EFFECT OF SPIRULINA (*Spirulina platensis*) AND VITAMIN E ON ARSENIC INDUCED TOXICITY IN QUAIL

A THESIS

BY

MOST. FAYZA KHATUN

Registration No.: 1705487

Session: 2017

Semester: January-June, 2019

MASTER OF SCIENCE (MS)

IN

PHARMACOLOGY

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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Submitted to the Department of Physiology & Pharmacology
Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh in partial fulfillment of the requirements for the degree of

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JUNE, 2019

DEDICATED
TO MY
BELOVED PARENTS

ACKNOWLEDGEMENTS

At first, all praises are due to the Almighty Allah, the creator and supreme authority of universe, who empowers me to complete the research work successfully and to materialize the dream for the degree of Master of Science (MS) in Pharmacology.

I wish to express the deepest sense of gratitude, sincere appreciation, indebtedness and best regards to my research supervisor Dr. Md. Mahmudul Hasan Assistant Professor, Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur for his scholastic and dynamic guidance, constant inspiration, cordial consistence, innovative suggestions, affectionate feeling, helpful comment, inspiration, sympathetic supervision and constructive criticism in all phases of this study and preparing the manuscript.

I express deep indebtedness to my Co-supervisor, Professor Dr. Rakibul Islam, Chairman, Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur for his scholastic guidance, untiring assistance and advice in preparing the thesis.

I am honored to express my deepest sense of gratitude and sincere appreciation to honorable teacher, Dr. Fahima Binthe Aziz, Associate Professor and Dr. Md. Bazlar Rashid, Associate Professor, Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, for their helpful advice and co-operation in providing facilities to conduct the experiments.

I humbly desire to express my profound respect and sincere appreciation to my respectable teacher Dr. Misrat Masuma Parvez, Assistant Professor and Dr. Sumon Sarker , Lecturer, Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, for their helpful advice and co-operation in preparing the thesis.

I like to express profound gratitude and thanks to my all reverend teachers of the Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur for their kind help, co-operation, encouragement and valuable suggestions.

With due pleasure I wish to acknowledge the healthy working relationship of the staff of the Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur.

I like to express cordial thanks to Ministry of National Science and Technology, for funding for this experiment.

Finally, I am very much grateful to my beloved parents, brother and sister for their sacrifice, inspiration, encouragement and endless love and continuous blessing for educating myself up to the postgraduate level.

The Author
June, 2019

ABSTRACT

Chronic arsenic (As) toxicity is a severe disease in men and animals which occurs severely in Bangladesh. Arsenic contamination in ground water used in drinking is the major concern because As is present in human and animal food chain. This work was done in quails with a view to observing the efficacy of spirulina (*Spirulina platensis*) and vitamin E for prevention of As toxicity. 60 quails were used in this study and animals were divided into control group (T_0), As treated group (T_1), As plus spirulina treated group (T_2) and As plus vitamin E treated group (T_3). Each group consists of 15 quails. Quails of T_0 group were given normal feed and water and kept as control. Quails of T_1, T_2 and T_3 were given 100 mg arsenic trioxide/L drinking water daily for 30 days. In addition to arsenic trioxide quails of group T_2 and T_3 were simultaneously fed with spirulina @ 1 gm/kg feed and vitamin E @ 400mg /kg body weight up to 30 days respectively. Five quails from each group (T_0, T_1, T_2 and T_3) were sacrificed at 15 days interval in order to determine haematological parameters. Result showed that in group T_1, body weight gain was minimum, whereas in group T_2 and T_3 the body weight gain in quails were better. Reduction of TEC and Hb values were observed in arsenic treated group T_1. Whereas in rest groups the TEC and Hb values were comparatively higher than arsenic treated group. Noticeable change observed in liver and kindey of As treated group in compare to the control group. Histopathological changes also observed in liver and kindey of As treated group in compare to the control group. In conclusion, spirulina and vitamin E have significant effect on body weight, hematological, postmortem and histopathological changes.

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CHAPTER I
INTRODUCTION

Arsenic (As) poisoning is one of the most sensitive environmental issue in Bangladesh even it is a major health concern in Asia. It creates a serious public health problem in developing countries. As can enter into food chain causing wide spread distribution throughout the plant and animal kingdoms. As is a shiny grayish non essential trace element that is widely distributed in nature. It appears in three allotropic forms in nature. The forms are yellow, black and gray; the stable form is a silver gray, brittle crystalline solid. As can be found in both organic and inorganic forms in water, food, air and soil. Inorganic forms of As are more toxic than organic forms. The most important inorganic forms of As compounds are arsenic trioxide, sodium arsenite, arsenic trichloride, arsenic acid and arsenites (trivalent forms) and lead and calcium arsenates (pentavalent forms). Common organic As forms are arsanilic acid monomethyl arsinate (MMA), dimethylarsinic acid (DMA) (David J. Thomas *et al.,* 1995) and arsenobetaine. The inorganic forms of As exhibit the highest toxicity level (FAO, WHO, 1983). As is a ubiquitous and one of the most potent toxic metalloids in environment. Globally millions of people are being exposed to inorganic arsenic through consumption of contaminated drinking water and food (Silbergeld *et al.,* 2008). As is a metalloid that occurs in organic and inorganic forms in water and soil throughout the world especially in Bangladesh, India and several other countries of Southeast Asia (Bhatacharya *et al.,* 2009). Although it is rare in nature as a pure element however, the organic form exists as arsinobetaine in most of the microbiota, plants and in other biological system. Reduced form of as As (As+3 and As+5) are frequently present in industrial products, agricultural wastes and in surface water. As and its compounds are considered as potent carcinogen (Wang *et al.,* 2006).

Natural sources of As include arsenate, sulfide, arsenite, arsenide, oxides, silicates, assinopyrites which result in ground water pollution. Iron arsenate is also found near mineral mines including coal mines and oil ore extraction. Many industries such as leather, textile, oil refineries, treated lumber, metal extraction and purification, fertilizer production, insecticides, herbicides and fossil fuel release considerable amount of arsenic into environment (Jomova *et al.,* 2011).High residual concentration of As has been found

in the tissues of many marine organisms, wild and sea birds (Fairbrother *et al.*, 1994). Arsenite reacts with the cells and cause free radical injury. Persistent exposure either due to geochemical enrichment or due to industrial process induces severe biochemical, toxicological and pathological changes (Liu *et al.*, 2000).

Ground water As contamination in Bangladesh is reported to be the biggest As calamity in the world in terms of the affected population (Talukder *et al.*, 1998). Chronic As exposure is associated with many human health conditions, including skin lesions and cancers of the liver, lung, bladder and skin (Uddin and Huda, 2011). Arsenicosis presents with significant changes in the Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), serum creatinine, uric acid levels and various hemato-logical parameters like TEC, TLC, Hb, blood sugar level in the Swiss albino rats (Yasmin *et al.*, 2011). Arsenic trioxide (As_2O_3) is the most prevalent inorganic arsenical found in air, while a variety of inorganic arsenates (AsO_4) or arsenites (AsO_2) occur in water, soil or food (Magalhaes, 2002; Chou *et al.*, 2007). In drinking water the main source of As is arsenic rich rocks through which the water has filtered. As is a well-known human carcinogen and has many other toxic effects. To produce a state of the art review on As in drinking water the World Health Organization (WHO) has worked with other UN system organizations (WHO, 1999). The safety limit of As accepted by Bangladesh government is 0.05 mg/liter for drinking water (WHO, 1999). The World Health Organization (WHO) limits for drinking water 0.01 mg/liter and far foodstuffs is 2mg/liter on a fresh weight basis (Robinson *et al.*, 2003). Now As creates a serious public health issue in different developing countries (Rahman, 2006), where the drinking water contaminated with inorganic arsenic (As). Chronic arsenic toxicity is a global health issue at present (Yoshida *et al.*, 2004). It is also a major health problem of Bangladesh and surrounding regions (Kalia, 2005; Khalequzzaman *et al.*, 2005; Guha Mazumder *et al.*, 2001). Chronic As poisoning can cause serious health problems including cancers, hyperkeratosis, restrictive lung disease, and ischaemic heart disease (Rossman 2003 and Mandal and Suzuki 2002;) 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 (Mazumder, 2003; Smith et al. 1998). About half of the total populations (more than 50 millions) of Bangladesh are consuming As through drinking and cooking. Among them, more than 40,000 people have already developed

the signs and symptoms of chronic As 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 water for drinking and cooking purposes. The general populations are exposed to As through drinking water, dust, fumes and dietary sources.

Clinical study suggests that algae having very high concentration of micronutrients and vitamins may have beneficial effects in heavy metal poisoning. Spirulina and vitamin E have been considered as a potential therapeutic supplement due to its ability to minimize several element induced toxicities in various species including man. Spirulina is a microscopic filamentous aquatic non-toxic blue-green algae belongs to the group cyanobacterium (genus *Spirulina*, especially *S. platensis* synonym *Arthrospira platensis*) (Spolaore *et al.,* 2006) that is rich in proteins, lipids, carbohydrates, β-carotene, riboflavin, α-tocopherol and α- linoleic acid (El-Desoky *et al.,* 2013). It is not only a whole food, but it seems to be an ideal therapeutic supplement. Spirulina has been shown to prevent cataract (Haque *et al.,* 2005), acute allergic rhinitis and vascular reactivity (Mao *et al.,* 2005) and cerebral ischemia (Khan *et al.,* 2005) has also been shown to be effective against cadmium and arsenic induced-toxicities (Saha *et al.,* 2005). Spirulina was found to be beneficial in goats of chronic As poisoning (Halim, 2007) and spirulina extract plus zinc was found to be beneficial in patients of chronic As poisoning (Misbahuddin *et al.,* 2006). Spirulina contains about 13.6% carbohydrates; (Shekharam *et al.,* 1987), vitamin B1, B2, B3, B6, folic acid, vitamin C, vitamin D, and vitamin E (Babadzhanov *et al.,* 2004). Spirulina reduced mercury and other toxic metal accumulation in the tissue (Johnson *et al.,* 1986). It reduced nephrotoxicity from mercury (Yamane, 1988). Spirulina have protective effect against As toxicity (M. Z. Islam *et al*. 2008). Spirulina reduces hepatic damage due to drug abuse and heavy metal exposure, inflammatory response (Richmond, 1984; González *et al.,* 1999). Spirulina alone or in combination with other vitamin and/or mineral was found to be effective in the removal of As from arsenic-loaded tissues in various species including man (Fariduddin *et al.,* 2001; Misbahuddin *et al.,* 2006; Awal, 2007), in the treatment of chronic As poisoning (Khan *et al.,* 2001), in reducing As toxicity induced skin manifestations of patients in Bangladesh (Karim *et al.,* 1999) .It is reported that administration of spirulina provide a protective mechanism against As induced toxicity in goats (Ghosh A, *et al.,* 2014).

Spirulina is helpful on toxic signs, body weight and hematological parameters in As induced toxicities in ducks (Islam *et al.*, 2009).

Vitamin E is a lipid soluble free radical scavenger that protects the membrane from lipid peroxyl radicals (Wagner *et al.*, 1996). It plays an important role in the body's enzyme function and may help to stimulate the production of antibodies. Vitamin E is also considered as an antioxidant (Ayaz *et al.*, 2007) and it may work with other antioxidant to protect the cell from damage. Many scientists from different countries are working on the As problem in Bangladesh, especially on ground water for human concern. Vitamin E (α-tocopherol) is considered as chain-breaking micronutrient antioxidant. Owing to antioxidant properties, vitamin E and selenium have major impact on immunity and enhance intracellular generation of super oxide dismutase and glutathione, thus; reduce free radical induced lethal injury (Selvaraj *et al.*, 2012).

In Bangladesh, elaborate data is available for As only on tube-well water; however, data on the specific treatment in prevention of As toxicity in both human and animals are very limited. Therefore, data on the effective prevention of arsenicosis with Spirulina and vitamin-E 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 know the effect of spirulina and vitamin E on the body weight of As fed quails.
2. To evaluate the effect of spirulina and vitamin E on the hematological parameters in As fed quails.
3. To observed the effect of spirulina and vitamin E on the post-mortem changes and histopathological changes on As induced quails.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Physical and chemical properties of As

As is representing with its symbol 'As', atomic number 33, valences 3 and 5, and atomic weight 74.9216. As is omnipresent in the biosphere and occurs naturally in both organic and inorganic forms in water, food, soil, dust, wood, and other materials. The most important inorganic As compounds are arsenic trioxide, sodium arsenite, arsenic trichloride (trivalent forms), and arsenic pentoxide, arsenic acid and arsenates, such as, lead and calcium arsenates (pentavalent forms). Common organic As 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.

Friberg *et al.,(*1986) and Lau *et al.,*(1987) studied that organo-arsenicals include arsenocholine, dimethyloxyarsyl-ethanol, trimethylarsonium lactate, As containing sugars and phospholipids have also been found in fish .

Oremland and Stolz, (2003) observed that arsenic can be found to a small extent in the elemental form. As can exist in the environment in redox states, arsenite (+3), arsenate (+5), arsine (-3) and elemental (0) forms. As 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. Such activities result in the release of the soluble inorganic arsenic oxyanions, arsenite and arsenate. Trivalent inorganic As is more toxic than pentavalent form and inorganic As is thought to be more toxic than organic one to the biological systems.

Vhater *et al.,(*2001) studied that methylation of inorganic As might be considered a detoxification mechanism, as the end metabolites, monomethyl arsonic 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. They also stated that inorganic As was highly reactive with tissue components, due to its strong affinity for sulfhydryl groups and thus, following exposure to pentavalent As , the first step in the biotransformation to trivalent, may be considered a bioactivation.

Metabolism of inorganic As to MMA and DMA, and this methylation facilitated urinary As excretion that had a lower risk of adverse As-related health outcomes (Heck *et al.,* 2007).

Mazumder, (2008) found that chronic As toxicity due to drinking of As contaminated ground water is a major environmental health hazard throughout the world. Organic As exposure can also occur by eating food. Organic As is 500 times less harmful than inorganic As. Inorganic arsenic trioxide is a component of geologic formations and can be washed out into the ground water. As poisoning can be related to human activities such as mining and ore smelting but is more often associated with dissolved solids naturally endemic in the aquifer environment.

2.2 Sources of arsenic contamination

Rahman *et al.,* (2007) observed that the concentrations of As in different parts of rice (*Oryza sativa* L; varieties of Bangladesh namely BRRI dhan 28, BRRI dhan 29, BRRI dhan 35, BRRI dhan 36, BRRI hybrid dhan 1) showed significant increased content of As ($p<0.05$) and the order of As contents in tissues of rice was: straw > husk > brown rice grain > polish rice grain.

Norra *et al.,* (2005); Duxbury *et al.,* (2003); Meharg and Rahman (2003;) shown that irrigation with As-contaminated water can lead to elevated As concentrations in rice-paddy soil, as well as in the rice root, stalk, and grain.

2.3 Risk of arsenic contamination

Hossain (2006), stated that Bangladesh is currently facing a serious threat to public health, with 85 million people at risk from As in drinking water and in food crops. In Bangladesh, the groundwater As contamination problem is the worst in the world. Ninety-seven percent of the population in the country uses groundwater for drinking and domestic purposes as surface water is mismanaged. High levels of As in groundwater are causing widespread poisoning in Bangladesh. Different studies have addressed various aspects of the As issue in Bangladesh. This review is undertaken to give an overview of the latest findings and statistical data on the issue especially on soil, water and food cycle. The World Health Organization (WHO) recommends a safe limit for As in drinking water of 10μg/L. A recent survey looked at the As concentrations of drinking

water from deep wells in 64 districts in the country and found that 59 had concentrations >10μg/L and 43 had concentrations >50μg/L. Contaminated groundwater is also used for irrigation of paddy rice, which is the main staple food for the population. This practice enhances the level of As in the soils rendering them unsuitable for agriculture. A few recent studies have reported that 85–95% of total As in rice and a vegetable was inorganic, which outlines the need for more studies for standardization. Arsenic concentration is higher in Bangladeshi soils, groundwater and plants (data based on 4% area of the country) than the permissible limits or normal range reported. This situation poses a serious threat on human and livestock health and highlights the need for scientific studies that would better describes the fate of As in the natural environment and identify all potential routes of exposure.

2.4 A serious public health issue

Mount and Feldman, (1984) estimated that acute As poisoning results in symptoms of gastro-intestinal pain, diarrhea, weakness and death. The diffuse toxic process of arsenic poisoning causes widespread endothelial cellular toxicity, resulting capillary damage and tissue hypoxia precipitating generalized vasodilatation and transudation of plasma. Gastrointestinal, cardiac, renal, bone marrow, central nervous system, and hepatic damage may be noted at different stages of arsenic poisoning (Donofrio *et al.*, 1987). 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).

Marafante (1982) showed that the highest accumulation of arsenic in the spleen followed by lung, liver, kidney, skin and lowest accumulation was in intestine. Arsenicosis is chronic and sub clinical or there is clinical toxicity due to high level of As in the body.

2.5 Cutaneous exposure caused by arsenicosis

Skin lesions are the most common outward sign of chronic arsenic exposure. One recent population study in West Bengal, India, published in the March 2003 issue of Epidemiology, showed that the lowest peak arsenic ingested by a confirmed case of

arsenic-induced skin lesions was 115µg/L. Nearly 2.9% of the study population (n=1654) in Bangladesh had clinical manifestations of skin lesions of As poisoning. 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). Typical skin pigmentation, as palmar and plantar keratosis in all patients (n=20) while gastrointestinal symptoms, anaemia and signs of liver disease and peripheral neuropathy were found in many of study people (Mazumder, 2005). Duration of arsenic symptoms was significantly associated with older age (P<0.001), male (P=0.002), married (P<0.001), smoking (P=0.002), longer duration of consuming tubewell water (P<0.001), complication of conjunctivitis (P=0.002), loss of appetite (P<0.001), wasting (P=0.006), and social problem faced having arsenicosis (P=0.040; Hossain *et al.,* 2005).

Mitra *et al., (*2004),Dietary micronutrient and macronutrient intake modulates the risk of arsenic-induced skin lesions, including alterations in skin pigmentation and keratosis.

2.6 Arsenicosis in human

Miller *et al.,* (2002) found that As exposure has been linked with various types of cancer, cardiovascular disease (Navas Acien *et al.,* 2005) and dermal effects (Cohen *et al.,* 2006). Low intake of calcium, animal protein, folate and fiber may increase susceptibility to As caused skin lesions (Mitra *et al.,* 2004). 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).

2.7 Dermal disease

McCarty *et al.,* (2007) studied that chronic exposure to arsenic leads to the development of lesions on the skin, including hyperkeratosis and hyperpigmentation, often used as diagnostic criteria for arsenicosis. Dermal effects following the exposure to arsenic are hallmarks of the early stages of arsenic poisoning.

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. 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).

2.8 Carcinogenicity

Rossman, (2003) described that As 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 As .It is thought that the mechanism by which these cancers originate may involve the promotion of oxidative stress by As 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).

2.9 Gastrointestinal disturbances

Uede and Furukawa (2003) and Vantroyen *et al.,*(2004) shown that 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 As .The gastrointestinal tract appears to be the critical target of toxicity following oral exposure to MMA.

Lee *et al.,* (1995) estimated that ingestion of 80 mg kg−1 of organic arsenicals causes vomitting, abdominal pain, hyperactive bowel and diarrhoea .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 Immunotoxicity

Gonseblatt *et al.,* (1992) observed that 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.But Bencko *et al.* (1988) found no abnormalities in serum concentrations of immunoglobulin in workers exposed to arsenic in a coal-burning power plant.

2.11 Hematotoxicity

Fowler and Weissberg (1974) examined that 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. 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.

2.12 Neurological disorders

Tsai *et al.,* (2003) studied that adult animals appear to be much less susceptible to the neurological effects of inorganic arsenic than humans. Recent findings indicate a possible association between arsenic in drinking water and neurobehavioral alterations in children .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 a typical feature of axonal degeneration. 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.

2.13 Reproductive health effects

Holson *et al.,* (1999) 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. 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.

2.14 Antioxidant protection against arsenic mutagenicity

Valko *et al.,* (2005) observed that oxidative stress to DNA is recognized as an underpinning component of the mechanism of arsenic carcinogenesis .Antioxidant enzymes are considered to be the first line of cellular defence against oxidative damage. 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 2010). The most effective known treatment for arsenic poisoning is chelation therapy; however such agents as British anti lewisite, sodium 2,3 dimercaptopropane1sulfonate, meso 2,3 dimercaptosuccinic acid etc. result in a number of undesirable side effects (Flora 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 2010).

2.15 Effect of arsenicosis on hematological parameters

Mondal *et al.,* (2016) studied that 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 (p0.05). The arsanilic acid treatments significantly decreased high density lipoprotein and increased very low density lipoprotein in plasma (p0.05). Activity of serum glutamate oxaloacetate

trasaminase (SGOT) was reduced by Arsenic alone and serum alkaline phosphatase was reduced by As (Mahaffey *et al.,* 1981). 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).

2.16 Composition of spirulina

2.16.1 Protein and amino acids

Vonshak *et al.,* (1997) and Fujisawa *et al.,* (2010), observed that platensis is the most useful microalgae for nutrition due to its components, especially protein. The nutritional level of protein is almost 70% of its dry weight and also has a high quantity and quality belonging to amino acids. *S. platensis* contains all of the essential amino acids. Researchers reported that although methionine and cysteine are found in a lower value, albumin and casein are found in a higher value, of animal proteins, respectively, ineggs and milk. *S. platensis* contains biliproteins, especially C-phycocyanin which is 20% of all protein fractions. C-Phycocyanin molecule has an antioxidant feature, which regulates immunity and protects the organism against disease.

2.16.2 Vitamins

Kapoor *et al.,* (1993) and Watanabe (2007) *S. platensis* has the richest vitamin source of vitamin A (beta-carotene), vitamin E, thiamin (vitamin B1), biotin (vitamin B7), and inositol (vitamin B8) in food. Beta-carotene is in a biotransformed state which can be absorbed by humans, and is also important for antioxidant processes in organisms. On the other hand, there is a conflict of cobalamin (vitamin B12) content in S. platensis. Some researchers reported that *S. platensis* has no reliable vitamin B12. They explain that it is a pseudo vitamin B12 which is inactive and in a form that the human organism cannot uptake at a cellular level.

2.16.3 Carbohydrates

Walter (1997) and Nielsen (2010), summarised that *S. platensis* contains 13.6% carbohydrates, which are glucose, mannose, galactose, and xylose. *S. platensis* easily digestible and a safe nutrient for human consumption. Nevertheless, it does not contain cellulose, which cannot be absorbed by humans and thereby this feature makes. It is significant for people who have intestinal mal absorption and for the elderly.

2.16.4 Lipids

Colla *et al.,* (2004) and Jubie *et al.,* (2012), total fatty acids tend to fluctuate around 6% by dry weight. Fatty acids such as γ-Linolenic acid (GLA) at up to 20.8mg/g (up to 25% of total fatty acids), Stearic Acid, Alpha Linoleic Acid, Palmitic Acid, and Linoleic Acid; exact composition varies depending on production. These components are also mediators of immune and cardiovascular system due to their precursor effects of prostaglandins and leukotrienes.

2.16.5 Minerals

Viswanadha *et al.,* (2011), *S. platensis* contains many minerals such as potassium, calcium(600–1,200mg/100g),chromium, copper, iron(50-150mg/100g), magnesium(200-600mg/100g), manganese, phosphorus, selenium(50-200mcg/100g), sodium, and zinc. This microalgae is a good component due to its iron, calcium, and phosphorus contents. The ferrous component in this microalgae can be easily digested and bioactive in an organism easily which is important for pregnant adult females. The utilization of calcium and phosphorus contents of *S. platensis* has an important impact on bone calcification and improves bone health.

2.16.6 Pigments

Babadzhanov *et al.,* (2004) studied that diets rich in carotenes are found to be important for human health due to its effects in reducing the risk of diseases. *S. platensis* has some natural pigments which color this microalgae, such as c-phycocyanin, chlorophyll, xanthophyle, beta-carotene, zeaxanthin, and allophycocyanin. The most important are phycocyanin, chlorophyll, and beta-carotene. C-Phycocyanin is the most important

pigment, which includes iron, and contains 14% of its dry weight. Also, *S. platensis* is one of the best nutrients that contains the highest chlorophyll value (1%). Chlorophyll is known as a detoxifier and purifier phyto-nutrient. It improves the carbohydrate, protein, and lipid metabolism and affects reproduction positively. Carotenes constitute half of this microalgae, especially beta-carotene. The carotenes and xanthophyle in *S. platensis* are demonstrated in different metabolism pathways in the body, and also better influence the function of vitamins and minerals in an organism.

2.17 Treatment with spirulina

Basshandy *et al.,* (2016) conducted a study to examine the protective role of *Spirulina platensis* against arsenic-induced testicular oxidative damage in rats. Arsenic (in the form of $NaAsO_2$ at a dose of 6.3mg/kg body weight for 8 weeks) caused a significant accumulation of arsenic in testicular tissues as well as a decrease in the levels of testicular superoxide dismutase (SOD), catalase (CAT), reduced glutathione, and zinc. Moreover, it significantly decreased plasma testosterone, luteinizing hormone (LH), triiodothyronine (T_3), and thyroxine (T_4) levels and reduced sperm motility and sperm count. AS led to a significant increase in testicular malondialdehyde (MDA) tumour necrosis factor alpha (TNF-α, nitricoxide (NO) and sperm abnormalities. *S. platensis* at a dose of 300mg/kg was found to attenuate As-induced oxidative stress, testicular damage, and sperm abnormalities by its potent antioxidant activity.

Dixit and Suseela (2013) found that pharmaceutical importance of bioactivities of cyanobacterium including anti proliferative, antitumor, antifungal, antibacterial, antimalarial, antiviral, antimycotics, cytotoxicity, multi-drug resistance reversers and immunosuppressive agents. Blue green algae, *Spirulina* display antitumor activity against many cancers both in human and animal systems.

Besednova *et al.,* (1979) shown that *Spirulina platensis* enhances functions of selected effect or cells of immune system of chicken. The available data suggesting that the *Spirulina platensis* exposure improves chickens immune performance without adversely affecting other performance characteristic. The whole cells of blue green algae, *Spirulina platensis* and its lipopolysaccarides were shown to stimulate production of macro and microglobulin antibodies in rabbits.

Islam *et al.,* (2009), observed that spirulina has protective effect against arsenic toxicity in rats. Thirty six female Long Evans rats were randomly divided into three equal groups (n=12) and marked as T_0, T_1 and T_2 groups. Rats of T_0 group were given normal feed and water and kept as control. Rats of T_1 and T_2 groups were given 5mg Sodium arsenite/kg body weight (BW) and 5mg Sodium arsenite/kg (BW) plus spinach extract 100 mg/kg body weight respectively daily for 30 days orally. This indicates that As causes liver injury. Chronic exposure of experimental animals to inorganic arsenic has been shown to produce various liver lesions, including inflammation and oxidative damage, fatty accumulation, parenchymal cell degeneration, hepatic fibrosis and liver proliferative lesions (Mazumder, 2005). From this findings it may be stated that spinach somehow prevent liver injury caused by arsenic.

Vedi *et al.,* (2013), conducted a study on to evaluate the protective properties of *Spirulina fusiformis* against galactosamine induced toxicity in swiss albino mice. Galactosamine injection significantly increased the levels of SGOT, SGPT, SBLN and TNF–α in the serum and caused depletion in the antioxidant status in the liver. Administration of *Spirulina fusiformis* (100mg/ kg body weight, i.p.) altered these parameters and brought them activity against galactosamine induced toxicity in mice.

Samah M.M. Fathy, *et al.,* (2015) conducted a study to investigate the effect of intra-peritoneal injection of purified exudates of arsenic *Spirulina platensis* on the mammalian endocrine and nervous systems. The intra-peritoneal injection of the cyanobacterial exudates in mice was applied line assay kit. A sharp disruption in the sex hormones levels of testosterone, progesterone, follicular stimulating hormone and luteinizing hormone was demonstrated in the serum of the treated mice. At the same time, a significant reduction in the endogenous antioxidant defence enzymes, superoxide dismutase, catalase and glutathione peroxidase was observed in the hippocampus region of the injected mice. Moreover, levels of dopamine, nor adrenaline, serotonin and acetyl choline neurotransmitter in the same region were significantly affected as a result of the treatment with spirulina filtrate. The gas chromatography–mass spectrometer and liquid chromatography mass spectrometry/mass spectrometry analysis showed the presence of some sterol-like compounds in the cyanobacterial filtrate. He demonstrated the capability of Spirulina to release detrimental bioactive metabolites into their surrounding that can disrupt the mammalian endocrine and nervous systems.

Rawshon Jamil *et al.,* (2015), conducted to evaluate the prebiotic effects of spirulina as a growth and immunity promoter for broiler chickens. Birds were randomly and equally distributed into four groups (T_0, T_1, T_2 and T_3) and fed on a diet containing 0, 2, 4 and 8 g Spirulina/kg feed respectively for 4 weeks. The body weight was increased in the treatment groups fed with spirulina diet from 7^{th} days to 28^{th} days old. FCR was also decreased among the treatment groups. Haematological parameters were increased except ESR which was decreased in the treatment group. Aspartate Amino transferase (AST) and Alanine Aminotransferase (ALT) level were decreased in all the treatment groups. The study suggests that, Spirulina is a good natural feed additive which has a tremendous effect to improve the broiler production and thereby may reduce the production cost.

2.18 Treatment with vitamin E

Zablotska *et al.,* (2008), conducted a study to clarify the effects of the vitamin B group (17 thiamine, riboflavin, niacin, pyridoxine, and cobalamin) and antioxidants (vitamins A, C, and E) on arsenic-related skin lesions. A total of 14,828 individuals meeting a set of eligibility criteria were identified among 65,876 users of all 5,996 tube wells in the 25-km^2 area of Araihazar, Bangladesh; 11,746 were recruited into the study. This analysis is based on 10, 628 subjects (90.5%) with non-missing dietary data. Skin lesions were identified according to a structured clinical protocol during screening and confirmed with further clinical review. Riboflavin, pyridoxine, folic acid, and vitamins A, C, and E significantly modified risk of arsenic-related skin lesions. The deleterious effect of ingested arsenic, at a given exposure level, was significantly reduced (ranging from 46% reduction for pyridoxine to 68% for vitamin E) for persons in the highest quintiles of vitamin intake.

Mashkoor *et al.,* (2013), Studied that As induced toxico-pathological alterations in broiler chicks and their attenuation with Vit E and Se. Dullness, depression, open mouth breathing, increased thirst; ruffled feathers, pale comb, skin irritation and watery diarrhea were the most striking clinical signs. The body weight and feed intake was significantly decreased in treated birds. The erythrocyte counts, hemoglobin concentration and packed cell volume decreased ($P<0.05$) in treated broilers with As or As with Se and Vit E. Grossly pale and hemorrhagic liver and swollen kidneys were observed in As treated birds. 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.

CHAPTER 3
MATERIALS AND METHODS

3.1 Statement of the experiment

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 1st November to 30 th December, 2018.

3.2 Experimental animal

Day old sixty quails were used in this experiment. All the 60 quails were collected from the local hatchery. The quails were observed for 30 days for the adjustment with the environment. All the quails were maintained by feeding poultry feed (Nourish Poultry feed) and clean water *ad libitum.*

3.3 Preparation of house

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 everyday. The excreta of birds were cleaned properly in daily. The animal room was well ventilated.

3.4 Experimental animal grouping

Sixty quails were collected for this experiment. The birds were randomly divided into 4 equal groups and eventually each group comprised of 15 quails. 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 E.

Fig 3.1: Different groups of experimental birds.

3.5 Body Weight (BW)

Following grouping, all the quails were weighed individually firstly on (day 0= immediate previous day of starting treatment) after grouping and marking, then on day 15 and finally on day 30 and the results of body weight were recorded.

Fig 3.2: Measuring body weight.

3.6 Layout of experiment

The layout of the experiment is presented below:

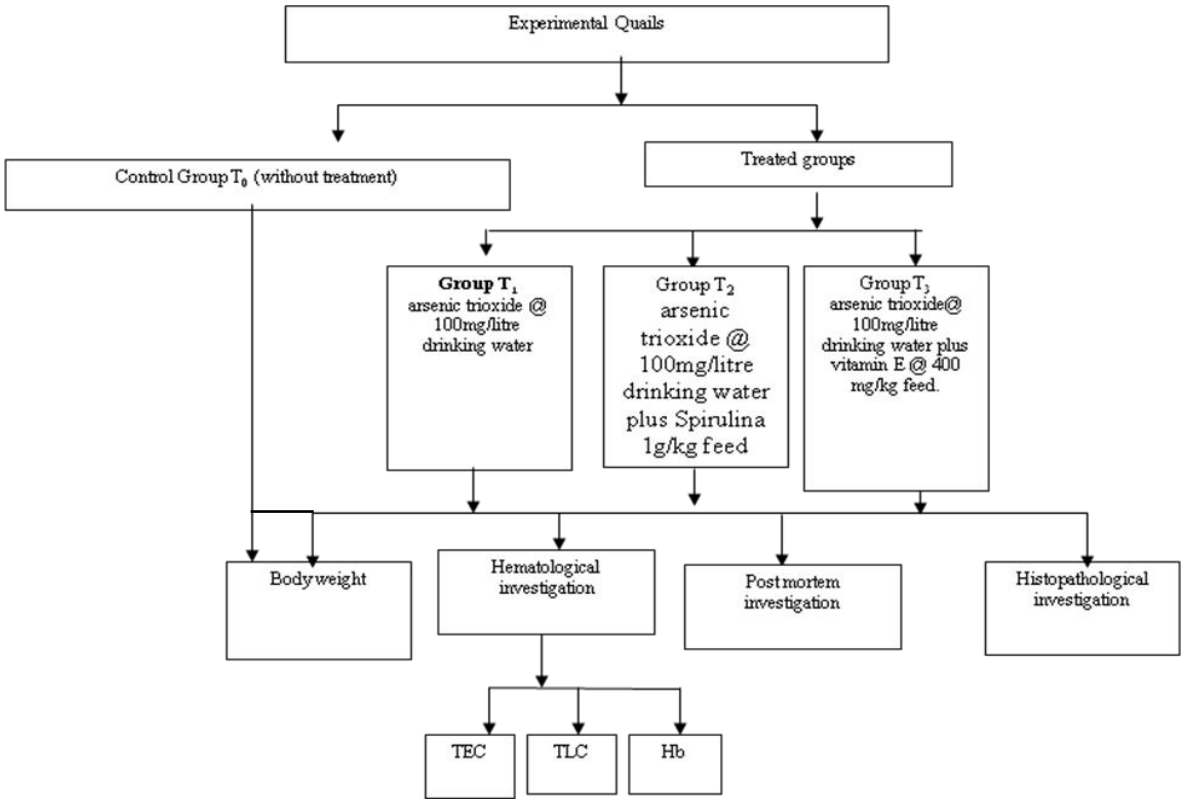


Fig. 3.3: Layout of the experiment (n=15 in each group)

3.7 Clinical signs

Quails were closely observed after feeding arsenic trioxide, spirulina and vitamin E daily for 3 times (morning, afternoon and evening) for the appearance of toxic clinical signs if any in them, during the entire experimental period (from Day 1 to Day 30) and the findings were recorded.

3.8 Experimental trial

Each of group is treated by different parameter and the experiment was concluded by 60 days. Quails of group T_0 were maintained on only normal feed and water as control group, quails of group T_1 were treated with arsenic trioxide @ 100mg/L of drinking water daily and normal feed and quails of group T_2 were treated with arsenic trioxide @ 100mg/L of drinking water plus feed with spirulina (Spirulina platensis) @ 1gm/kg feed.

The spirulina (Navit) used in this experiment was collected from Square Pharmaceuticals Limited; as a capsule form, quails of group T_3 were treated with arsenic trioxide @ 100mg/L of drinking water plus Vitamin–E Tablet (Square Pharmaceuticals Limited; Bangladesh) simultaneously at a dose of 400mg/kg body weight. All treatments were given for 30 days.

3.9 Preparation of treatment materials

3.9.1 Arsenic trioxide solution

The respective required amount of arsenic trioxide @ 100mg/L of drinking water was weighted. 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 group was allotted for mixing As_2O_3 to ensure that they take full time of arsenic trioxide for their requirement.

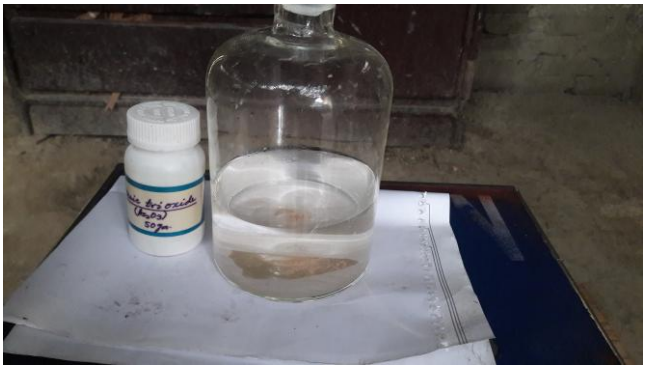


Fig 3.4: Arsenic trioxide solution.

3.10 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 distilled 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 airtight plastic container.

Fig 3.5: Preparation of spirulina mixed feed.

3.11 Sampling procedure

After every 15 days from each group of quails blood samples were collected for haematological test and 1 ml of blood for each was taken separately in EDTA coated tube. Bloods samples for hematological investigation were preserved at refrigerator temperature. All blood were taken 1st on Day 15 and 2nd on Day 30.

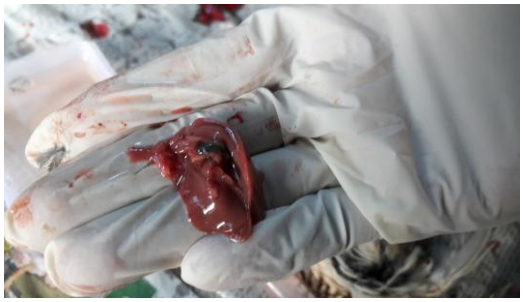
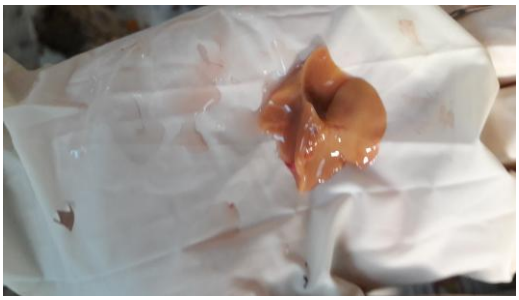
3.12 Examination of blood for determination of hematological parameters

From each group, 5 birds were selected randomly and killed humanely on experimental days 15 and 30 and blood was collected for TEC, TLC and Hb concentration determination following standard protocol.

Fig 3.6: Blood samples

3.13 Postmortem examination

At the end of the experiment, 16 birds (4 birds) for each treatment group were randomly selected and slaughtered after fasting to collect the viscera (liver, kidney, lung) and muscle samples and to find the gross changes of those organs.

The total lung, liver, and kidney were collected aseptically, washed with physiologic saline and were kept in the pre-marked 10 percent formalin solutions.

3.14 Histopathology of sample

Histopathology of liver and kidney samples from each group were performed by following method.All histological procedures can be divided into a similar series of steps-

3.14.1 Collection

After slaughtering samples were collected from the quail as soon as possible to avoid autolysis and prepare for fixation.

3.14.2 Fixation

Pieces of organs should be promptly adequately fixed as soon as possible after removal from the animal's body. To avoid tissue digestion by enzymes present within the cell (autolysis) or by bacteria and to preserve the cell structure and molecular composition. This process is called fixation, and the resulting specimen is described as fixed. After collection of sample we cut the tissues into small fragments to facilitate the penetration of fixative. The samples were fixed in 10% formalin.

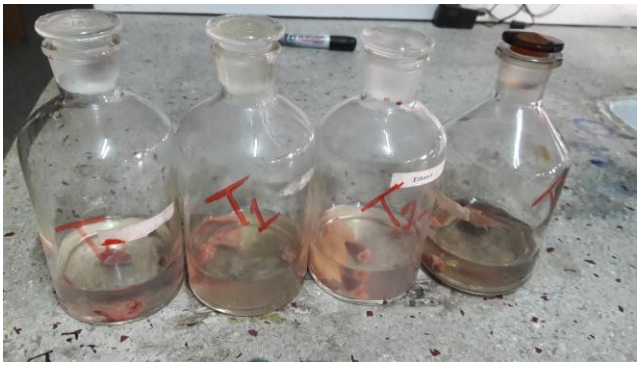


Fig. 3.7: Fixing in formalin

3.14.3 Tissue processing

Once the tissue has been fixed, it was processed into a form in which it can be made into thin microscopic sections. The technique of getting fixed tissue into paraffin is called tissue processing. The main steps in this process are dehydration and clearing. Wet fixed tissues (in aqueous solutions) cannot be directly infiltrated with paraffin. First, the water from the tissues was removed by dehydration. This was usually done with a series of alcohols; viz. 70% to 95% to 100%. The next step is called "clearing" and consisted of removal of the dehydrant with a substance that was miscible with the embedding medium (paraffin). The commonest clearing agent is xylene.

3.14.4 Embedding

Once the tissue was impregnated with solvent, it was placed in melted paraffin in oven, typically at 58- 60°C. Heat causes the solvent to evaporate, and the space within the tissues becomes filled with paraffin. The tissue together with its impregnating paraffin hardens after being taken out of the oven.

Fig. 3.8 Block ready for microtomy

3.14.5 Sectioning

Once the tissues were embedded, they were cut into sections to a thickness of 5-10 microns that was placed on a slide. This was done with a microtome. The microtome is nothing more than a knife with a mechanism for advancing a paraffin block standard distances across it. In this study the microtome here used was from the brand Leica RM 2135, Germany.

3.14.6 Mounting of sections on microscope slides

In this procedure, the sections were permanently attached to microscope slides. Before mounting the microscope slides were washed with soap and water and rinsed free of soap with tap water. Then placed the slides in a coplin jar and rinsed several times with H_2O. Handling the slides only by their edges, the slides were placed in the slide storage box for drying. The sections became flattened by floating them on water held at 45^0C. The solution also contained an adhesive, gelatin agar which causes the tissue section to bind to the slide.

3.14.7 Staining

The embedding process was reversed in order to get the paraffin wax out of the tissue and allow water soluble dyes to penetrate the sections. Before the staining, the slides were "deparaffinized" by running them through xylenes (or substitutes) to alcohols to water. There are no stains that can be done on tissues containing paraffin. Here used routine Hematoxylin and Eosin stain.

Fig.3.9. Staining of sample

Fig.3.10 Cover slipping

Fig.3.11. Microscopic observation

3.14.8 Cover slipping

After staining cover slip was placed on the slides using one drop of DPX, taking care to leave no bubbles and dry overnight to make the permanent slide and finally observed the slide under microscope in 10x objectives.

3.15 Statistical analysis

Data were expressed as mean ± standard error (SE) and analyzed using one way analysis of variance (ANOVA) followed by Duncan's test as a post-hoc test using IBM SPSS Statistics 20.0 software package and the chart was created by Microsoft Excel 2007 software. Results were considered to be statistically significant when P values are less than 0.01 ($P<0.01$).

CHAPTER 4
RESULTS

The experiment was conducted to determine the efficacy of spirulina and Vitamin E on arsenic toxicity in quails. It was also undertaken to observe the effects of spirulina and vitamin E on body weight, hematological, postmortem and histopathological parameters in arsenic fed quails. Sixty quails were randomly divided into four equal groups to conduct the experiment. T_0 group served as negative control and fed with normal diet. Group T_1 were treated with arsenic trioxide at a dose of 100mg/L drinking water and this group were kept positive control. Group T_2 were treated with same dose of arsenic trioxide and spirulina (*Spirulina platensis*) simultaneously at a dose of 1 gm/kg feed. Group T_3 were treated with same dose of arsenic trioxide and vitamin E simultaneously at a dose of 400 mg/kg feed. All the treatment were continued for 30 days and treated quails were closely observed through the entire period.

4.1 Clinical signs

There were no significant change in clinical signs of arsenic toxicity were observed in trial quails for the first 7 days. After 7 days, clinical symptoms (depression, reduced feed intake and ruffled feathers) were observed in birds treated with arsenic during the entire experimental period.

4.2 Body Weight (BW) of the Quails

BW of quails of all groups were taken fifteen days interval on day 30, day 45 and day 60. Table 1 showed that the body weight gain was highest the (130.10 ± 1.03) gm in control group quails at 60 days but the body weight gain was lowest (96.60 ± 0.62) gm in As treated T_1 group at 60 days whereas body weight gain in T_2 and T_3 were (116.50±0.91) and (107.50±0.65) gm respectively which were better than arsenic treated T_1 group. The BW of initial groups were not significant ($p > 0.05$) but in 45 days and 60 days mean value of BW were significant ($p<0.01$).

The BW of treated group were increased with their age but in T_1 group it decreased compared to other groups. In the present study As reduced the body weight with their increasing age. The highest body weight gain was found in control group where supplied normal feed and diet.

Table 4.1 Effects of spirulina and vitamin E on the body weight (gm) of quails

Treatment	T_o	T_1	T_2	T_3	P. value
Initial (30 days)	45.30 ± 0.75	43.50 ± 0.91	43.90 ± 0.90	44.90 ± 0.82	NS
45 days	84.60 ± 0.76^{c}	64.50 ± 0.69^{a}	80.80 ± 0.74^{b}	79.70 ± 0.67^{b}	**
60 days	130.10 ± 1.03^{d}	96.60 ± 0.62^{a}	116.50 ± 0.91^{c}	107.50 ± 0.65^{b}	**

Figures indicate the Mean ± SE (standard error); NS means not significant

** = $p<0.01$

*= $p<0.05$

In a row figures with same or without superscripts do not differ significantly as per DMRT.

4.3 Hematological parameter

4.3.1 Total Erythrocyte Count (TEC)

In Table 2, TEC values were the highest ($3.00 \pm 0.006^{d} \times 10^{12}$) million/$mm^3$ found in T_2 group at 60 days where the birds were treated with spirulina against As toxicity but the lowest ($2.87 \pm 0.003^{a} \times 10^{12}$) million/$mm^3$ was in T_1 group exposed to As.

Table 4.2: Effects of spirulina and vitamin E on Total Erythrocyte Count (TEC) million/mm^3 values of quails

Treatment	T_0	T_1	T_2	T_3	P. Value
45 Days	$2.93 \pm 0.003^{b} \times 10^{12}$	$2.85 \pm 0.003^{a} \times 10^{12}$	$2.97 \pm 0.003^{d} \times 10^{12}$	$2.96 \pm 0.003^{c} \times 10^{12}$	**
60 Days	$2.95 \pm 0.003^{b} \times 10^{12}$	$2.87 \pm 0.003^{a} \times 10^{12}$	$3.00 \pm 0.006^{d} \times 10^{12}$	$2.98 \pm 0.003^{c} \times 10^{12}$	**

Figures indicate the Mean ± SE (standard error); NS means not significant

** = $p<0.01$
*= $p<0.05$

In a row figures with same or without superscripts do not differ significantly as per DMRT.

4.3.2 Total Leukocyte Count (TLC)

In Table 3, TLC on Day 60 was the highest ($258.23 \pm 0.15^{c} \times 10^{9}$) in control group quails and the lowest in T_2 group quails ($254.04 \pm 0.56^{a} \times 10^{9}$) where birds were treated with spirulina and the difference were statistically significant among all group of quails ($p<0.01$). Spirulina decrease the TLC level.

Table 4.3: Effects of spirulina and vitamin E on Total Leukocyte Count (TLC) Thousand/mm$_3$ values of quails

Treatment	T_0	T_1	T_2	T_3	P. Value
45 Days	$256.20 \pm 0.21^{c} \times 10^{9}$	$254.80 \pm 0.15^{b} \times 10^{9}$	$253.00 \pm 0.32a \times 10^{9}$	$255.73 \pm 0.32^{c} 10^{9}$	**
60 Days	$258.23 \pm 0.15^{c} \times 10^{9}$	$256.77 \pm 0.24^{b} \times 10^{9}$	$254.04 \pm 0.56^{a} \times 10^{9}$	$256.71 \pm 0.33^{c} 10^{9}$	**

Figures indicate the Mean ± SE (standard error); NS means not significant

** = $p<0.01$
*= $p<0.05$

In a row figures with same or without superscripts do not differ significantly as per DMRT.

4.3.3 Hemoglobin (Hb)

The highest ($20.34 \pm .17^{c}$) Hb concentration was found in T_2 group at 60 days and the lowest concentration was found in T_0 group ($15.63 \pm .09^{a}$) (Table 4). Difference among values of 60 days of Hb concentration were statistically significant ($p<0.01$). It might be concluded that spirulina might slightly increase the values of Hb against arsenic toxicity in quails.

Table 4.4: Effects of spirulina and vitamin E on Hemoglobin concentration (Hb) (gm/dl) values of quails

Treatment	T_0	T_1	T_2	T_3	P. Value
45 Days	14.73 ± 0.12^{a}	15.53 ± 0.15^{b}	19.70 ± 0.26^{d}	18.63 ± 0.09^{c}	**
60 Days	15.63 ± 0.09^{a}	15.90 ± 0.06^{a}	20.34 ± 0.17^{c}	19.37 ± 0.24^{b}	**

Figures indicate the Mean ± SE (standard error); NS means not significant

** = $p<0.01$

*= $p<0.05$

In a row figures with same or without superscripts do not differ significantly as per DMRT.

4.4 Postmortem findings

At the end of experiment, post-mortem examination showed diffuse congestion (Fig. 4.2), pale color, fragile and granular appearance focus showing possible As deposition on liver. Kidney showed congestion (Fig. 4.5) and lung (Fig. 4.7) were haemorrhage on liver (Fig. 4.3). Gross examination of control group (group T_0) revealed normal liver (Fig. 4.1), lung (Fig. 4.6) and kidney (Fig. 4.4).

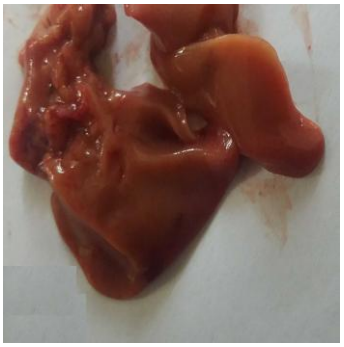


Fig.4.1: Normal liver

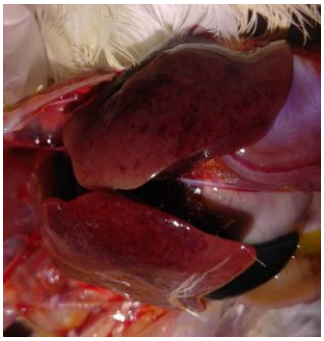


Fig.4.2: Congestion on liver

Fig.4.3: Haemorrhage on liver

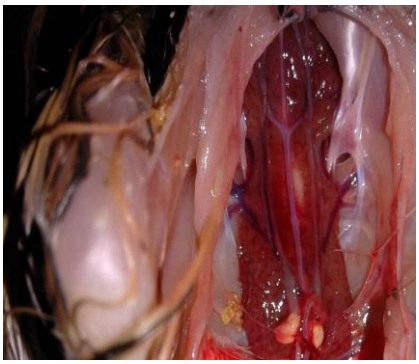


Fig.4.4: Normal kidney

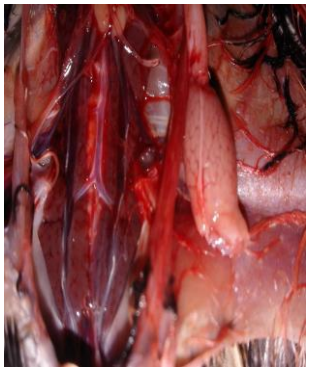


Fig.4.5: Congestion of kidney

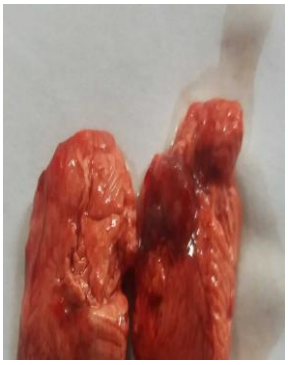


Fig.4.6: Normal Lung

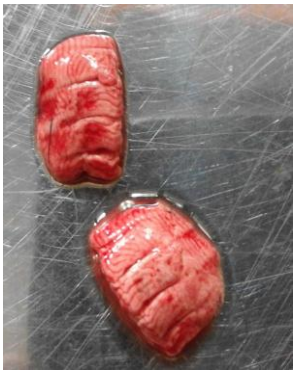


Fig.4.7: Haemorrhage on lung

4.5 Histopathological observations

Six bird's form each group were sacrificed at the end of the experiment. The organs viz. liver, kidney, muscle and heart were collected and preserved. Liver and kidney were examined under microscope after processing. Histopathological changes in different groups are described below:

4.5.1 Liver

Microscopical examination of liver from control group (T_0), birds treated with arsenic plus spirulina (T_2) and arsenic plus vitamin E (T_3) revealed as normal histological picture (Fig. 4.8). However liver of arsenic treated group birds showed fatty changes and congestion. Cirrhosis (Fibrous tissue proliferation in the liver parenchyma), severe congestion of hepatic vessels and fatty changes were observed in the arsenic treated group (T_1) (Fig. 4.9 and 4.10).

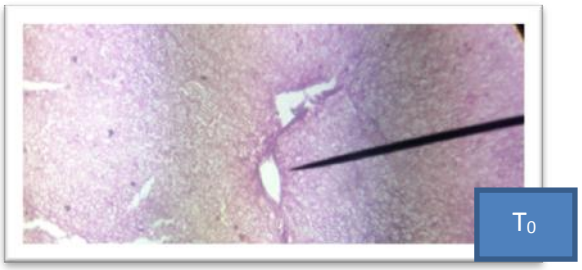


Fig. 4.8: Microscopic view of liver: showing regular pattern of hepatic cord in group T_0

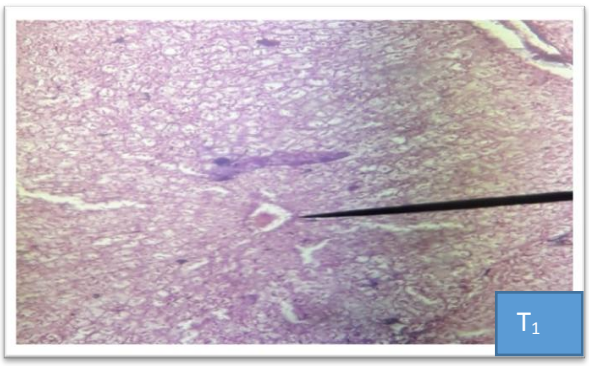


Fig.4.9: Microscopic view of liver: group T_1 showed congestion in liver (H and E, Dimension- 947×619)

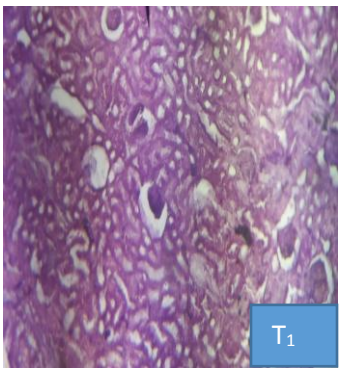


Fig.4.10: Microscopic view of liver: group T_1 showed cirrhosis in liver (H and E, Dimension-947×619)

4.5.2 Kidney

Microscopically, kidneys from control (T_0), arsenic plus spirulina (T_2) & arsenic plus vitamin E (T_3) showed as normal architecture with normal glomeruli, Proximal Convoluted Tubules (PCT) and Distal Convoluted Tubules (DCT) (Fig.4.11). Fatty

degeneration, cytoplasmic vacuoles were observed in the section of kidneys from the birds receiving arsenic trioxide (T_1) group (Fig. 4.12).

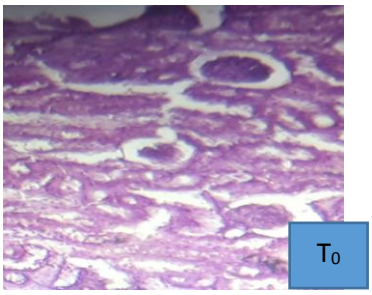


Fig.4.11: Microscopic view of kidney: group T_0 showed no remarkable change in kidney tubules (proximal and distal convoluted tubules & Henle's loop (H and E, Dimension- 947×619)

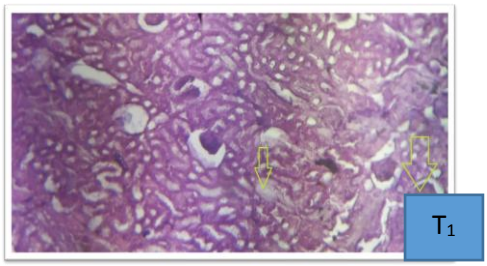


Fig.4.12: Microscopic view of kidney: group T_1 showed Fatty changes in kidney tubules (H and E, Dimension- 947×619)

CHAPTER 5
DISCUSSION

The As toxicity emerged as a 'real disaster' in Bangladesh and affecting thousands of people, particularly in villages (Islam *et al.,* 2004). With greater public awareness of As poisoning in animal and human nutrition, there has been a growing interest in developing regulatory guidelines for mitigating As-contaminated ecosystems (Mahimairaja *et al.*, 2005).

As induced quails showed several clinical signs during the experimental period but slightly increased body weight was observed in all groups because the quails were in growing stage. On the other hand, the body weight gain in quails of As treated group was found lower compared to other treated groups. The presented findings were in partial agreement with previously conducted study by Islam *et al*. (2009), who reported that ducks of only arsenic trioxide group showed the percentage of decrease body weight was maximum (14.93%) whereas, in arsenic plus spirulina treated groups rate of decrease body weight in ducks (4.08-11.26%) were lower than only As treated groups. Moderate weakness was also observed in the quails of arsenic treated group compared to As plus spirulina, As plus vitamin E and control groups. Hence, it could be said that feeding of arsenic trioxide caused chronic toxicity in quails, although for the manifestation of more pronounced symptoms of As toxicity would take more time.

There was significant difference on TEC, TLC and Hb was found among the groups, the value of TLC was the highest in control group quails and the lowest in As treated quails. The cause of change in hematological values might be due to the toxic effect of As on haematopoietic system which is responsible for such alterations in hematological parameters. The value of TEC was the highest in spirulina treated group T_2 and the lowest in As treated group T_1.The value of Hb concentration was the highest in spirulina treated group T_2 However, the findings might suggest that chronic As toxicity possibly decrease TEC level in the quails and that could be recovered by spirulina (1g/kg feed) with feed within 60 days.

Islam *et al*., (2005) assumed that toxic effects of arsenic trioxide on bone marrow may be responsible for erythrocytopenia. Treatment of chronic As toxicity with spirulina/vitamin E for a longer time might give a clear picture in this regard. Increased levels of As found in the lung, liver and kidney following feeding of arsenic trioxide (100 mg/L drinking water) to the quails of As treated group compared to control group during the whole study period and was increased with the length of exposure period. This finding agreed with the findings of Nabi *et al*., (2005) and Kamaluddin and Misbahuddin (2006). They showed that administration of As in quails for different periods induced a significant increase in As accumulation.

Spirulina reduced As level significantly from the tissues compared to only As treated quails which was in agree with finding of Fariduddin *et al*., (2001), who stated that spirulina was effective in lowering As level from the arsenic loaded tissues in quails, Ghosh *et al*. (2014), who stated that spirulina was effective in lowering of As level from blood of induced arsenicosis in goats.

Vitamin E reduced As level significantly from the tissues compared to only arsenic treated quails that agreed with the findings of Islam *et al*. (2005), who stated that supplementation of vitamin E, iron and zinc reduced the arsenic concentration in tissues like liver, kidney, spleen, heart, intestine, stomach, muscle and dermis of rats as well as lower the tissue damage caused by arsenic.

However, it is known that spirulina is an enriched source of nutrients like protein, amino acid, iron, β-carotene, phycocyanin, γ-lenolenic acid, vitamin B1, B2, B3, B6, B12 and essential fatty acid which are very much helpful to maintain the normal health and vitamin E influences the body's enzyme functions and may help to stimulate the production of antibodies.

Microscopical examination of liver from control group (T_0) and birds treated with arsenic plus spirulina (T_2) and birds treated with arsenic plus vitamin E (T_3) revealed as normal histological picture, however liver of arsenic treated groups (T_1) birds showed fatty changes and congestion. Cirrhosis (Fibrous tissue proliferation in the liver parenchyma), severe congestion of hepatic vessels and fatty changes were observed in the T1 group (100 mg arsenic trioxide/L drinking water). Cirrhosis and congestion in the blood vessels of liver also reported by PK. Singh *et al.,* (2011) in albino rat treated by As containing ground water. Dutta (2004) also observed fatty changes, congestion of

hepatic vessels, thrombi in central vein and coagulative necrosis in his study carried out on rat with as treatment. M.S. Islam *et al.,* (2013) observed that the distribution of arsenic concentration was highest in liver and lowest in faces of chickens. Such histological changes in the liver of treatment group birds might be due to toxic effect of arsenic trioxide on liver because most of the toxicants (like arsenic) are metabolized in liver & thereby affect its morphology.

Microscopically, kidneys from control (T_0), arsenic plus spirulina (T_2) & arsenic plus vitamin E (T_3) showed as normal architecture with normal glomeruli, Proximal Convoluted Tubules (PCT) and Distal Convoluted Tubules (DCT).Fatty degeneration, cytoplasmic vacuoles were observed in the section of kidneys from the birds receiving arsenic trioxide/L drinking water (T_1) group. These findings are in agreement with previous study of Dutta *et al.*,(2004). Since kidney is involved with ultra-filtration, selective reabsorption and tubular secretion, As may be accumulated in the kidney. The histopathological changes in arsenic treated group birds could be due to accumulation of arsenic in kidney & thereby exert harmful effect.

CHAPTER 6
CONCLUSION

From this study it may be concluded that spirulina alone and vitamin E alone lowered As toxicity in quails but spirulina was more efficacious than vitamin E in reducing arsenic load in quails. As toxicity caused reduce the body weight of quails. Treatment with spirulina and vitamin E might increase the body weight. Spirulina and vitamin E alone can reduce the effects of As toxicity As toxicity has adverse effect in hematological parameters in quails. This study suggested that spirulina and vitamin E has significantly reduced the As concentration of inorganic As toxicity in quails. Further investigation in this line may make more clear evidence to use spirulina as a therapeutic treatment for As toxicity. More study is also needed to determine the level of As in blood and to optimize the dose of spirulina and vitamin E to minimize arsenicosis in animals.

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