells in the colon and rectum. Squamous metaplasia was also observed in the colon of mice chronically exposed to 67 mg MMA kg^{-1} per day (Gur *et al.,* 1991; Arnold *et al.,* 2003).

2.10 Liver disease

2.10.1 Inorganic arsenicals

A number of studies revealed symptoms of hepatic injury after oral exposure of humans to inorganic arsenic. These effects were most frequently observed after repeated exposure to doses of 0.01–0.1 mg As kg^{-1} per day. Clinical examination confirmed liver damage (Liu *et al.,* 2002) and blood tests showed elevated levels of hepatic enzymes. Histological examination of the livers has revealed a consistent finding of portal tract fibrosis (Mazumder *et al.,* 2005).

Levels of malondialdehyde and glutathione were decreased in the livers of rats receiving 200 mg As kg^{-1} as GaAs (Flora *et al.,* 1998). An increase in peroxidation markers was reported in rats administered with 0.02 mg As kg^{-1} per day for 60 days from drinking water containing 2.5 mg sodium arsenite l^{-1} (Bashir *et al.,* 2006).

2.10.2 Organic arsenicals

Rats exposed to a dose of 72.4 mg MMA kg^{-1} per day for 104 weeks showed a decrease in absolute liver weight. While rats exposed to DMA (Siewicki, 1981) did not exhibit any effect, mice exposed to oral doses of 720 mg DMA kg^{-1} exhibited decreased liver glutathione and cytochrome P-450 content and reduced serum ornithine decarboxylase activity (Ahmad *et al.,* 1999).

2.11 Renal disease

Inorganic arsenicals do not cause any significant renal injury in humans. In some cases elevated levels of creatinine or bilirubin have been reported (Moore *et al.,* 1994). Similarly, animal studies indicated that the kidney is not a major target for inorganic arsenic. However, at high levels of exposure, mild histological changes in the renal tubules of monkeys have been noted. Animal studies have reported renal and urinary bladder effects following oral exposure to organic arsenicals. The urinary system is a more sensitive target for DMA than for MMA (Cohen *et al.,* 2001). Prevalence of diabetes mellitus among chronic As-exposed subjects was about 2.8 times higher than the unexposed subjects (Nabi *et al.,* 2005). In Taiwan and Bangladesh, five studies reported that As interfere with transcription factors involved in insulin related gene expression. Other studies assessed the effect of As on glucose uptake, typically using very high concentration of arsenite and arsenate (Navas-Acien *et al.,* 2006).

2.12 Immunotoxicity

The response to phytohemagglutinin (PHA) stimulation of peripheral blood lymphocytes from healthy human volunteers incubated with arsenate or arsenite at concentrations of 10^{-7} M, 10^{-8} M, or 10^{-9} M. showed delayed onset in cell-cycle kinetics at all concentrations of both arsenicals in a dose-dependent pattern (Gonseblatt *et al.,* 1992). But Bencko *et al.* (1988) found no abnormalities in serum concentrations of immunoglobulin in workers exposed to arsenic in a coal-burning power plant.

2.13 Hematotoxicity

A number of arsenic compounds are toxic to blood cells. Exposure to arsenic can result in anemia and leukopenia, Arsine gas (AsH3) is a severe hemolytic toxicant that can be acutely fatal (Fowler and Weissberg, 1974).

The sequence of toxic events in arsine-induced hemolysis was studied in human erythrocytes *in vitro* by Winski *et al.* (1997), and Winski and Carter (1998) evaluated arsenate toxicity in human erythrocytes and its possible role in vascular disease. Human erythrocytes were incubated *in vitro* with sodium arsenate (AsV) or sodium arsenite (AsIII), and assessed for damage. After five hours incubation with 10 mMAsV or AsIII, significant cell death (hemolysis) only occurred in the AsV treated cells.

The exposed group with mean 0.41 mg/L As concentration in drinking water for about 18 years showed a significant decrease in whole blood nonprotein sulfhydryl levels (4.3 vs. 7.5 µmol/g Hb, $P < 0.01$) observed and this result supports a link between ingested arsenic via contaminated well water and the induction of oxidative stress. Islam *et al.* (2004) reported that the relationship of clinical complications with nutritional status and the prevalence of leukopenia among arsenic exposed patients living in the rural villages they showed that the poor nutritional status of patients increases the complications of chronic arsenic toxicity and establish a higher prevalence of leukopenia and lymphocytosis in arsenicosis patients.

2.14 Neurological disorders

2.14.1 Inorganic arsenicals

Inorganic arsenic can cause serious neurological effects, after both inhalation (Lagerkvist and Zetterlund, 1994; Calderon *et al.,* 2001) and oral exposure (Uede and Furukawa, 2003). This conclusion is based mainly on clinical observations and neurological examinations of exposed individuals.

Animal studies have shown that neurological effects following rat exposure to arsenic in the form of sodium arsenite involve changes in levels of neurotransmitters such as dopamine, norepinephrine and 5-hydroxytryptamine (Kannan *et al.,* 2001). Since adult animals appear to be much less susceptible to the neurological effects of inorganic arsenic than humans, studies in adult animals would probably not help to estimate a safe human exposure limit.

Recent findings indicate a possible association between arsenic in drinking water and neurobehavioral alterations in children (Tsai *et al.,* 2003).

The most typical neurological feature of arsenic neurotoxicity is peripheral neuropathy which may last for several years (Mathew *et al.,* 2010). Studies on patients with As neuropathy have shown a reduced nerve conducting velocity in their peripheral nerves, and this has become a hallmark of As-induced neurotoxicity, as is is a typical feature of axonal degeneration. The majority of the unfavorable effects of Arsenic are caused by the inactivation of enzymes that are important for cellular energy metabolism, whereby As reacts with the thiol groups of proteins and enzymes and inhibits their catalytic activity. In a similar fashion to other neurodegenerative diseases, arsenic-induced neurotoxicity causes changes in cytoskeletal protein composition and hyper phosphorylation. These changes may lead to disorganization of the cytoskeletal structure, which is a potential cause of As-induced neurotoxicity.

2.14.2 Organic arsenicals

No neurological symptoms or brain lesions were observed following chronic exposure of rats to MMA (72.4 mg kg^{-1} per day) or mice to MMA kg^{-1} per day (67.1 mg; Arnold *et al.,* 2003).

2.15 Reproductive health effects

Animal studies have shown that reproductive activity was unaffected in rats receiving doses of 8 mg of As_2O_3 from 14 days prior to mating. The evaluation of reproductive activity included a mating index, a fertility index and the precoital interval (time before mating) index (Holson *et al.,* 1999).

This conclusion was recently confirmed by another study which showed changes in several reproductive system end points, including reduced weights of the uterus and ovary and reduced ovarian and uterine peroxidase activities; inhibition of steroidogenic enzymes and decreased estradiol levels relative to the controls (Chattopadhyay *et al.,* 2001, 2003).

2.16 Antioxidant protection against arsenic mutagenicity

Oxidative stress to DNA is recognized as an underpinning component of the mechanism of arsenic carcinogenesis (Valko *et al.,* 2005). Antioxidant enzymes are considered to be the first line of cellular defense against oxidative damage. Superoxide dismutase (SOD) and catalase (CAT) are the most important, first line antioxidant defense in cells exposed

to oxygen. SOD catalyses the dismutation of superoxide into oxygen and hydrogen peroxide, while CAT catalyses the decomposition of hydrogen peroxide to water and oxygen. Arsenic-intoxicated rat's revealed reduced activity of SOD which was attributed to the enhanced production of superoxide radical anions.

A second line of cellular defense system against free radical-induced damage is provided by a thiol-based antioxidant system (Manna *et al.,* 2008). Decreased GSH pools and increased levels of lipid peroxidation due to arsenic toxicity were found to lead to a decrease in the activities of GST and GPx with a concomitant decrease in the activity of the GSH-regenerating enzyme GR.

A field trial was undertaken in West Bengal (a region whose population is exposed to high levels of arsenic in drinking water), to evaluate the role of the phytochemical, curcumin, from turmeric for its antioxidant and antimutagenic activity (Biswas *et al.,* 2010). Blood samples taken from volunteers in the region showed notable DNA damage and depleted antioxidant activity. However, following dosage with curcumin capsules for 3 months, the DNA damage was reduced, ROS generation and lipid peroxidation were suppressed, and the antioxidant activity of blood plasma was raised, thus offering the hope of some protective role for curcumin against DNA damage by arsenic.

The most effective known treatment for arsenic poisoning is chelation therapy; however such agents as British anti lewisite, sodium 2,3-dimercaptopropane-1-sulfonate, meso 2,3-dimercaptosuccinic acid etc. result in a number of undesirable side-effects (Flora *et al.,* 2007). It has been shown that supplementation of the chelating agent with antioxidants may be beneficial in achieving optimum effects.

Another study reported genotoxic effects of sodium arsenite (known for its genotoxic effects through ROS generation) in forming micronuclei in the polychromatic erythrocytes in the bone marrow cells of Wistar rats (Balakumar *et al.,* 2010). Supplementation by orally administered α-tocopherol (400 mg kg^{-1} of body weight) and ascorbic acid (200 mg kg^{-1} of body weight) to rats given 100 ppm of sodium arsenite in their drinking water for 30 days suggested a protective effect on the cellular antioxidant system and a modulation of arsenic-induced micronuclei formation.

2.17 Effect of arsenicosis on hematological and biochemical parameters

2.17.1 Biochemical parameters

Mondal *et al.* 2016 studied the protection against arsenic-induced hematological and hepatic anomalies by supplementation of vitamin C and vitamin E in adult male rats. They observed that Arsenic exposure caused significant reduction of erythrocyte counts, leukocyte counts and hemoglobin (Hb) levels while arsenic exposure also led to marked echinocytic transformation of erythrocytes resulting in increased morphological index. They also found altered serum oxidative balance was observed with a higher oxidative stress index. Again, they showed a significant increase of serum cholesterol, low-density lipoprotein and triglycerides, and decreased high-density lipoprotein along with total protein. A marked elevation of hepatic thiobarbituric acid reactive substance along with decreased reduced glutathione levels were also observed by them. Interestingly, they found that co-administration of VC and VE significantly prevented all the arsenic-induced alterations except Hb content and serum protein.

Oral exposure to As for a period of 12 weeks significantly ($p<0.05$) increased As burden in blood, liver and kidney from arsenic treated rats. This was associated with exposure duration-dependent rise ($p<0.05$) in lipid peroxidation in these tissues (Nandi *et al.,* 2006).

Serum cholesterol levels, triacylglycerol, total protein, and albumin were significantly higher in the groups treated with As_2O_3 or As_2O_5 as compared to control ($p<0.05$). The arsanilic acid treatments significantly decreased high density lipoprotein and increased very low density lipoprotein in plasma ($p<0.05$). The creatine kinase level in the roxarsone, As_2O_3, and As_2O_5 groups was significantly higher compared to control ($p<0.05$; Chen *et al.,* 2001).

Activity of serum glutamate oxaloacetate trasaminase (SGOT) was reduced by Arsenic alone and serum alkaline phosphatase was reduced by As (Mahaffey *et al.,* 1981). No change in serum glutamate pyruvate trasaminase (SGPT) was observed with As supplementation for 0, 45 and 90 days (Kaur *et al.,* 2005). Dietary As increased liver glutathione peroxidase activity only when rats were fed 2.0 µg /g selenium diet as selenite. Dietary As did not influence liver cytosolic concentrations. Blood As

concentrations were significantly ($p<0.0001$) elevated when rats were fed high dietary As (Davis *et al.,* 2000).

Patients (n=10) with higher serum creatinine (>2.0 milligram /deciliter; mg/dl), urea (>0.70 g/L) and urinary protein (mean standard error; mean ± SD: 1.12 ± 0.82 g/L) showed higher As concentrations (5.8 ± 3.3 µg/L in serum and 18.0 ± 16.7 µg/kg in packed cells) compared with those with lower creatinine (<1.6 mg/dl), urea (<0.6 g/L) and urinary protein (mean ± SD: 0.27 ± 0.82 g/L). The significant differences ($p<0.001$) implied a relationship between the As level and the degree of chronic renal insufficiency (Zhang *et al.,* 1995). The patients of arsenicosis had significantly lower levels of serum creatinine (0.97 mg/dl) compared to the controls (1.15 mg/dl); but had significantly elevated levels of total protein, 84 g/L (patients) and 77 g/L (controls) respectively.

2.18 Spirulina

The cyano-bacterium Spirulina is a filamentous blue-green alga belonging to the *Oscillatoriaceae* family that is generally found in tropical and subtropical regions in warm alkaline water (Samir *et al.,* 2016). Spirulina is characterized by high nutritional value where it contains high protein content (60–70% by dry weight), plenty of vitamins, amino acids, gamma-linoleic acid, and minerals (Hoseini *et al.,* 2013). The consumption of Spirulina as a diet supplement has health benefits in preventing or managing hypercholesterolemia (Ferreira-Hermosillo *et al.,* 2010), hyperglycerolemia (Deng *et al.,* 2010), obesity, inflammation (Coskun *et al.,* 2011), cancer (Ismail *et al.,* 2009), and cardiovascular disease (Khan *et al.,* 2005). In addition, Spirulina has antidiabetic effect (Karkos *et al.,* 2011). Spirulina provides protection against mercuric chloride-induced oxidative stress and alteration of antioxidant defense mechanism in the liver. These activities were largely related to phycocyanin, an active protein of Spirulina (Romay *et al.,* 1998). Phycocyanin (Pc) is a biliprotein of the blue-green alga. This protein contains a tetrapyrrolephycocyanobilin, which is responsible for antioxidant properties of Pc (Bhat *et al.,* 2001). It has been reported that Pc has significant antioxidant and radical scavenging properties, offering protection against oxidative stress (Lissi *et al.,* 2000). Antioxidants can reduce arsenic toxicity through chelating it and scavenging free radicals (Rana *et al.,* 2007). Spirulina and thankuni significantly lowered the arsenic burden of rats whereas spirulina is more effective than thankuni against arsenic burden in rats (Hasan *et al.,* 2015).

The protective actions of *S. platensis* against arsenic are believed to originate from its free radical scavenging, antioxidant activities, maintenance of antioxidant enzymes, and a decrease in the production of inflammatory mediators that are implicated in the pathogenesis of arsenic-induced testicular injury. Therefore, *S. platensis* represents a potential agent to prevent testicular injury and dysfunction induced by arsenic exposure.

Hossain *et al.,* 2012 conducted a controlled experiment to assess the effect of chronic arsenicosis on hematological and biochemical changes in Long-Evans rats and to assess the protective role of Spirulina combined with vitamin A following experimental arsenicosis, using daily oral doses of sodium arsenite for 63 days. They showed that the values of SGOT (Serum glutamate oxaloacetate transaminase) and SC (Serum creatinine) increased significantly in all the treated groups of rats compared to the control group, but *Spirulina* combined with Vitamin A produced values significantly comparable to the untreated control group. They also found SGPT (Serum glutamate pyruvate transaminase) showed slight significance differences among the treatment groups, *Spirulina* combined with Vitamin A appeared most effective in managing arsenic treatment. *Spirulina*+ Vitamin A increased the values of TEC, TLC and Hb (Total erythrocyte count, Total leukocytecount and Hemoglobin) against arsenic toxicity in rats but showed no significance differences. They concluded that the combination of *Spirulina* and vitamin A were found more effective in the prevention of chronic arsenicosis in rat than using these substances (*Spirulina* or Vitamin A) alone.

Millions of people in Bangladesh, India, Taiwan, and Chile are consuming high concentration of arsenic through drinking water, and thousands of them have already developed chronic arsenic poisoning. (Misbahuddin *et al.,* 2006).

It is clearly evidenced that spirulina could play consistent role in the treatment of arsenicosis patients. The spirulina intake caused more improvements in patients of age group 15-35 years (66.66%) than patients of 35 to 55 years (50%). The impact of spirulina improvement represents a different phenomenon on the vulnerability of social taboos. It occurred 71.42% in middle class, while in poor class this was 69.29%. The greatly vulnerable poverty related malnourished arsenicosis patients responded to improvement equally as attained by well-nourished patients. The overall response revealed that 60% patients showed considerable improvement with spirulina treatment (Rahman *et al.,* 2006).

Some of the pharmacological properties of spirulina (*Spirulina platensis*) may be linked to its antioxidant potential, which mitigates oxidative stresses. (Ghosh *et al.,* 2014).

2.18.1 Composition of spirulina

Proteins: Spirulinais useful in human nutrition, due to the high quality and quantity of its protein. Spirulina has a high protein concentration (60%-70% of its dry weight) (Ciferri, 1983). The nutritive value of a protein is related to the quality of amino acids, digestibility coefficient, as well as by its biological value (Richmond, 1992; Dillon and Phan, 1993). Spirulina contained essential amino acids; the highest values are leucine (10.9%), valine (7.5%), and isoleucine (6.8%), (Cohen, 1997).

Vitamins: Among foods, spirulina has a relative high provitamin-A concentration (Belay, 1997). An excessive dose of β-carotene may be toxic, but when the β-carotene was ingested from the spirulina or another vegetable it was usually harmless since the human organism converted it into vitamin A as the quantity needed (Henrikson, 1994). Spirulina is found very rich in vitamin B_{12}, and thus, spirulina is of great value for people needing supplements in the treatment of pernicious anemia (Becker, 1984; Richmond, 1992; Belay, 1997).

Lipids: Spirulina contains 4-7% lipids and had essential fatty acids: linoleic acid and gamma-linolenic acid (Othes and Pire, 2001). The latter is claimed to have medicinal properties and is required for arachidonic acid and prostaglandin synthesis (Dubacq and Pham-Quoc, 1993). Low-density lipoprotein is lowered by gamma-linolenic acid, being 170-fold more effective than linoleic acid (Cohen, 1997).

Minerals: Iron in some nutritional complements is not appropriately absorbed but that in spirulina is 60% better absorbed than ferrous sulfate and other complements. Consequently, it can represent an adequate source of iron in anemic pregnant women (Puyfoulhoux, *et al.,* 2001).

Carbohydrates: Spirulina (*Spirulina platensis*) contains about 13.6% carbohydrates; some of these are glucose, rhamnose, mannose, xylose and galactose (Shekharam *et al.,* 1987). Spirulina do not have cellulose in its cell wall, a feature that makes it an appropriate and important foodstuff for people with problems of poor intestinal absorption, and geriatric patients (Richmond, 1992). A new high molecular weight polysaccharide, with immunostimulatory activity has been isolated from spirulina and

called "Immulina". This highly water-soluble polysaccharide represented between 0.5% and 2.0% (w/w) of the dry microalgae (Pugh *et al.,* 2001).

Nucleic acids content: Spirulina contained 2.2%-3.5% of RNA and 0.6 %-1% of DNA, which represented less than 5% of these acids, based on dry weight. These values were smaller than those of other microalgae like Chlorella and Scenedesmus (Ciferri, 1983).

Pigments: Some natural pigments are found in spirulina. These pigments are responsible for the characteristic colors of certain flamingo species that consume these cyanobacteria in the African Valley. This knowledge has promoted the use of this microorganism as source of pigmentation for fish, eggs (Ciferri, 1983; Saxena *et al.,* 1983; Henrikson, 1994) and chicken. Spirulina also increase the yellowness and redness of broiled chicken due to accumulation of zeaxanthin (Toyomizu *et al.,* 2001).

2.18.2 Benefits of spirulina consumption

Lactobacillus population in human gastrointestinal tract is increased by spirulina consumption. This means food digestion and absorption improvement, intestinal protection against bacterial infections and immune system stimulation (Henrikson, 1994; Schiffrin *et al.,* 1997). Immune system modulation is due to interference on production and NK cytotoxicity (Hirahashi *et al.,* 2002). Hasan *et al.,* 2015 conducted an experiment to study the prophyalactic contributions of spirulina and thankuni on rats experimentally induced with arsenic toxicity were tested and the comparative efficacies of both spirulina and thankuni. They showed that no visible clinical signs were observed in any groups of experimental rats. They also found that sodium arsenite feeding caused arsenic burden in rats but Spirulina and thankuni significantly lowered the arsenic burden of rats whereas spirulina is more effective than thankuni against arsenic burden in rats. Finally they concluded that the combination of spirulina and thankuni were found more effective in prevention of arsenic burden in rat.

Other benefits are attributed to spirulina: anti-arthritic effect due to the anti-inflammatory and antioxidative properties of phycocyanin (Ramirez *et al.,* 2002); anti-atherogenic property (Kaji *et al.,* 2002), tumor burden inhibition (Dasgupta *et al.,* 2001); chemo protective and radio-protective effect (Zhang *et al.,* 2001); and antioxidant activity on lead-induced toxicity in rats (Upasani *et al.,* 2001).

An extract of sulfated polysaccharides, called Calcium-Spirulan, made up of rhamnose, ribose, mannose, fructose, galactose, xylose, glucose, glucuronic acid, galacturonic acid, and calcium sulfate, obtained from spirulina, showed activity against HIV, Herpes Simplex Virus, Human Cytomegalovirus, Influenza A Virus, Mumps Virus and Measles Virus (Henrikson, 1994; Hayashi, 1996).

Studies have shown that spirulina consumption during 4 weeks reduces serum cholesterol levels in human beings by 4.5% (Henrikson, 1994) and significantly reduces body weight by 1.4 ± 0.4 kg after four weeks (Becker *et al.,* 1986). These reports indicated no changes in blood pressure or in hematocrite, hemoglobin, white blood cells, sedimentation rate, and absence of adverse effects. Cholesterol reduction is partly owed to the gamma-linolenic acid cyanobacteria high content (Henrikson, 1994).

Spirulina reduces hepatic damage due to drug abuse and heavy metal exposure, inflammatory response (Richmond, 1984; González *et al.,* 1999), cells degeneration (Bulik, 1993), anaphylactic reaction (Yang *et al.,* 1997), Bitot's spots, and Cesium-137 and Strontium-90 radiation in Chernobyl children (Henrikson, 1994).

Spirulina contains vitamin A, important in preventing eye diseases; iron and vitamin B_{12}, useful in treating hypoferric anemia and pernicious anemia, respectively; gamma-linolenic acid, appropriate in treatment of atopic child eczema therapy; to alleviate premenstrual syndrome, and in immune system stimulation (Pascaud, 1993). Spirulina also has a positive effect on cardiac disease, Parkinson's disease, malnutrition, sclerosis (Fox, 1993; Thein, 1993; Fox, 1998) and wounds cure (Richmond, 1992).

Spirulina extract induces the tumor necrosis factor in macrophages, suggesting a tumor destruction mechanism (Shklar and Schwartz, 1988).

2.18.3 Uses of spirulina as food and drugs

Spirulina is an animal cell-growth stimulant (Kerby and Rowell, 1992) and in the treatment of residual waters using alginate (Cañizares *et al.,* 1993; Patnaik *et al.,* 2001). Cattle and horse breeders affirm that when adding spirulina to silage, the quantity of sperms in males and the fertility in females are increased (Henrikson, 1994).

Spirulina has therapeutic effects ranging from reduction of cholesterol and cancer to enhancing the immune system, increasing intestinal lactobacilli, reducing nephrotoxicity

by heavy metals and drugs, and radiation protection (Belay and Ota, 1993). Spirulina reduced mercury and other toxic metal accumulation in the tissue (Johson *et al.,* 1986). It reduced nephrotoxicity from mercury and three pharmaceutical drugs in laboratory rats in Japan (Yamane, 1988). A protective effect on the kidneys apparently associated with the phycocyanin present in spirulina (Fukino, 1990).

2.18.4 Toxicological aspect of spirulina

Due to the use of fertilizers, possible water and environmental pollution optimal quality control and periodic revisions of this cyanobacteria culture is necessary to detect high metal concentration values (Chamorro *et al.,* 1996). Studies in Mexico showed that the administration of *Spirulina platensis* to mice does not cause embryonic or fetal damages (Chamorro *et al.,* 1989; Chamorro and Salazar, 1990). Absence of phycotoxins in spirulina is an advantage with respect to *Microcystis*, *Anabaena* and *Aphanizomenon*, fresh water cyanobacteria that have caused death in livestock and allergic or gastrointestinal reactions in human beings (Chamorro *et al.,* 1996). Chronic and sub-chronic toxicity studies revealed no toxic effects by spirulina. The lethal dose (LD_{50}) of spirulina has not been determined, since it would be necessary to dispense high quantities in one single dose (Switzer, 1980; Chamorro *et al.,* 1996).

2.18.5 Spirulina in the treatment and prevention of arsenic toxicity

Administration of 10 g of spirulina per day for 3 months was reported to be effective in the treatment of chronic arsenic poisoning (Khan *et al.,* 2001). And it was found to be effective in the removal of arsenic from the arsenic loaded tissues in rats as it contained ample of protein, vitamin, amino acids, minerals and other nutrients (Fariduddin *et al.,* 2001). Ten grams of spirulina daily for 4 months improvement in reducing skin manifestation of patient in Bangladesh (Karim *et al.,* 1999). Spirulina extract (250 mg) plus zinc (2 mg) twice daily for 16 weeks may be useful for the treatment of chronic arsenic poisoning with melanosis and keratosis where they showed that the clinical scores (median) for melanosis before and after treatment with placebo was not statistically significant ($p>0.05$), whereas in spirulina extract plus zinc- treated group, it was statistically significant ($p<0.01$; Misbauddin *et al.,* 2006). Spirulina significantly reduced ($p<0.01$) As burden in different organs of chicken (Awal, 2007). Spirulina treatment lowered arsenic contents in blood and milk of goats (Halim, 2007).

2.19 Vitamin C

Ascorbic acid and n-aceltyl-l-cysteine as well as other antioxidants have different reactivates for specific oxidative species, such as hydroxyl radicals, singlet oxygen, hydrogen peroxide, peroxyl radicals, or superoxide anion (Childs A *et al.,* 2001; Ramanathan *et al.,* 2002). Understanding how each of these two compounds reacts with ATO may provide new insights into their potential influence on the clinical outcome of APL patients receiving ATO chemotherapy. The specific aim of this research was to determine whether co-exposure to AA or NAC modulates oxidative stress associated with ATO toxicity in human leukemia (HL-60) cells. Many antioxidants have been reported to enhance or inhibit ATO-mediated apoptosis in tumor cells (Yi *et al.,* 2004). Ascorbic acid (AA) is an anti-oxidant and free radical scavenger effective against peroxyl- and hydroxyl-radicals, superoxide, singlet oxygen and peroxynitrite. Many researchers believe that AA, known as vitamin C, prevents cancer by deactivating free radicals before they can damage DNA and initiate tumor growth (Frei Balz. 1994). Some scientists have claimed that AA can cure anything from the common cold to cancer by stimulating the immune system and protecting the body against free radicals (Pauling *et al.* 1970). However, other studies have reported that vitamin C may act as a pro-oxidant that helps the body's own free radical defense mechanism destroy tumors in their early stages (Uddin *et al.,* 1995, Schwartz *et al.,* 1996). N-acetyl-L-cysteine (NAC) is an antioxidant/free radical scavenger or reducing agent that protects against cell death (Mayer *et al.,* 1994; Cotgreave *et al.,* 1997). The protective action of NAC, a thiol-containing compound that acts as a nucleophile, and a precursor of reduced glutathione, has been widely demonstrated (Yedjou *et al.,* 2008; Cotgreave *et al.,* 1997).

Karasavvas *et al.* (2005) studied the circumvent of the extracellular in vitro pro-oxidant effects of AA, by loadeding HL60, U266, and RPMI-8226 cells with vitamin C by incubation with dehydroascorbic acid (DHA). They found loading cells in this manner resulted in prominent, dose-dependent protection of As_2O_3-treated cells as measured by viability, colony formation, and apoptosis assays. Glutathione depletion enhanced cell sensitivity to the cytotoxic effects of As_2O_3 and vitamin C loading provided protection. They also reported that AA was found to generate cytotoxic concentrations of H_2O_2 in culture medium without cells and copper/iron chelators inhibited this reaction but AA did not generate H_2O_2 in simple buffer or human plasma. Direct incubation with AA resulted in increased intracellular ROSs, whereas DHA incubation decreased it. These

results clarify an apparent paradox and indicate that vitamin C loading in HL60, U266, and RPMI-8226 cells ameliorates As_2O_3 cytotoxicity.

The reduction of arsenic induced higher blood glucose level by folic acid and vitamin C demonstrates that folic acid and vitamin C has significant effect in preventing arsenic induced disease (Ali *et al.,* 2008). Folic acid supplementation total blood arsenic levels were reduced by 13.6 and by 2.5% in the placebo group (Gamble *et al.,* 2007).

CHAPTER III

MATERIALS AND METHODS

The experiment was accomplished under the Department of Physiology and Pharmacology, Faculty of Veterinary and Animal Science of Hajee Mohammed Danesh Science and Technology University, Dinajpur during the period of 12th September to 10th November, 2016.

3.1 Design of the experiment

Sixty male rats of about one months of age were used in this experiment. All the 60 rats were bred in the laboratory of International Center for Diarrheal Disease Research Bangladesh (ICDDRB). At first all the rats were selected and marked by using different color for identification such as red, yellow and black. The rats were observed for fifteen days for the adjustment with the environment. All the rats were maintained by feeding pellet poultry feed (Nourish Poultry feed) and tap water. All feeds and water were with admissible levels of arsenic. They were kept in different cage in a pre-disinfected and well ventilated room with controlled ambient temperature and natural relative humidity. The room underwent disinfection with 5% phenol following detergent washing at every day. The excreta of animals were cleaned properly daily in the morning and evening. The animal room was well ventilated with electricity-run ventilator.

The animals were randomly divided into 5 equal groups and eventually each group comprised of 12 male rats. Groups were identified as T_0 for control, T_1 for arsenic treated group, T_2 for arsenic plus spirulina treated group, T_3 for arsenic plus Vitamin C extract treated group and T_4 for arsenic plus vitamin-C extract plus spirulina group.

Following grouping, all the rats were weighed individually firstly on day 0, then on day 15, day 30 and finally on day 45 and the results were recorded.

3.2 Layout of experiment

The layout of the experiment is presented below:

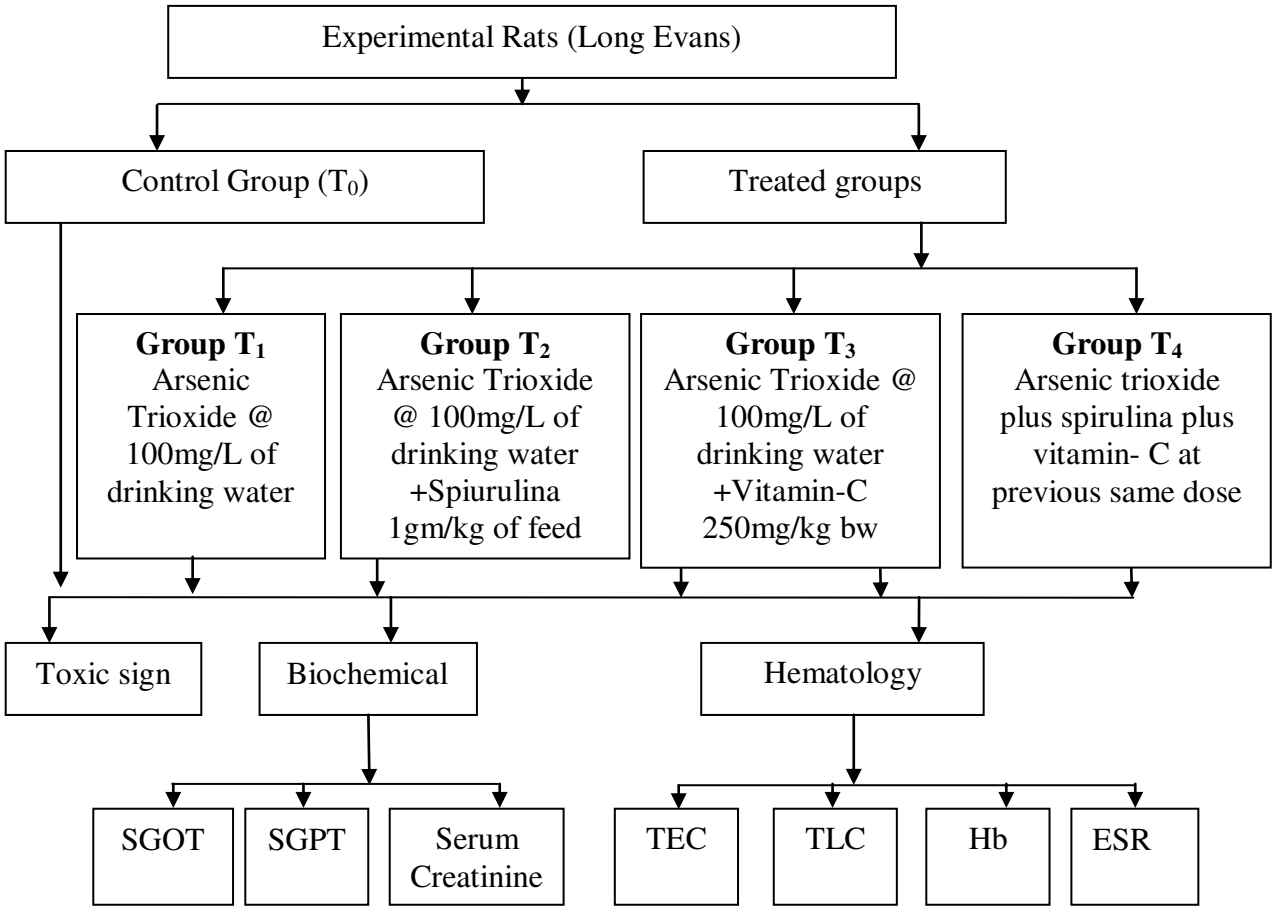

Fig. 1: Layout of the experiment (n=12 in each group)

3.3 Body weight

The rats were individually weighed firstly on Day 0 (Day 0= immediate previous day of starting treatment, after grouping and marking), D_{15}, D_{30} and finally D_{45} and the results were recorded.

Plate 1: Weighing of body weight of rat

3.4 Clinical signs

Rats were closely observed daily for 3 times (morning, afternoon and evening) for toxic clinical signs if any in them, during the entire experimental period (from Day 1 to Day 60) and the findings were recorded.

3.5 Experimental trial

Each of group is treated by different parameter and the experiment was concluded by 45 days. Rats of group T_0 were maintained on only normal feed and water as control group, rats of group T_1 were treated with Aresnic Trioxide @ 100mg/L of drinking water daily and normal feed and rats of group T_2 were treated with Arsenic Trioxide @ 100mg/L of drinking water plus feed with spirulina @ 1gm/kg feed, rats of group T_3 were treated with Arsenic Trioxide @ 100mg/L of drinking water plus Vitamin–C Tablet (Cevit®; of Square Pharmaceuticals Limited; Bangladesh) simultaneously at a dose of 250mg/kg bodyweight and the rats of T_4 were treated with Arsenic Trioxide @ 100mg/L of drinking water plus Vitamin–C at a dose of 250mg/ kg bodyweight plus spirulina @ 1gm/kg feed. All treatments were given for 45 days.

3.6 Preparation of treatment materials

3.6.1 Arsenic trioxide solution

On the basis of the total body weight of the rats, the respective required amount of Arsenic Trioxide @ 100mg/L of drinking water for a day was weighted separately for each group of rats. The respective pre-weighed As_2O_3 was mixed with the drinking water daily for that particular group. Generally, required amount of arsenic trioxide (100mg/L

of drinking) water per rat was allotted for mixing As_2O_3 to ensure that they take full time of arsenic trioxide for their requirement.

Plate 2: Weighing of arsenic trioxide

3.6.2 Preparation of spirulina mixed feed

Each capsule of Spirulina was purchased from Square Pharmaceuticals Company that containing 500mg of *Spirulina platensis.* The capsule was made to a homogeneous powder with the help of pestle and mortar. Then the required amount of spirulina was measured with the help of electric balance. The powder Spirulina was kept in a desiccators so that no change in quality of the Spirulina. For proper mixing small amount of distill water was added to make it a suspension and then the suspension was added drop by drop to the feed with the help of a small stainless steel spoon and simultaneously the feed was stirred by a glass rod for homogenous mixing. After completion of the mixing, the mixed feed was dried in an electric oven at 50ºC for 24 hours and kept in air-tied plastic container.

3.6.3 Vitamin C mixed feed

Each vitamin Tablet Cevit® was purchased from Square Pharmaceuticals Limited; Bangladesh which contained 250mg of vitamin C. The tablet was made to a homogeneous powder with the help of pestle and mortar. Then the powder was mixed with required amount of distilled water and simultaneously the water was stirred by a glass rod for homogenous mixing. After completion of the mixing, the mixed solution was added into drinking water and provided to rats.

Plate 3: Feed

3.7 Sampling

At every 15 days interval 4 rats were sacrificed and about six milliliters (ml) of blood samples were collected from hearts of each rats using disposable plastic syringe for determination of biochemical parameters, hematological test and for the determination of arsenic concentration in blood. For the biochemical test, blood sample was taken into pre-marked centrifuge glass test tubes immediately after collection. The blood samples were kept at room temperature for 1 hour without agitation to let it clot with a view to collecting serum. For the hematological test and for the detection of arsenic concentration in blood 1 ml of blood for each was taken separately in EDTA coated tube.

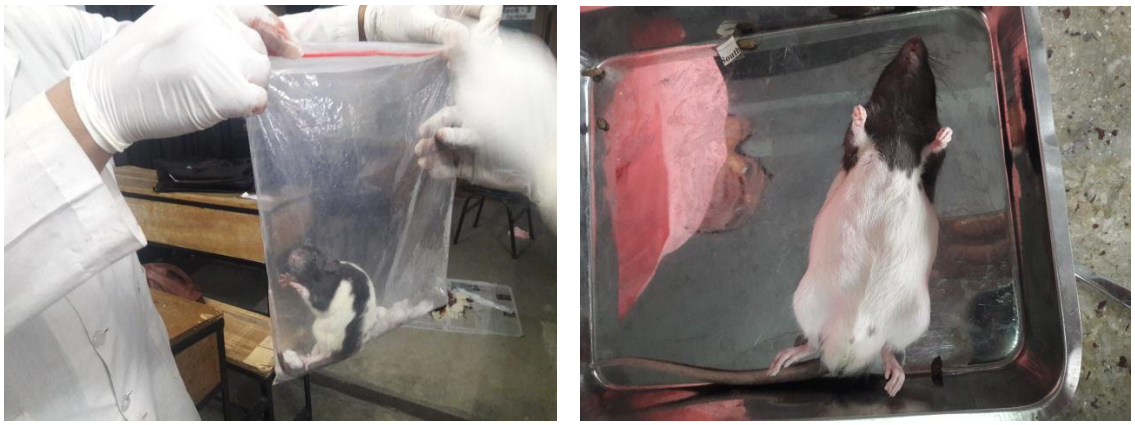

Plate 4: Anaesthesia of rat

Plate 5: Collecting blood sample

3.8 Examination of blood for determination of hematological parameters

3.8.1 Total Erythrocyte Count (TEC)

Total erythrocyte count was done following the method described by Lamberg and Rothstein (1977). Well-mixed blood sample was drawn with red blood cell diluting pipette exactly up to 0.5 marks. Outside of the tip of the pipette was wiped with cotton. Then the pipette was immediately filled with the red cell diluting fluid (Hayem's solution) up to 101 marks. The free end of the pipette was wrapped around with the rubber tube stretching to both the ends and held with thumb and middle finger. The content of the pipette was mixed thoroughly by shaking with 8-knot motion for 3-5 minutes. Then the counting chamber was placed with special cover glass under microscope using low power (10x) objectives. After discarding 2 or 3 drops of fluid from the pipette, a small drop was placed to the edge of the cover glass on the counting chamber as the entire area under the cover glass was filled by the fluid. One-minute time was spared to allow the cells to settle on the chamber under the cover glass. Taking 5 larger squares (4 in the 4 corners and the central one) of the central large square, the cells were counted from all the 80 small squares (16x5) under high power objectives (45x). After completion of counting, the total number of RBC was calculated as number of cells counted x 10, 000 and the result was expressed in million/µl of blood.

3.8.2 Total Leukocyte Count (TLC)

Well mixed blood sample was drawn up to the 0.5 mark of white blood cell pipette. The pipette was filled up to the 11 mark with the diluting fluid (N/10 HCl) by steady sucking and the content was thoroughly mixed for 2 minutes and then 2 or 3 drops of the content

was discarded and counting chamber was then filled in the same way as done in the red blood cell count. At least 1 minute was allowed for lysis of RBCs. The leukocytes in the 4 large corner squares (each 1 square mm) of the counting chamber were counted. The counting and calculation of leukocyte were performed as per methods described by Lamberg and Rothstein (1977). The result was expressed as thousand/µl.

3.8.3 Determination of hemoglobin concentrations (Hb)

The N/10 hydrochloric acid was taken in a graduated tube up to 2 marks with the help of a dropper. Well-homogenized blood sample was then drawn into the Sahli pipette up to 20 cm. mark. The tip of the pipette was wiped with sterile cotton and the blood of the pipette was immediately transferred into the graduated tube containing hydrochloric acid. This blood and acid were thoroughly mixed by stirring with a glass stirrer. There was a formation of acid hematin mixture in the tube by hemolysing red blood cells with the action of hydrochloric acid (HCL). The tube containing acid hematin mixture was kept standing in the comparator for 5 minutes. After that distilled water was added drop by drop. The solution was mixed well with a glass stirrer until the color of the mixture resembled to the standard color of the comparator. The result was read in daylight by observing the height of the liquid in the tube considering the lower meniscus of the liquid column. The result was then expressed in g %. The above procedure was matched by the Hellige hemometer method as described by Lamberg and Rothstein (1977).

3.9 Serum collection

After 1 hour, each clot of properly clotted blood samples (without anticoagulant) was detached from the inner wall of the respective test tube with a separate long fine needle by moving it slowly between the clot and the inner wall of the test tube, and the tubes were then kept overnight at 4^0C in a refrigerator. In the next morning, tubes with blood clot were kept outside the refrigerator for matching to the room temperature. Following reaching the room temperature, the test tubes containing blood clot were centrifuged in centrifuge machine (Hettich, Universal II, Germany) at 2000 rpm (rotation per minute) for 15 minutes. Then the serum was come out over the each clot within the test tube following centrifugation. The supernatant serum was collected gently in the correspondingly marked screw capped sterile test tubes with separate sterile Pasteur pipette and kept in deep freeze at -20^0C until tested.

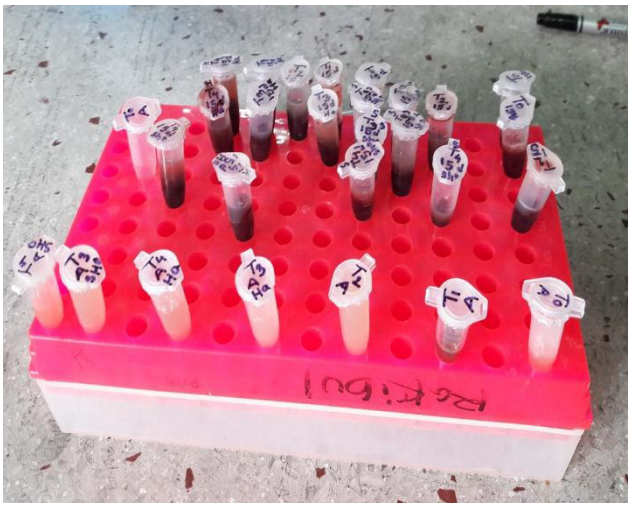

Plate 6: Collection of serum

3.10 Examination of blood sample (serum) for biochemical parameters

3.10.1 Serum Glutamate Oxaloacetate Transaminase (SGOT)

In brief, serum of the sample was 4-fold diluted in Phosphate Buffered Solution (PBS) with p^H 7.4. Twenty five micro liters of diluted serum was placed on the centre of the red application zone (xx marked) of the glutamic oxaloacetic transaminase (GOT) test strip with the help of micropipette after opening the sliding cover of the test strip. The strip was then placed on to the strip guide within 15 seconds from placing of serum on the strip and the slide was forwarded until it locks into place. The sliding cover was closed. The SGOT level was displayed on the monitor in 75 seconds in Unit/L.

3.10.2 Serum Glutamate Pyrovate Transaminase (SGPT)

SGPT is determined following the same procedure as done in case of SGOT. Serum of the sample was 4-fold diluted in Phosphate Buffered Solution (PBS) with p^H 7.4. Twenty five micro liters of diluted serum was placed on the centre of the red application zone (xx marked) of the glutamate pyrovate transaminase (GPT) test strip with the help of micropipette after opening the sliding cover of the test strip. The strip was then placed on to the strip guide within 15 seconds from placing of serum on the strip and the slide was forwarded until it locks into place. The sliding cover was closed. The GPT level was displayed on the monitor in 75 seconds in Unit/L.

3.10.3 Serum creatinine

Serum creatinine is determined following the same procedure as done in case of SGOT by Reflotron® Plus. Briefly, serum of the sample was diluted in PBS. Twenty five micro liters of diluted serum was placed on the centre of the red application zone of the creatinine test strip with the help of micropipette after opening the sliding cover of the test strip. The strip was then placed on to the strip guide within 15 seconds from placing of serum on the strip and the slide was forwarded until it locks into place. The sliding cover was closed. The creatinine level was displayed on the monitor in 75 seconds in mg/dl.

3.11 Statistical analysis

The experimental data were designed in CRD and analyzed statistically using one way ANOVA with the help of the SPSS software. The mean comparisons of the treatments were made by the Duncan's Multiple Range Test (DMRT).

CHAPTER IV

RESULTS

4.1 Clinical signs

There are no significant clinical signs were observed in rats during the entire period of the experimental trial but highly increases in the body weight were observed in all groups except arsenic treated group.

4.2 Body weight of rats

Body weights (BWs) of rats of all groups were taken on 0, 15, 30 and 45 days. The BWs taken on day 45 were found highest in rats of control group. BWs were slightly increased in arsenic plus spirulina treated rats, whereas that was lost by the rats of arsenic T_1 treated group, on the other hands arsenic plus spirulina (T_2) treated group; arsenic plus Vitamin C (T_3) treated rats and arsenic plus Vitamin C plus spirulina (T_4) treated rats gained higher body weight than control group compared to their initial weights (Table 1). On day 30 BWs were found slightly increased in all groups except in group T_1 and the highest BWs gain was found in arsenic plus spirulina treated group, arsenic plus Vitamin C group and arsenic plus Vitamin C plus spirulina treated group. On day 45 BWs were found slightly increased in T_2, T_3, T_4 and control groups except arsenic T_1 groups (Fig. 2).

Table 1: Effects of arsenic, spirulina and Vitamin C on the body weight of against rats

Treatments	Body weight (gm)					
	Group T_0	Group T_1	Group T_2	Group T_3	Group T_4	Level of significance
Initial	78.20 ±3.28	82.20 ± 2.63	87.67 ± 3.08	83.06 ± 2.96	83.14 ± 3.70	NS
Day 15	171.00^{b} ±5.56	87.40^{a} ± 4.01	181.80^{b} ± 5.21	179.60^{b} ± 4.71	187.60^{b} ± 6.98	**
Day 30	220.20^{b} ± 4.31	94.80^{a} ± 2.63	261.20^{c} ± 2.92	250.30^{c} ± 4.01	259.60^{c} ± 3.92	**
Day 45	255.80^{b} ± 5.12	97.00^{a} ± 2.43	299.40^{c} ± 3.70	297.80^{c} ± 4.68	303.40^{c} ± 2.06	**

Figures indicate the mean ± SE (standard error), NS = Not significant, * = 5% level of significant, ** = 1% level of significat

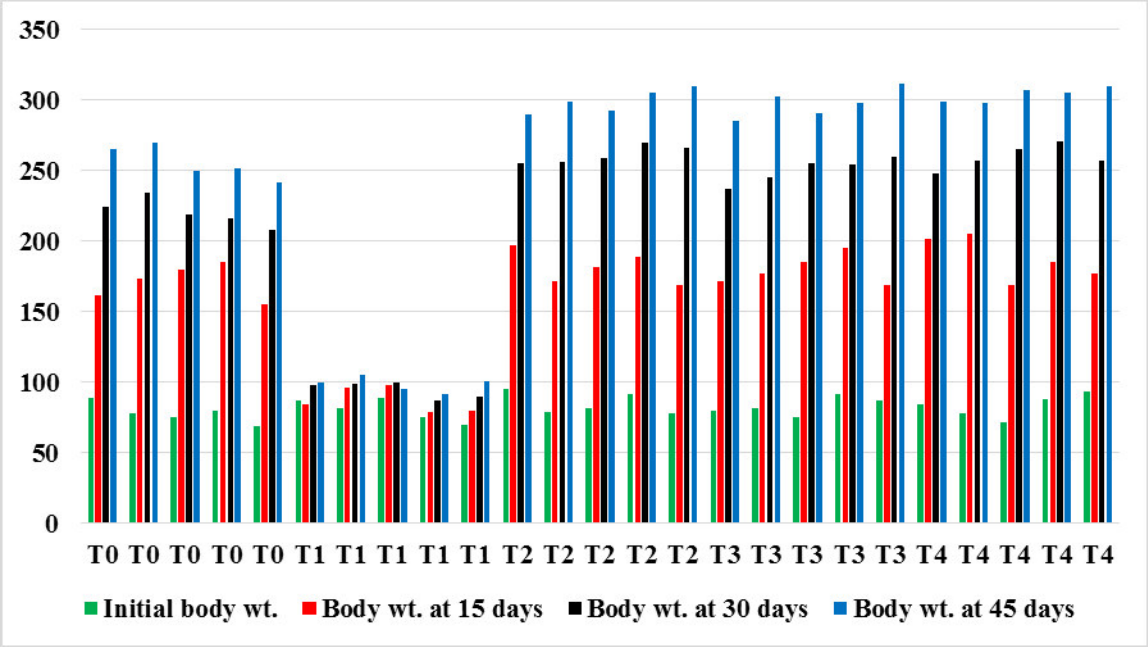

Fig. 2: Effects of different treatment on body weights of rats on different day

4.3 Hematological parameter

4.3.1 Total Erythrocyte Count (TEC)

Onday 15, total erythrocyte counts were found highest in arsenic plus spirulina (T_2) group and lowest in arsenic (T_1) group and arsenic plus Vitamin C (T_3) treated rats and this decrease was not statistically significant. In T_4 treated group of rats were slightly decrease and the value of Total erythrocyte count (TEC) was not significantly compared to control (Table 2).

On Day 30, highest TEC were recorded in T_4 (arsenic plus spiruluna plus Vitamin C) groups of rats and lowest TEC was recorded in arsenic (T_1) treated animals compared to control group. The value of TEC in others groups are gradually increase compared to control group which are statistically significant.

On day 45, the TEC was found highest in arsenic plus spirulina plus vitamin C (T_4) treated rats and lowest in arsenic (T_1) group. Total erythrocyte counts on day 45 in rats of other groups of rats are gradually increases which are statistically significant (Fig. 3).

Table 2: Effects of arsenic, spirulina and vitamin-C on TEC of rats

Treatment	TEC *(Thousand/µl)*					
	Group T_0	Group T_1	Group T_2	Group T_3	Group T_4	Level of significance
15 Days	6.40± 0.13	6.20 ± 0.04	6.47± 0.13	6.27 ± 0.08	6.43± 0.09	NS
30 Days	6.47 ± 0.17^{ab}	6.22 ± 0.05^{a}	$6.97 \pm 0.18b^{c}$	7.20 ± 0.25^{c}	7.25 ± 0.30^{c}	*
45Days	6.71 ± 0.25^{ab}	6.35 ± 0.25^{a}	$7.35\pm 0.26b^{c}$	7.90 ± 0.37^{c}	7.95 ± 0.19^{c}	*

Figures indicate the mean ± SE (standard error), NS = Not significant, * = 5% level of significant, ** = 1% level of significat

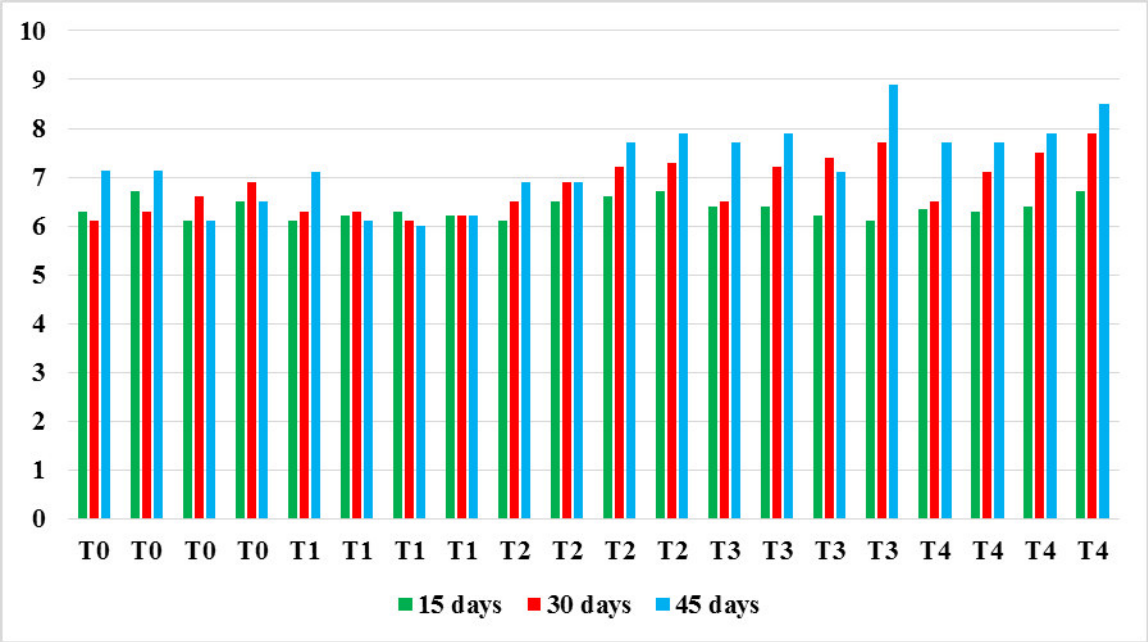

Fig. 3: Effects of different treatment on Total Erythrocyte Count (TEC) of rats on different day

4.3.2 Total Leukocyte Count (TLC)

Total leukocyte count on Day 15 in rats was found highest in control group (T_0) and lowest in arsenic (T_1) treated group and this change was statistically significant. The value of TLC in others group is gradually increased which are statistically significant (Table 3).

The value of TLC on Day 30 was highest in rats of control group (T_0) and lowest in arsenic treated group and arsenic plus vitamin C (T_3) treated group but the change was statistically significant in between these two groups. The value of TLC in others group is gradually increased which are statistically significant.

On day 45, the value of TLC was highest in control (T_0) group and slightly increased in arsenic treated groups but lowest in arsenic plus spirulina plus vitamin C (T_4) treated group of rats. Others two groups of rat's arsenic plus spirulina and arsenic plus vitamin C were changed slightly and the change was statistically significant (Fig. 4).

Table 3: Effects of arsenic, spirulina, vitamin-C on TLC of rats

Treatment	*TLC (Thousand/µl)*					
	Group (T_0)	Group T_1	Group T_2	Group T_3	Group T_4	Level of significance
15 Days	9.56± 0.00^{d}	9.09 ± 0.00^{a}	9.41± 0.00^{c}	9.33± 0.04^{b}	9.55± 0.01^{d}	**
30 Days	10.87± 0.01^{d}	10.24± 0.01^{a}	10.42 ± 0.01^{c}	10.26± 0.01^{b}	10.76± 0.01^{e}	**
45Days	10.82± 0.00^{d}	9.79± 0.01^{a}	9.87± 0.01^{b}	9.87± 0.01^{b}	10.71± 0.1^{c}	**

Figures indicate the mean ± SE (standard error), NS = Not significant, * = 5% level of significant, ** = 1% level of significat

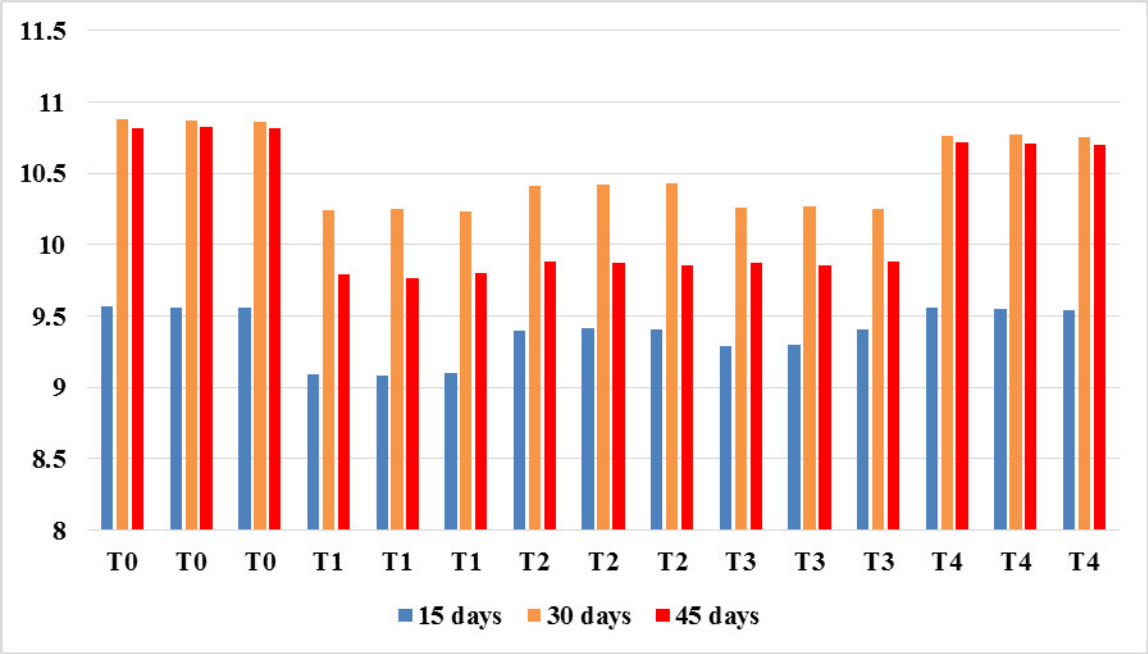

Fig. 4: Effects of different treatment on Total Leukocyte Count (TLC) of rats on different day

4.3.3 Hemoglobin concentration (Hb)

Hemoglobin concentrations on Day 15 was found highest in arsenic plus spirulina plus vitamin C (T_4) treated group rats and lowest in arsenic (T_1) treated group of rats compared to control group and others group of rats HB increase gradually compared to arsenic group and the change was statistically significant (Table 4).

On day 30, the Hb concentration was highest in arsenic plus spirulina (T_2) group of rats and lowest in arsenic (T_1) treated group compared to control (T_0) group, and the difference was statistically significant. The values of Hb in other group are slightly increased which are significant. Rats of arsenic plus spirulina group were found highest with Hb concentration and lowest in that of arsenic treated group.

Total Hemoglobin concentration (Hb) counts on day 45 was found highest in arsenic plus spirulina plus vitamin C (T_4) treated group rats and lowest in (T_1) group rats but the difference were statistically significant among all group of rats (Fig. 5).

Table 4: Effects of arsenic, spirulina, vitamin-C on Hb

Treatments	Hb (gm/dl)					
	Group T_0	Group T_1	Group T_2	Group T_3	Group T_4	Level of significance
15 Days	13.50 ± 0.54^{b}	11.12 ± 0.43^{a}	14.00 ± 0.40^{b}	13.37 ± 0.37^{b}	14.35 ± 0.90^{b}	*
30 Days	11.03 ± 0.89^{ab}	9.42 ± 0.70^{a}	15.25 ± 0.78^{d}	13.02 ± 0.41^{bc}	14.55 ± 0.61^{cd}	**
45 Days	15.22 ± 0.96^{b}	7.75 ± 1.78^{a}	15.27 ± 0.83^{b}	15.20 ± 0.96^{b}	16.75 ± 1.37^{b}	*

Figures indicate the mean ± SE (standard error), NS = Not significant, * = 5% level of significant, ** = 1% level of significat

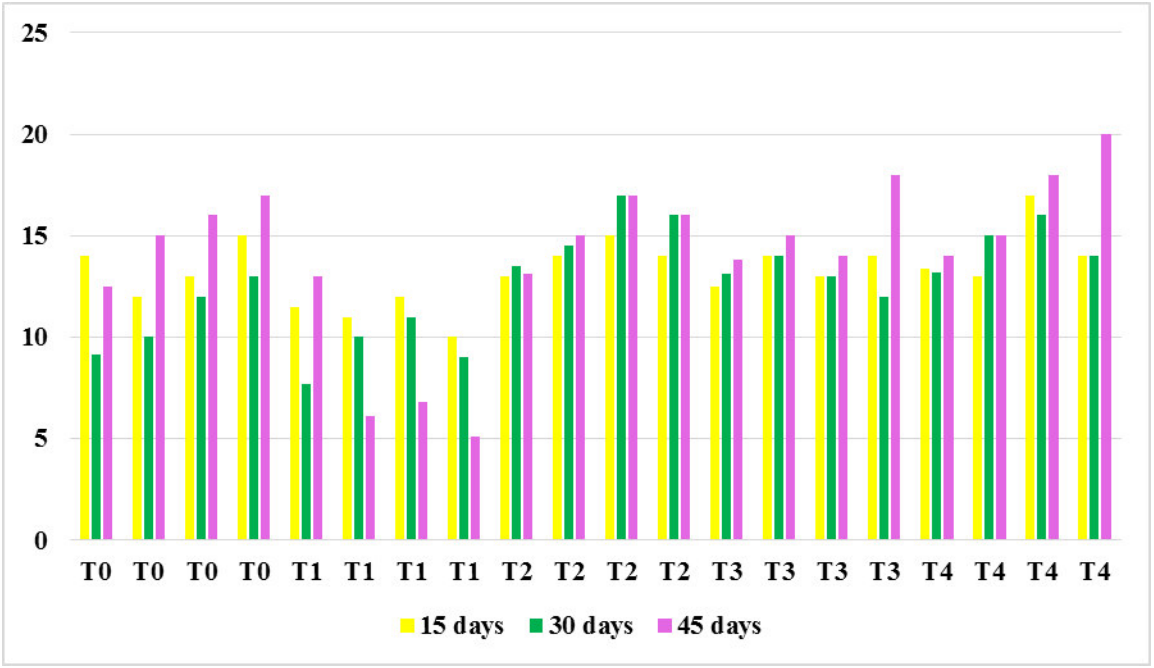

Fig. 5: Effects of different treatment on Hemoglobin Concentration (Hb) of rats on different day

4.4 Biochemical parameters

4.4.1 Serum Glutamate Oxaloacetate Transaminase (SGOT/AST)

The values of SGOT on day 15 were lowest in Group T_2 Spirulina+ Arsenic followed by Group T_4 (Arsenic+ vitamin C + spirulina) and and highest value were found in control (T_0) group and among other groups it was found not significantly (Table 5).

On day 30, SGOT value was lowest in Group T_2 (Spirulina+ Arsenic) treated group and highest in arsenic (T_1) treated group compared to control group. The differences between the mean values of different groups were found not significant.

On day 63, SGOT value was lowest in arsenic, Group T_3 (Arsenic+ vitamin C) treated group and highest in Group T_1 arsenic treated group. Arsenic plus spirulina plus vitamin C group value was found lower than that of arsenic treated group (Fig. 6). The difference between the mean values of different groups were found significant ($p<0.01$).

Table 5: Effects of arsenic, spirulina, vitamin-C on SGOT of rats

Treatments	Biochemical SGOT(U/L)					
	Group T_0	Group T_1	Group T_2	Group T_3	Group T_4	Level of significance
15 Days	115.00 ± 2.89	110.00± 2.89	100.00 ± 2.52	105.33 ± 2.60	101.67 ± 6.67	NS
30 Days	109.00 ± 3.79	117.00 ± 8.50	97.33 ± 1.45	102.33 ± 3.84	103.00 ± 4.00	NS
45 Days	109.67 ± 3.18^{c}	141.67 ± 6.23^{d}	106.67 ± 1.67b^{c}	94.00 ± 2.08^{a}	98.00 ± 1.53^{ab}	**

Figures indicate the mean ± SE (standard error), NS = Not significant, * = 5% level of significant, ** = 1% level of significat

In a column figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT)

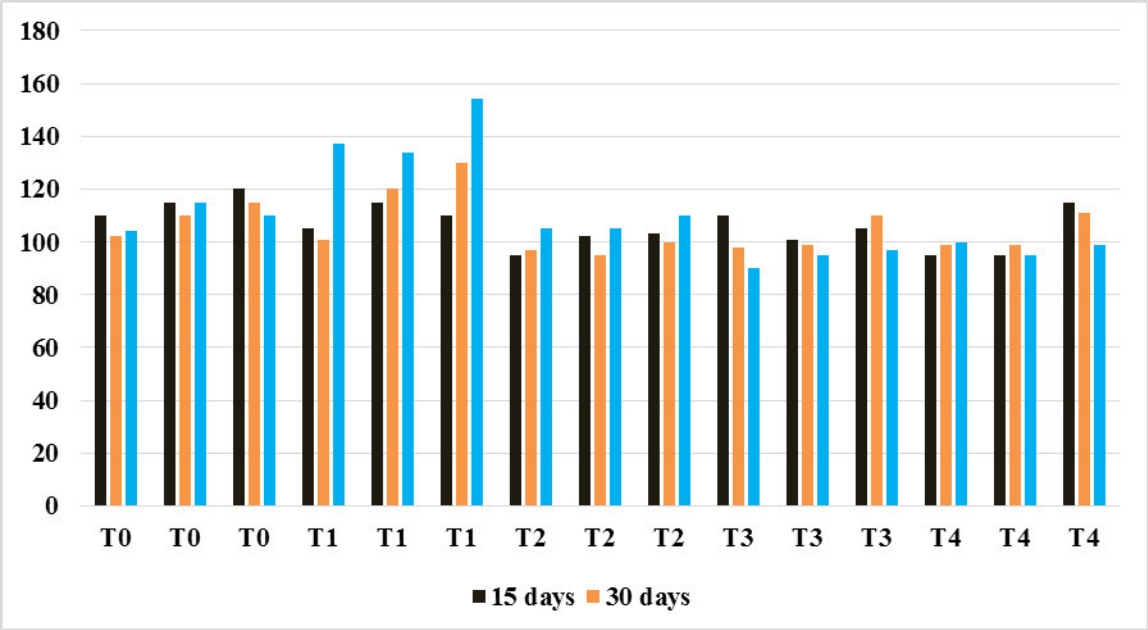

Fig. 6: Effects of different treatment on Serum Glutamate Oxaloacetate Transaminase (SGOT/AST) of rats on different day

4.4.2 Serum Glutamate Pyruvate Transaminase (SGPT/ALT)

The values of SGPT on day 15 were lowest in arsenic plus spirulina (T_2) treated group and highest value was found in arsenic (T_1) treated group (Table 6) compared to control (T_0) group. The mean values of SGPT of different groups were found not significant.

On day 30, SGPT value was lowest in Arsenic+ vitamin C (T_3) group followed by arsenic plus spirulina treated group and highest in arsenic (T_1) treated group. The difference between the mean values of different groups was found not significant.

On day 45, SGPT value was lowest in Arsenic+ vitamin C (T_3) treated group and highest values were found in arsenic (T_1) treated group (Fig. 7). The difference between the mean values of different groups were found significant ($p<0.05$).

Table 6: Effects of arsenic, spirulina, vitamin-C on SGPT of rats

Treatments	SGPT(U/L)					
	Group T_0	Group T_1	Group T_2	Group T_3	Group T_4	Level of significance
15 Days	71.67± 0.89	77.00 ± 1.53	64.33 ± 7.17	73.00 ± 2.52	68.00 ± 4.93	NS
30 Days	73.00 ± 0.58	76.00 ± 3.51	68.67 ± 8.95	68.33 ± 2.67	69.00 ± 2.08	NS
45Days	74.00 ± 0.00[a]	89.33 ± 4.84[b]	68.67 ± 7.36[a]	63.67 ± 5.24[a]	64.00 ± 2.08[a]	*

Figures indicate the mean ± SE (standard error), NS = Not significant, * = 5% level of significant, ** = 1% level of significat

In a column figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT)

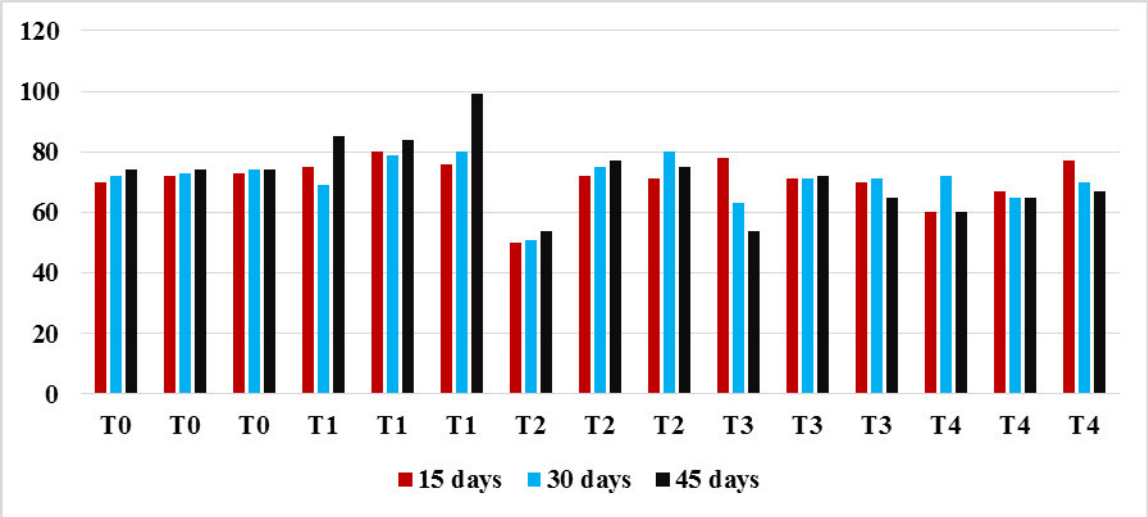

Fig. 7: Effects of different treatment on Serum glutamate pyruvate transaminase (SGPT/ALT) of rats on different day

4.4.3 Serum creatinine

The values of serum creatinine was found lowest in control group. The values of arsenic plus spirulina treated group and arsenic and vitamin C plus spirulina (T_4) group are very similar than that of control (T_0) group (Table 7). The differences between the mean values of different groups were found significant ($p<0.01$).

On day 30 the mean values of control group and arsenic plus spirulina (T_2) a treated group and arsenic plus spirulina plus vitamin C (T_4) were found somewhat similar and highest in the arsenic (T_1) treated group. . The differences between the mean values of different groups were found significant ($p<0.01$).

On day 45 lowest mean values was found in arsenic plus spirulina (T_2) group and the highest mean value was found in arsenic (T_1) and others are near about similar to control group or arsenic treated group (Fig. 8). The differences between the mean values of different groups were found significant ($p<0.01$).

Table 7: Effects of arsenic, spirulina, vitamin-C on Serum creatinine of rats

Treatments	Serum creatinine (mg/dl)					
	Group T_0	Group T_1	Group T_2	Group T_3	Group T_4	Level of Significance
15 Days	0.51 ± 0.01^{a}	0.62 ± 0.00^{b}	0.52 ± 0.01^{a}	0.53 ± 0.003^{a}	0.52 ± 0.01^{a}	**
30 Days	0.52 ± 0.00^{a}	0.65 ± 0.01^{d}	0.54 ± 0.01^{b}	0.61 ± 0.003^{c}	0.51 ± 0.003^{a}	**
45Days	0.52 ± 0.006^{a}	0.67 ± 0.003^{c}	0.51 ± 0.005^{a}	0.62 ± 0.005^{b}	0.52 ± 0.005^{a}	**

Figures indicate the mean ± SE (standard error), NS = Not significant, * = 5% level of significant, ** = 1% level of significat

In a column figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT)

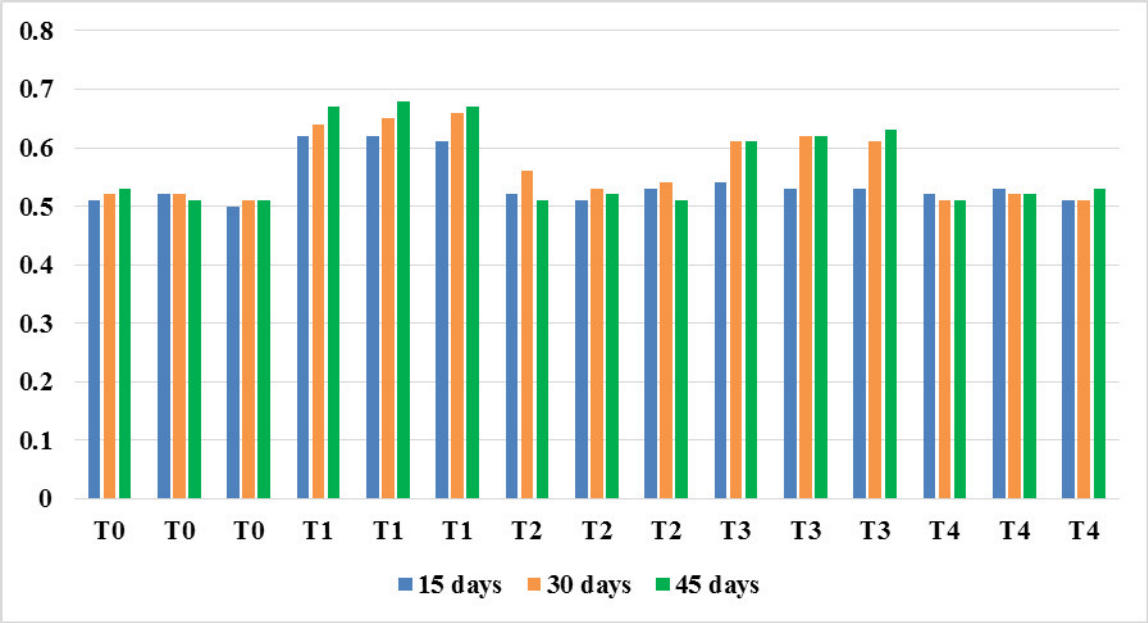

Fig. 8: Effects of different treatment on Serum Creatinine of rats on different day

CHAPTER V

DISCUSSION

The treated rats did not show any clinical signs/lesion during the entire study period but slight increase in body weight in rats of all groups because the rats were in growing stage. On the other hand, the body weight gain in rats of arsenic treated group was found higher compared to rats of arsenic plus spirulina and control groups (Table 1). Moderate weakness and dullness were also observed in rats of arsenic treated group compared to arsenic plus spirulina, arsenic plus Vitamin C and control groups. Hence, it could be said that feeding of As_2O_3 caused chronic arsenic toxicity in rats, although the manifestation of more pronounced symptoms of arsenic toxicity may take more time. This experiment should be continued for a longer period of time to observe the full manifestation of clinica1 symptoms of chronic arsenic toxicity, but the limited time did not allow reaching this target.

In this study, the values of TEC (Table 2) were increased significantly ($P<0.05$) at day 30 and day 45 but there was no significant difference by day 15 in the blood samples of the treated groups of rats (T_1, T_2, T_3 and T_4) compared to control (T_0). On day 15, day 30 and day 45 the values of TEC were increased in Arsenic plus Spirulina plus vitamin C treated group (T_4) compared to arsenic treated group (T_1). However the findings suggest that the chronic arsenic toxicity might cause decreased in TEC values and Spirulina plus vitamin C treatment might be recovering it. The findings also revealed that the TEC in the T_1 group was lower compared to control and other treated groups also. The experiment result also confirmed that treatment with vitamin C and Spirulina either alone or in combination improved the TEC, although the combination produced a better output. Chronic arsenic toxicity might cause decreased in TEC and anemia. The effects observed in this study and outcomes of treatment using Spirulina plus vitamin C treatment had earlier been corroborated by Breton *et al.,* 2006; Halim *et al.,* 2007; Gupta *et al.,* 2007; Juruli and Katsitadze, 2007; Hossain *et al.,* 2012. They reported decreasing RBC level with increased concentration of arsenic due to arsenic metabolism and its methylating activity.

Incase of TLC values there was significant difference observed among all the groups during the entire study period but slight decrease in TLC values in rats of all other

treated groups (T_1, T_2, T_3 and T_4) compared to control treated group (T_0) was observed (Table 3). The result is dissimilar to the findings of Yasmin *et al.* (2011); but contradicts that of Rousselot *et al.,* 2004; Islam, 2008; Hossain *et al.,* 2012; Hasan *et al.,* 2015 where they found WBC level decreased when rat were given higher dose of arsenic and that might be due to apoptotic effect of arsenic on plasma cells. Whether Spirulina and Vitamin C had an influence on the values of TLC against arsenic toxicity in rats cannot be established in this study. A more carefully planned research targeting this objective is required to be undertaken.

In all the groups, a significant difference was observed in Hb values ($P<0.01$) during the study period but little increase in Hb values in rats of all other treated groups (T_2, T_3 and T_4) compared to arsenic treated group (T_1) (Table 4). It may be due to that Spirulina and vitamin C might be slightly increasing the values of Hb against arsenic toxicity but it does not fulfill the real picture of findings. More research is necessary to fulfill the proper findings.

In this study, the values of SGOT were decreased significantly ($P<0.01$) at day 45 and there was insignificant difference by day 15 and day 30 in the blood samples of the treated groups of rats (T_1, T_2, T_3 and T_4) compared to control (Table 5). Although this finding agreed with the previous findings that SGOT was reduced by as alone (Mahaffey *et al.,* 1981) disagreed with the findings of Vutukuru *et al.,* 2007; Islam, 2008; Hossain *et al.,* 2012; Hasan *et al.,* 2015. It concurred with the findings of Yasmin *et al.* (2011) who indicated similar results. In Spirulina treated (T_2), Vitamin C treated (T_3) and Spirulina plus Vitamin C treated (T_4) experimental arsenicosis groups, there were significantly decreased values of arsenic recorded ($P<0.01$) compared with the arsenic treated group of rats (T_1). Since arsenic toxicity can cause hepatic insufficiency and Spirulina and Vitamin C treatment improved the hepatic functions apart from decreasing the level of arsenic in blood, it can be concluded that Spirulina improve liver function by reducing hepatic damage due to heavy metal exposure and drug abuse (González *et al.,* 1999; Guha Mazumder, 2001).

Although the levels of SGPT in serum differ significantly at day 45 and day ($P<0.05$), it normalized in all groups and there was no significant difference by day 15 and day 30 except for the arsenic group (Table 6). It is possible that individual treatment using Vitamin C or Spirulina or a combination of these two will produce a significantly

different result in long term arsenicosis. Interestingly, the level of SGPT did not change drastically in arsenic treated group (T_1) between days 15 and 30. It may be that once a peak level of arsenicosis is reached, the SGPT level will adjust to it and maintain a peak value. Kaur *et al.* (2005) had earlier found that no change was observed in SGPT level associated with arsenicosis over a 90-day period. However, the result of this finding were not corroborated with the finding of Sharma *et al.,* 2007; Hossain *et al.,* 2012; Hasan *et al.,* 2015 who observed a highly significant ($P < 0.001$) elevation in ALT activity of arsenic-intoxicated mice with respect to control animals for 30 days treatment. On the other hand, Kumar *et al.* (2005) observed that Swiss albino mice treated with spirulina @ 800 mg/kg bwt orally before and after mercury intoxication showed a significant decrease in AST and ALT activity for 30 days treatment.. Yasmin *et al.* (2011) had also recently reported a 16.67% increase in SGPT level of arsenic treated mice as compared to the control but this are disagree of my research.

There were significant differences were found in serum creatinine levels among the control group and all other treatment groups throughout the study period (Table 7). The findings disagreed with the results of Nabi *et al.* (2005) in human being which showed that patients of arsenicosis had significantly lower level of serum creatinine compared to the control. It is possible that the differences observed in these two studies are related to the differences in species used for the study. Zhang *et al.* (1995) had also observed that there is a relationship between arsenic level and degree of chronic renal insufficiency in men. The result of this study was dissimilar with the findings of Roger *et al.,* 2000; Islam *et al.,* 2009; Yasmin *et al.,* 2011 who concluded that there were no significant rises in the serum creatinine levels of arsenic treated mice. Although this finding agreed with the previous findings that serum creatinine was reduced by As alone (Hossain *et al.,* 2012; Hasan *et al.,* 2015) and slight reduction in serum creatinine was however observed with the time progression in our study for the Spirulina and Vitamin C treated group (T_4), suggesting that Spirulina and Vitamin C may improve liver function.

CHAPTER VI

CONCLUSIONS

The results of this study lead to the following conclusions:

- Spirulina and Vitamin C were found moderately effective in prevention of arsenic toxicity in rats.
- Arsenic toxicity causes hepatic and renal dysfunction and thereby increases SGOT, SGPT and Serum creatinine level in blood but spirulina improved the hepatic and renal function by recovering their normal level.
- The combined effect vitamin C and spirulina were found more effective compared to their single effect. The effect of spirulina was found better in recovering body weight and biochemical parameters in blood compared to Vitamin C alone.
- It can be concluded that arsenic induction increased the arsenic content in tissues and might cause hepatic distress and simultaneous feeding of spirulina could reduce burden of arsenic and improved the growth in rats.

This study is a preliminary work to study the effect of Spirulina and Vitamin C in arsenic induced toxicity in Bangladesh.

For further detailed, study on pharmacokinetics of spirulina and Vitamin C or other treatment the result of this research work will certainly help for future researchers who carry out for better therapeutic use in arsenicosis in man and animals.

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