STUDY ON THE PREVENTION OF ARSENIC TOXICITY IN QUAIL BY USING SPIRULINA AND GARLIC

A THESIS
BY

MST. AZMERY SHATHY
Registration No.: 1705488
SEMESTER: January-June/2019
SESSION: 2017

MASTER OF SCIENCE (MS)

IN

PHARMACOLOGY

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR

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Submitted to the

Department of Physiology and Pharmacology
Faculty of Veterinary and Animal Science
Hajee Mohammad Danesh Science and Technology University, Dinajpur
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE (MS)

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Approved as to style and contents by

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DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
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JUNE, 2019

Dedicated to

My

Beloved Parents

ABSTRACT

Chronic arsenic (As) toxicity is a severe disease in men and animals which occurs severely in Bangladesh. As contamination in ground water used in drinking is the major concern because arsenic is present in human and animal food chain. This work was done in quails with a view to study the comparative efficacy of Spirulina (*Spirulina platensis*) and Garlic (*Allium sativum*) for prevention of arsenic toxicity. Forty quails were used in this study and animals were divided into control group (T_0), Arsenic treated group (T_1), Arsenic (As) plus Spirulina treated group (T_2) and Arsenic (As) plus Garlic treated group (T_3). Each group consists of 10 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 (As) trioxide/L drinking water daily for 30 days. In addition to arsenic (As) trioxide quails of group T_2 and T_3 were simultaneously fed with Spirulina @ 1 gm/kg feed and T_3 were simultaneously feed with Garlic @ 1gm /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 hematological parameters. Result showed that in group T_1, body weight gain was minimum, whereas in groupT_2 and T_3 the body weight gain in quails were better. Reduction of TEC, Hb values were significant ($P<0.01$) in T_1 group. Whereas in rest groups reduction of TEC, Hb were less than arsenic treated groups. Noticeable change observed in liver and kindey of arsenic (As) treated group in compare to control group in postmortem and histopathological changes. In conclusion, spirulina and garlic have significant effect on body weight, hematological, postmortem and histopathological changes.

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 respected teacher and 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 Dr. Fahima Binthe Aziz, Associate Professor, Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur for her 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, Professor Dr. Rakibul Islam, Chairman 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 Sarkar Setu, 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***

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CHAPTER-I
INTRODUCTION

Access to safe drinking water is the basic human right. Now-a-days, one of the most serious worldwide environmental problems is drinking water polluted by arsenic. Arsenic poisoning from underground drinking water in Bangladesh was first identified in 1993 (Smith *et al.*, 2000). Almost 57 of 140 million people (Mahmood, 2002) and 61 of total 64 districts of Bangladesh are reported to have dangerous levels of inorganic arsenic (>50 μg/L) in most of the tube wells (DPHE *et al.*,1999; BAMWSP, 2001). Arsenic can enter into food chain (Ulman *et al.*, 2004) causing wide spread distribution throughout the plant and animal kingdoms.

Groundwater arsenic contamination in Bangladesh is reported to be the biggest arsenic calamity in the world in terms of the affected population (Talukder *et al.*, 1998). Chronic arsenic exposure is associated with many human health conditions, including skin lesions and cancers of the liver, lung, bludder 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, urea, uric acid levels and various hemato- logical parameters like TEC, TLC, Hb, blood sugar level in the quail (Yasmin *et al.*, 2011).

Arsenic can enter into the blood circulation (Ulman *et al.*, 2004). Recently arsenic intoxication in experimental animals has been found to be associated with hepatic tumors, spermatogenesis (Sukla and Pandey, 1984), inhibition of testicular steroidogenic function (Sarker *et al.*, 1991) and severe metabolic disorders such as diabetes in human. The natural source of human exposure to arsenic occurs through consumption of drinking water sourced from groundwater that contains dissolved inorganic arsenic (Nandi *et al.*, 2006). Significantly elevated standard mortality from cancer of the bladder, lung, liver, kidney, skin and colon were found in the population living in an area of Taiwan, China and some part of Africa where arsenic contamination of the water supply was endemic (Azcue and Nriagu, 1995).

Arsenic 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. Chronic arsenic toxicity due to drinking of arsenic contaminated ground water is a major environmental health hazard throughout the world (Mazumder, 2008). Chronic arsenic poisoning results from drinking contaminated well water over a long period of time. This is due to arsenic contamination of aquifer water.

According to the World Health Organization, the U.S. is one of the countries affected by inorganic arsenic that is naturally present at high levels. Arsenic toxicity is an even bigger problem in the Far East. “Millions of people in Bangladesh, India, Taiwan and Chile are consuming a high concentration of arsenic through drinking water, and thousands of them have already developed chronic arsenic poisoning.” (Misbahuddin *et al.*, 2006).

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 and Chow, 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 tetrapyrrole phycocyanobilin, which is responsible for antioxidant properties of Pc (Bhat and Madyastha, 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, 2007). It was reported that Pc can bind with heavy metals (Gelagutashvili *et al.*, 2003); hence, it can chelate and remove them. In view of the above concerns, the present study was designed to evaluate the antioxidant action of S. platensis enriched with phenolic compounds in ameliorating testicular dysfunction and oxidative stress induced by arsenic.

Garlic is well known as a folk remedy for a variety of ailments since ancient times, however, very few studies are available suggesting its beneficial role against arsenic toxicity pertaining to its ability to eliminate arsenic from the blood and soft tissues and in reversal of arsenic-induced oxidative stress in affected tissues. Further, an attempt to understand the mechanism of arsenic in inducing hepatic apoptosis was also studied. Results of the present study suggested that arsenic administration in quails caused generation of reactive oxygen species (ROS) causing apoptosis through mitochondria-mediated pathway. The ROS generation in hepatic tissue reverted to normal values after co-administration of garlic (Flora *et al.,* 2009).

In studies on garlic supplementation in quail feeds, the effects of garlic on cholesterol levels in the egg yolk (Lim *et al.*, 2006), plasma, and meat (breast and leg) and the level of microbial fermentation in the gastrointestinal tract (Choi *et al.,* 2010) were studied. In quail, the effects of garlic on the levels of lead tissues (Hanafy *et al.,* 1964), live weight (Al Homidan, 2005), performance and carcass traits (Raeesi *et al.,* 2010) were evaluated. In addition, in quail, the effects of garlic on enteropathogen counts, ileal histological structure, and productive parameters (Peinado *et al.,* 2012), antioxidant characteristics (Jakubcova *et al.,* 2014) and ascites incidence (Varmaghany *et al.,* 2015) were studied. In quail, the effects on the parasitic disease histomoniasis (infectious enterohepatitis caused by Histomonas meleagridis) were evaluated by Hafez & Hauck (2006). In quails and layers the effects of dietary garlic addition on egg weight, feed intake, feed conversion ratio, body weight gain, serum and egg yolk cholesterol levels and egg productivity (Chowdhury *et al.*, 2002; Yalcin *et al.*, 2006; Yalcin *et al.*, 2007; Ao *et al.*, 2010; Canogullari *et al.*, 2010), as well as on fecal bacterial load and on egg quality (Olobatoke & Mulugeta, 2011) were studied.

The effects of inclusion garlic (*Allium sativum*) at different levels and copper into diets of quail performance, egg quality traits, yolk and serum cholesterol content. Treatment groups were fed diets containing a standard commercial quail diet, basal diet plus 200 ppm copper (CuSO4.5H2O), 2% garlic , 2% garlic + 200 ppm copper, 4% garlic, 4% garlic + 200 ppm copper, 6% garlic and 6% garlic + 200 ppm copper from week 38th to 50th. Feed conversion efficiency and cracked egg were not affected by feeding garlic and copper. Egg weight, egg production and feed consumption decreased with garlic powder and copper supplementation. There were no differences in the egg quality traits except for shell stiffness and yolk index. Egg yolk cholesterol concentration decreased linearly with increased levels of garlic powder but serum cholesterol concentration increased. The supplementation of 200 ppm copper and combinations of garlic powder and copper did not have a significant effect on cholesterol and triglyceride concentrations of egg and serum. Consequently, without having a significant effect on laying performance and egg quality characteristics, oven dried garlic powder can be used up to 6% as a hipocholesterolemic agent in practical layer diets without copper (Hatice Kaya and Muhlis Macit, 2012).

Objective of the Study

- To know the effect of spirulina and garlic on the body weight in arsenic fed quails.
- To investigate the effect of Spirulina and garlic on haematological changes against arsenic toxicity in quails.
- To know the postmortem changes and histopathological examination in arsenic induced toxic quails.

CHAPTER-II
REVIEW OF LITERATURE

Review of related literature is necessary in the sense that it provides scope for reviewing the stock of knowledge and information relevant to the proposed research. Despite the fact that a few numbers of works have been done in Bangladesh related to this research, there are some published reports and related activities. However, the limited numbers of works so far published are mentioned here along with other related works. A short description on the available literature relevant to the present investigation has been presented below.

2.1 Physical and Chemical Properties of Arsenic

Habashi, 2013. Arsenic is a bright silver-gray metalloid; its outermost electrons are not free to move in the crystal structure because they are fixed in position in a covalent bond. It has metallic luster but is brittle with no useful mechanical properties. In addition to the metallic-like form, there are other modifications which change into the metallic-like form above 270°C. Black arsenic, formed as a mirror when arsenic hydride is passed through an incandescent glass tube and also when the vapor is rapidly cooled. Yellow arsenic, formed by the sudden cooling of arsenic vapor and consists of transparent, waxy crystals. It is unstable and changes into metallic-like arsenic on exposure to light or on gentle heating. Brown arsenic, is either a special modification or simply a more finely divided form is obtained in the reduction of solutions of arsenic trioxide in hydrochloric acid with tin(II) chloride or hypophosphorous acid.

Mazumder, (2008). Chronic arsenic toxicity due to drinking of arsenic contaminated ground water is a major environmental health hazard throughout the world. Organic arsenic exposure can also occur by eating food. Organic arsenic is 500 times less harmful than inorganic arsenic. Arsenic is a component of geologic formations and can be washed out into the ground water. Arsenic 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

Ahmad *et al.*, 2018. Arsenic contamination of groundwater in Bangladesh has been recognized as a major public problem. The arsenic contamination was first identified in the tubewell water in 1993 in a northern district of Bangladesh. Tubewells are the main source of drinking water in rural areas, and except hilly and terrace upland throughout the Bangladesh, the arsenic-contaminated tubewells are distributed. Fifty million people of Bangladesh were estimated to be at risk of exposure to arsenic through consumption of water from contaminated tubewells. Chronic exposure to arsenic causes arsenicosis and may include multi-organ pathologies. Many of the health effects of chronic toxicity are evident in Bangladesh. Besides dermatological manifestations, noncommunicable diseases including cancer, adverse pregnancy outcomes, and decreased intelligence quotient among the children are reported to be increasing. Cancer due to long-term low-dose arsenic exposure through consumption of contaminated water is now an important concern of Bangladesh as it is being increasingly reported from arsenic-exposed individuals. Stoppage of consumption of the arsenic-contaminated water is the mainstay of arsenicosis prevention and case management. At present, a higher proportion of the people are still consuming arsenic-contaminated water because of the lack of sustainable arsenic-safe water supply. In providing sustainable arsenic-safe water options, any option advocated should be cheap, easy to use, locally maintainable, and owned by the community. In addressing arsenic-related health issues, arsenic-exposed population needs to be brought under the coverage of the regular surveillance program for detection and subsequent management of noncommunicable diseases and cancers.

Janga *et al.*, 2016. Arsenic (As), a mobilizing metalloid, is found in almost every fraction of the environment. Arsenic levels in the environment have become a global concern due to its toxicity and adverse effects on human health; even at low concentrations, it is highly toxic and classified as a carcinogen. Although it often exists in soils and plants from natural sources such as igneous and sedimentary rocks, the largest anthropogenic source of arsenic is the use of chromated copper arsenate (CCA)-treated wood. As the wood comes in contact with both soil and water, arsenic can be released into the environment. Atmospheric releases of arsenic may also occur when mining, smelting and refining, industrial processes, coal combustion, and waste incineration take place. Once released, it may undergo cycling processes in the environment, depending upon its oxidation state, speciation, concentrations, and the presence of organic matter, competing ions, and other environmental factors (e.g., pH, redox).

Shankar *et al.*, 2014. Arsenic contamination of groundwater in different parts of the world is an outcome of natural and/or anthropogenic sources, leading to adverse effects on human health and ecosystem. Millions of people from different countries are heavily dependent on groundwater containing elevated level of As for drinking purposes. As contamination of groundwater, poses a serious risk to human health. Excessive and prolonged exposure of inorganic as with drinking water is causing arsenicosis, a deteriorating and disabling disease characterized by skin lesions and pigmentation of the skin, patches on palm of the hands and soles of the feet. Arsenic poisoning culminates into potentially fatal diseases like skin and internal cancers. This paper reviews sources, speciation, and mobility of as and global overview of groundwater As contamination. The paper also critically reviews the as led human health risks, its uptake, metabolism, and toxicity mechanisms. The paper provides an overview of the state-of-the-art knowledge on the alternative as free drinking water and various technologies (oxidation, coagulation flocculation, adsorption, and microbial) for mitigation of the problem of as contamination of groundwater.

Jin-Yong *et al.*, 2014. Arsenic is a ubiquitous, naturally occurring metalloid that may be a significant risk factor for cancer after exposure to contaminated drinking water, cigarettes, foods, industry, occupational environment, and air. Among the various routes of arsenic exposure, drinking water is the largest source of arsenic poisoning worldwide. Arsenic exposure from ingested foods usually comes from food crops grown in arsenic-contaminated soil and/or irrigated with arsenic-contaminated water. According to a recent World Health Organization report, arsenic from contaminated water can be quickly and easily absorbed and depending on its metabolic form, may adversely affect human health. Recently, the US Food and Drug Administration regulations for metals found in cosmetics to protect consumers against contaminations deemed deleterious to health; some cosmetics were found to contain a variety of chemicals including heavy metals, which are sometimes used as preservatives. Moreover, developing countries tend to have a growing number of industrial factories that unfortunately, harm the environment, especially in cities where industrial and vehicle emissions, as well as household activities, cause serious air pollution. Air is also an important source of arsenic exposure in areas with industrial activity

Garelick *et al.*, 2008. Arsenic is a widely dispersed element in the Earth's crust and exists at an average concentration of approximately 5 mg/kg. There are many possible routes of human exposure to arsenic from both natural and anthropogenic sources. Arsenic occurs as a constituent in more than 200 minerals, although it primarily exists as arsenopyrite and as a constituent in several other sulfide minerals. The introduction of arsenic into drinking water can occur as a result of its natural geological presence in local bedrock. Arsenic-containing bedrock formations of this sort are known in Bangladesh, West Bengal (India), and regions of China, and many cases of endemic contamination by arsenic with serious consequences to human health are known from these areas. Significant natural contamination of surface waters and soil can arise when arsenic-rich geothermal fluids come into contact with surface waters. When humans are implicated in causing or exacerbating arsenic pollution, the cause can almost always be traced to mining or mining-related activities. Arsenic exists in many oxidation states, with arsenic (III) and (V) being the most common forms. Similar to many metalloids, the prevalence of particular species of arsenic depends greatly on the pH and redox conditions of the matrix in which it exists. Speciation is also important in determining the toxicity of arsenic.

Garelick *et al.*, 2008. Arsenic is a group V element, together with nitrogen, phosphorus, antimony and bismuth. Its electronic configuration is [Ar]3d104s24p3; it has an atomic weight of 75 and commonly occurs naturally in two oxidation states, +5, +3, and, more rarely, in the 0 or –3 state. Arsenic is classified as a metalloid in that it has a chemical nature intermediate between that of metals and nonmetals. The environmental presence of arsenic derives from both natural and anthropogenic sources. Many natural processes contribute to environmental background concentrations of arsenic, including pedogenesis, dust storms, volcanic eruptions, geothermal/ hydrothermal activity, and forest fires. Arsenic is widely distributed and is present in the Earth's crust at an average abundance of about 5 mg/kg. It occurs naturally in more than 200 mineral forms, of which approximately 60% are arsenates, 20% sulfides and sulfosalts, with the remaining 20% comprising.

Wang *et al.*, 2006. The main natural arsenic sources are weathering and erosion of arsenic-containing rocks and soil, while tailings from historic and recent gold mine operations and wood preservative facilities are the principal anthropogenic sources. Across Canada, the 24-h average concentration of arsenic in the atmosphere is generally less than 0.3μg/m3. Arsenic concentrations in natural uncontaminated soil and sediments range from 4 to 150 mg/kg. In uncontaminated surface and ground waters, the arsenic concentration ranges from 0.001 to 0.005 mg/L. As a result of anthropogenic inputs, elevated arsenic levels, above ten to thousand times the Interim Maximum Acceptable Concentration (IMAC), have been reported in air, soil and sediment, surface water and groundwater, and biota in several regions. Most arsenic is of toxic inorganic forms. It is critical to recognize that such contamination imposes serious harmful effects on various aquatic and terrestrial organisms and human health ultimately. Serious incidences of acute and chronic arsenic poisonings have been revealed. Through examination of the available literature, screening and selecting existing data, this paper provides an analysis of the currently available information on recognized problem areas, and an overview of current knowledge of the principal hydrogeochemical processes of arsenic transportation and transformation. However, a more detailed understanding of local sources of arsenic and mechanisms of arsenic release is required. More extensive studies will be required for building practical guidance on avoiding and reducing arsenic contamination. Bioremediation and hyperaccumulation are emerging innovative technologies for the remediation of arsenic contaminated sites. Natural attenuation may be utilized as a potential in situ remedial option. Further investigations are needed to evaluate its applicability.

National Research Council, 1999. The Safe Drinking Water Act (SDWA) directs the U.S. Environmental Protection Agency (EPA) to establish national standards for contaminants in public drinking-water supplies. Enforceable standards are to be set at concentrations at which no adverse health effects in humans are expected to occur and for which there are adequate margins of safety. Enforceable standards are standards that can be achieved with the use of the best technology available. Arsenic is a naturally occurring element present in the environment in both inorganic and organic forms. Inorganic arsenic is considered to be the most toxic form of the element and is found in groundwater and surface water, as well as in many foods. A wide variety of adverse health effects, including skin and internal cancers and cardiovascular and neurological effects, have been attributed to chronic arsenic exposure, primarily from drinking water. EPA's interim maximum contaminant level (MCL) for arsenic in drinking water is 50 micrograms per liter (µg/L).

2.3 Metabolism of arsenic

Zuzana *et al.,* 2009. It is likely that at least some of the toxic and carcinogenic effects associated with exposure to inorganic arsenic are, in fact, due to actions of its methylated metabolites. Here, we provide an overview of current models for the biological methylation of arsenicals. This information provides a context for understanding the chemical, biochemical, and genetic approaches to elucidation of the formation and function of metahylated arsenicals which are presented in the following manuscripts.

Roy and Saha, 2002. Inorganic arsenic is considered the most potential human carcinogen, and humans are exposed to it from soil, water, air and food. In the process of arsenic metabolism, inorganic arsenic is methylated to monomethylarsonic acid and finally to dimethylarsinic acid, followed by excretion through urine. Thus, arsenic exposure may cause DNA hypomethylation due to continuous methyl depletion, facilitating aberrant gene expression that results in carcinogenesis. Further, though arsenic is nonmutagenic, it interacts synergistically with genotoxic agents in the production of mutations, and also induces chromosome abnormalities and cell proliferation. Few epidemiological investigations in the arsenic endemic regions of West Bengal (India) have established that inorganic arsenicals have the potential to cause skin and lung cancers in humans. Studies on the genetic polymorphism in the arsenic methyltransferase (s) with the population exposed to arsenic and characterization in the arsenic-induced mutational spectra may be useful for the development of molecular markers and therapeutics and for furthering the knowledge of arsenic-induced carcinogenesis.

Vahter and Concha, 2001. In humans, as in most mammalian species, inorganic arsenic is methylated to methylarsonic acid (MMA) and dimethylarsinic acid (DMA) by alternating reduction of pentavalent arsenic to trivalent and addition of a methyl group from S-adenosylmethionine. The methylation of inorganic arsenic may be considered a detoxification mechanism, as the end metabolites, MMA and DMA, are less reactive with tissue constituents, less toxic, and more readily excreted in the urine than is inorganic arsenic, especially the trivalent form (AsIII, arsenite). The latter is highly reactive with tissue components, due to its strong affinity for sulfhydryl groups. Thus, following exposure to AsV the first step in the biotransformation, i.e. the reduction to AsIII, may be considered a bioactivation. Also, reactive intermediate metabolites of high toxicity, mainly MMAIII, may be formed and distributed to tissues. Low levels of MMAIII and DMAIII have been detected in urine of individuals chronically exposed to inorganic arsenic via drinking water. However, the contribution of MMAIII and DMAIII to the toxicity observed after intake of inorganic arsenic by humans remains to be elucidated. The major route of excretion of arsenic is via the kidneys. Evaluation of the methylation of arsenic is mainly based on the relative amounts of the different metabolites in urine. On average human urine contains 10-30% inorganic arsenic, 10-20% MMA and 60-80% DMA.

2.4 Contamination of arsenic

Mazumder *et al.*, 2010. Out of 10469 participants examined, prevalence rate of arsenicosis was found to be 15.43%. Out of 0.84 million people suspected to be exposed to arsenic, 0.14 million people are estimated to be suffering from arsenicosis in the district. Highest level of arsenic in drinking water sources was found to be 1362 μg/l, and in 23% cases it was above 100 μg/l. Majority of the population living in the arsenic affected villages were of low socio-economic condition, inadequate education and were farmers or doing physical labour. Chronic lung disease was found in 207 (12.81%) subjects among cases and 69 (0.78%) in controls. Peripheral neuropathy was found in 257 (15.9%) cases and 136 (1.5%) controls.

Hossain, 2006. Bangladesh is currently facing a serious threat to public health, with 85 million people at risk from arsenic (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 10 μg L−1. 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 >10 μg L−1 and 43 had concentrations >50 μg L−1. 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.

2.5 Health risk for arsenic

Rasheed *et al.*, 2016. Millions of people are exposed to arsenic resulting in a range of health implications. This paper provides an up-to-date review of the different sources of arsenic (water, soil, and food), indicators of human exposure (biomarker assessment of hair, nail, urine, and blood), epidemiological and toxicological studies on carcinogenic and no carcinogenic health outcomes, and risk assessment approaches. The review demonstrates a need for more work evaluating the risks of different arsenic species such as, arsenate, arsenite monomethylarsonic acid, monomethylarsonous acid, dimethylarsinic acid, and dimethylarsinous acid as well as a need to better integrate the different exposure sources in risk assessments.

Shameem *et al.*, 2015. Arsenic (As) is ubiquitous in nature and humans being exposed to arsenic via atmospheric air, ground water and food sources are certain. Major sources of arsenic contamination could be either through geological or via anthropogenic activities. In physiological individuals, organ system is described as group of organs that transact collectively and associate with other systems for conventional body functions. Arsenic has been associated with persuading a variety of complications in body organ systems: integumentary, nervous, respiratory, cardiovascular, hematopoietic, immune, endocrine, hepatic, renal, reproductive system and development. In this review, we outline the effects of arsenic on the human body with a main focus on assorted organ systems with respective disease conditions. Additionally, underlying mechanisms of disease development in each organ system due to arsenic have also been explored. Strikingly, arsenic has been able to induce epigenetic changes (in utero) and genetic mutations (a leading cause of cancer) in the body. Occurrence of various arsenic induced health effects involving emerging areas such as epigenetic and cancer along with their respective mechanisms are also briefly discussed.

Hong *et al.*, 2014. Arsenic is a unique element with distinct physical characteristics and toxicity whose importance in public health is well recognized. The toxicity of arsenic varies across its different forms. While the carcinogenicity of arsenic has been confirmed, the mechanisms behind the diseases occurring after acute or chronic exposure to arsenic are not well understood. Inorganic arsenic has been confirmed as a human carcinogen that can induce skin, lung, and bladder cancer. There are also reports of its significant association to liver, prostate, and bladder cancer. Recent studies have also suggested a relationship with diabetes, neurological effects, cardiac disorders, and reproductive organs, but further studies are required to confirm these associations. The majority of research to date has examined cancer incidence after a high exposure to high concentrations of arsenic. However, numerous studies have reported various health effects caused by chronic exposure to low concentrations of arsenic. An assessment of the health effects to arsenic exposure has never been performed in the South Korean population; thus, objective estimates of exposure levels are needed. Data should be collected on the biological exposure level for the total arsenic concentration, and individual arsenic concentration by species. In South Korea, we believe that biological exposure assessment should be the first step, followed by regular health effect assessments.

Charles *et al.*, 2003. Humans can be exposed to arsenic (As) through the intake of air, food and water. Although food is usually the major source of As exposure for people, most adverse effects are seen after As exposure from drinking water. The two main reasons for this situation are that most food arsenicals are organic and have little or no toxicity, and in many cases, As exposures from drinking water sources are to the more toxic inorganic form and occur at relatively high doses, e.g., hundreds of micrograms per day. In various parts of the world, As in drinking water is associated with such effects as gastroenteritis, neurological manifestations, vascular changes, diabetes and cancers (bladder, lung, liver, kidney and prostate). After reviewing the As database, the

U.S. Environmental rotection Agency promulgated a maximum contaminant level for As in drinking water of 10 g/L. J. Nutr. 133: 1536S–1538S, 2003.

2.6 Composition of spirulina

Vicky Jocelyne Ama Moor1 *et al.*, 2016. The extraction yield obatined was 16.84%. The results showed that S platensis contains protein (375.5+0.7 g/kg dw), lipids (301.2+11.9 g/kg dw), carbohydrates (243.9+9.9g/kg dw) and fibers (313.2 g/kg dw). The HPLC profile revealed the presence of polyphenols (21.2 + 1.18 mg eq. QE/g Ext.), flavonoids (56.4 + 6. 47 mg eq. QE/g Ext.) and phenolic acid like caffeic and coumaric acidss. Iron was the most micronutrients found but we also found copper, manganese, zinc selenium. The percentage of phycocyanin was 16.15% while carotenoids were 3.8%.

Gabriela Gutierrez-Salmeanm *et al.*, 2015. Undernutrition constitutes a public health problem particularly in developing countries. The utilization of algae, particularly Spirulina, as a functional food was suggested decades ago due to the fact that it is not only a protein-dense food source, but because its amino acid profile is considered as of high biologic-value protein content. Spirulina provides essential fats (e.g., gamma-linolenic oleic acids), concomitant to low content nucleic acids. It also has an exceptionally high content of vitamin B12, is a good source of beta-carotene, iron, calcium and phosphorous. Moreover, Spirulina has also proven to have good acceptance as of its organoleptic properties (thus making it a possible rospect for food or a nutrition supplement) and it has not exhibited neither acute nor chronic toxicities, making it safe for human consumption.

Renata Ribeiro Alvarenga *et al.*, 2011. The objective of this study was to determine the chemical and energy composition of spirulina (Spirulina platensis), the nutrient metabolizability coefficients, and the values of apparent metabolizable energy (AME) and the apparent metabolizable energy corrected for nitrogen balance (AMEn) in broilers. A digestibility trial was carried out by using total excreta collection method, with 90 Cobb 500 lineage chicks, with initial weight of 256 ± 5 g at 11 days of age. Birds were allotted in metabolic cages for 10 days, distributed in a completely randomized design, with three treatments and six repetitions with five birds each. Diets consisted on a reference-ration based on corn and soybean meal and two test diets, one containing spirulina (30%) and the other one with soybean meal (30%). Spiruline was superior to soybean meal for contents of dry matter (DM), gross energy (9.60%), crude protein (26.56%), ether extract (54.45%), mineral matter (42.77%), calcium (100%) and total phosphorus (130.77%) and also for most amino acids, except lysine, glutamate, histidine and proline. Nevertheless, spiruline presented lower values of gross fiber (83.95%), acid detergent fiber (85.12%) and neutral detergent fiber (6.15). The AME and AMEn values (kcal/kg of DM) were, respectively, 2,906 and 2,502 for the spirulina and 2,646 and 2,340 for the soybean meal and AMEn of spirulina was 6.92% higher than soybean meal.

Babadzhanov *et al.*, 2004. Conditions for growing spirulina (*Spirulina platensis*) were developed. The amino-acid and carbohydrate compositions were determined. Lipids and vitamins of the culture biomass.

Ortega-Calvo *et al.*, 1993. Three *Spirulina* and five eukaryotic algal food products available in the Spanish market have been extensively studied. Results are given for their gross chemical composition (water content, crude protein, total carbohydrates, lipids, nucleic acids etc.) and contents of macrominerals, trace elements, fatty acids, amino acids and neutral sugars. The results are compared to those from other studies on natural or laboratory-produced populations. An overall nutritional and toxicological evaluation of these products is included.

2.6.1 Protein and amino acids

Marrez *et al.*, 2014. Under controlled conditions in four different culture media, BG-11, modified BG-11, Zarrouk´s (ZM) and synthetic human urine (SHU). The effect of culturing media on chemical composition, amino acids content, fatty acids profile and minerals content were determined. The highest amount of protein (59.8%) was recorded when grown in BG-11. Whereas, modified BG-11 was the best medium in regard to both amino acids contents and maximum total lipid (8.13%). The most important unsaturated fatty acid γ-linolenic, was found at maximum percentage (4.7%) when grown in SHU medium. Whereas ZM was the best medium to obtain the highest percentage of arachidoic acid (17.63%).The highest percentage of ash in S. platensis was recorded when grown in ZM. Regarding to the minerals content, the maximumP, Ca, Mg, Zn and Cu (182.7, 155.8, 8.4, 5.1 and 5.5 mg/100g DW, respectively) were recorded in BG-11, while growing in ZM displayed the highest amount of K, Na and Fe (593.4, 766.7 and 39.9 mg/100g, respectively).

Leyla *et al.*, 2009. The purpose of this study was to clarify the seasonal variation of protein content and amino acid composition of Spirulina platensis grown in summer and winter. During the study, while the light intensity, pH and salinity were measured daily, the temperature and dissolved oxygen were measured during daytime and at night. While the mean day temperatures were recorded as 33.9±0.4 ºC and 18.6±0.5 ºC, the mean night temperatures were found to be 29.9±0.2 ºC and 14.4±0.2 ºC in summer and winter, respectively. The mean light intensity of 848.3 µmol m-2s-1 was determined in summer. It was 506.26±48 µmol m-2s-1 in winter. The protein amount (72.9±03 %) and the amino acid concentrations of S. platensis grown in summer were found to be higher than in winter.

2.6.2 Vitamins

Tang and Suter, 2011. Spirulina, chlorella, and dunalliella are unicellular algae that are commercially produced worldwide. These algae are concentrated sources of carotenoids (especially provitamin A carotenoids) and other nutrients, such as vitamin B12. Their health benefits as a complementary dietary source for macro and micro nutrients have been studied and confirmed in various populations. The safety of human consuming these algae and products derived from these algae by humans has been widely studied. It is generally concluded that these algae and its products are safe if cultivated properly in a non-contaminated environment, and if consumed in moderation.

Karkos *et al.*, 2010. Spirulina or Arthrospira is a blue-green alga that became famous after it was successfully used by NASA as a dietary supplement for astronauts on space missions. It has the ability to modulate immune functions and exhibits anti-inflammatory properties by inhibiting the release of histamine by mast cells. Multiple studies investigating the efficacy and the potential clinical applications of Spirulina in treating several diseases have been performed and a few randomized controlled trials and systematic reviews suggest that this alga may improve several symptoms and may even have an anticancer, antiviral and antiallergic effects. Current and potential clinical applications, issues of safety, indications, side-effects and levels of evidence are addressed in this review. Areas of ongoing and future research are also discussed.

Habib *et al.*, 2008. Spirulina appears to have considerable potential for development, especially as a small-scale crop for nutritional enhancement, livelihood development and environmental mitigation. FAO fisheries statistics (FishStat) hint at the growing importance of this product. Production in China was first recorded at 19 080 tonnes in 2003 and rose sharply to 41 570 tonnes in 2004, worth around US$7.6 millions and US$16.6 millions, respectively. However, there are no apparent figures for production in the rest of the world. This suggests that despite the widespread publicity about spirulina and its benefits, it has not yet received the serious consideration it deserves as a potentially key crop in coastal and alkaline areas where traditional agriculture struggles, especially under the increasing influence of salination and water shortages.

2.6.3 Carbohydrates

Puspanadan *et al.*, 2018. Freshwater microalgae that contained high starch, arbohydrates and lipids contents are beneficial to human being in many industrial especially in food and food packing industries. There are a few freshwater microalgae that contained high starch and carbohydrates content such as Chlorella vulgaris, Chlorella emersonii, Chlorella sorokiniana, Nannochloropsis salina and Spirulina sp. Spirulina sp. is chosen in this research because of its strong adaptation to the environment changes, short life cycle and able to produce high intracellular starch. In this project, results have shown that microalgae at late exponential phase contained the highest starch and carbohydrate yield of 0.491±0.046 mg/L and 0.090±0.046 mg/L, respectively. Thus, the harvesting time for microalgae with high starch production and yield is phase dependent rather than time dependent. Under optimized cultivation conditions of aeration at 5 L/min, using white wavelength and 32ºC, Spirulina sp. produced the highest starch and carbohydrate yield of 0.664±0.03 mg/L, and 1.019±0.025 mg/L, respectively. In conclusion, compared to before optimization, starch and carbohydrate yield after optimizations were increased. In conclusion, compared to before optimization, starch and carbohydrate yield after optimization were 435% and 221% increased, respectively.

Kent *et al.*, 2015. This study investigated the biochemical suitability of Australian native microalgal species Scenedesmus sp., Nannochloropsis sp., Dunaliella sp., and a chlorophytic polyculture as nutritional supplements for human health. The four microalgal cultures were harvested during exponential growth, lyophilized, and analysed for proximate composition (moisture, ash, lipid, carbohydrates, and protein), pigments, and amino acid and fatty acid profiles. The resulting nutritional value, based on biochemical composition, was compared to commercial Spirulina and Chlorella products. The Australian native microalgae exhibited similar, and in several cases superior, organic nutritional properties relative to the assessed commercial products, with biochemical profiles rich in high-quality protein, nutritious polyunsaturated fats (such as α-linolenic acid, arachidonic acid, and eicosapentaenoic acid), and antioxidant pigments. These findings indicate that the microalgae assessed have great potential as multi-nutrient human health supplements.

Markou *et al.*, 2013. In the present study the potential of bioethanol production using carbohydrate-enriched biomass of the cyanobacterium Arthrospira platensis was studied. For the accharification of the carbohydrate-enriched biomass, four acids (H2SO4, HNO3, HCl and H3PO4) were investigated. Each acid were used at four concentrations, 2.5 N, 1 N, 0.5 N and 0.25 N, and for each acid concentration the saccharification was conducted under four temperatures (40 °C, 60 °C, 80 °C and 100 °C). Higher acid concentrations gave in general higher reducing sugars (RS) yields (%, gRS/gTotal sugars) with higher rates, while the increase in temperature lead to higher rates at lower acid concentration. The hydrolysates then were used as substrate for ethanolic fermentation by a salt stress-adapted Saccharomyces cerevisiae strain. The bioethanol yield (%, gEtOH/gBiomass) was significantly affected by the acid concentration used for the saccharification of the carbohydrates. The highest bioethanol yields of 16.32% ± 0.90% (gEtOH/gBiomass) and 16.27% ± 0.97% (gEtOH/gBiomass) were obtained in hydrolysates produced with HNO3 0.5 N and H2SO4 0.5 N, respectively.

Karanth and Madaiah, 2011. Calothrix fusca, Gloeocapsa livida, Lyngbya limnetica and Scytonema bohneri isolated from Panekal sulfur spring. The species namely, Oscillatoria acuminata from petrochemical refinery, O. calcuttensis from dairy effluent and O. foreaui from a sewage drain located in the Western Ghats of Southern India under laboratory culture conditions. The biochemical constituents were analyzed in terms of total carbohydrates, total protein, total free amino acid, total lipid, fatty acid and mineral contents. The analysis showed that maximum amount of total carbohydrate in S. bohneri (28.4% dry weight) and minimum in O. foreaui (8.0% of dry weight). Maximum amount of total protein and total free amino acid were in O. foreaui (7% of dry weight).

O. calcuttensis showed higher amount of total lipid (20% dry weight). A total of 12 types of fatty acids were detected among which lauric acid was in highest quantity in all the seven species. Among the polyunsaturated fatty acids, oleic acid was present in all the species ranging from 1.68 to 3.89%. O. foreaui showed highest quantities of copper, manganese, ferrous and zinc. Nickel was maximum in S. bohneri (11.05 µgmL-1). O. acuminata showed highest quantity of magnesium (21.050 mg g-1) and it was least in O. foreaui (12.812 mg g-1).

2.6.4 Lipids

Liu *et al.*, 2018. Microalgae are one of the most promising feedstocks for biodiesel production due to their high lipid content and easy farming. However, the extraction of lipids from microalgae is energy intensive and costly and involves the use of toxic organic solvents. Compared with organic solvent extraction, supercritical CO2 (SCCO2) has demonstrated advantages through lower toxicity and no solvent-liquid separation. Due to the nonpolar nature of SCCO2, polar organic solvents such as methanol may need to be added as a modifier in order to increase the extraction ability of SCCO2. In this paper, pilot scale lipid extraction using SCCO2 was studied on two microalgae species: Spirulina sp. and Schizochytrium sp. For each species, SCCO2 extraction was conducted on 200 g of biomass for 6 h. Methanol was added as a cosolvent in the extraction process based on a volume ratio of 4%. The results showed that adding methanol in SCCO2 increased the lipid extraction yield significantly for both species. Under an operating pressure of 4000 psi, the lipid extraction yields for Spirulina sp. and Schizochytrium sp. were increased by 80% and 72%, respectively. It was also found that a stepwise addition of methanol was more effective than a one-time addition. In comparison with Soxhlet extraction using methylene chloride/methanol (2:1, v/v), the methanol-SCCO2 extraction demonstrated its high effectiveness for lipid extraction. In addition, the methanol-SCCO2 system showed a high lipid extraction yield after increasing biomass loading fivefold, indicating good potential for scaling up this method. Finally, a kinetic study of the SCCO2 extraction process was conducted, and the results showed that methanol concentration in SCCO2 has the strongest influence on the lipid extraction yield.

Elisabete da Costa *et al.*, 2016. In recent years, noteworthy research has been performed around lipids from microalgae. Among lipids, glycolipids (GLs) are quite abundant in microalgae and are considered an important source of fatty acids (FAs). GLs are rich in 16- and 18-carbon saturated and unsaturated fatty acids and often contain polyunsaturated fatty acids (PUFAs) like *n*-3 α-linolenic (ALA 18:3), eicosapentaenoic (EPA, 20:5) and docosahexaenoic (DHA, 22:6). GLs comprise three major classes: monogalactosyldiacyl glycerolipids (MGDGs), digalactosyl diacylglycerolipids (DGDGs) and sulfoquinovosyl diacylglycerolipids (SQDGs), whose composition in FA directly depends on the growth conditions. Some of these lipids are high value-added compounds with antitumoral, antimicrobial and anti-inflammatory activities and also with important nutritional significance. To fully explore GLs' bioactive properties it is necessary to fully characterize their structure and to understand the relation between the structure and their biological properties, which can be addressed using modern mass spectrometry (MS)-based lipidomic approaches. This review will focus on the up-to-date FA composition of GLs identified by MS-based lipidomics and their potential as phytochemicals.

Nugraha *et al.*, 2015. In this study, addition of urea was varied in the growth medium of Spirulina platensis. Effect of urea addition was observed on growth of Spirulina platensis. It was used for production of lipids and omega-3 fatty acids. Medium at concentration of 0.200 g/L of urea gave the best growth of Spirulina platensis in the culture. Growth characteristics showed that the generation time, growth rate, and productivity occurred in culture with 0.200 g/L of urea were 2.767, 0.226, and 0.099, respectively. Biomass of Spirulina platensis was harvested at the exponential phase and the lipid content was extracted method of solvent extraction method. The lipid content of Spirulina platensis biomass was 12.92%. Gas Chromatography Mass Spectroscopy (GC-MS) analysis lipid of Spirulina platensis contained 3.03% of eicosapentaenoic methyl ester as one of the omega-3 fatty acids.

Chaiklahan *et al.*, 2008. Lipid extraction from Spirulina using a single stage extraction at 30 °C showed that a sample-solvent ratio of 1:10 and an extraction time of 120 min gave the highest total fatty acid (TFA) yield, whereas a ratio of 1:5 was suitable for a multistage extraction. An increase in extraction temperature resulted in higher lipid and TFA yields. Increasing the extraction temperature from 30 °C to 60 °C decreased the extraction time and increased the yield of lipid and TFA in the solvent by approximately 69% and 55%, respectively. In order to increase the extraction yield of the lipid and TFA, multistage cross-current extraction was implemented. Seven-stage extraction at 30 °C resulted in 85% TFA recovery, whereas only a three-stage extraction was needed to obtain the same level of TFA at 60 °C. Lipid extracted from Spirulina contained approximately 21% linoleic acid and 18% γ-linolenic acid.

2.6.5 Minerals

Suliburska, *et al.*, 2016. The effects of Spirulina maxima supplementation on calcium, magnesium, iron, and zinc status were studied in a double-blind placebo-controlled trial of 50 obese subjects with treated hypertension, each randomized to receive 2 g of spirulina or a placebo daily for 3 months. At baseline and after treatment, the calcium, magnesium, iron, and zinc concentration in plasma was assessed. It was found that 3 months of S. maxima supplementation resulted in a significant decrease in the iron level in the plasma of obese patients. In conclusion, this is the first clinical study on the influence of spirulina supplementation on mineral status in obese patients with hypertension. Spirulina supplementation affects the iron status of obese Caucasians with well-treated hypertension.

Rosario and Josephine, 2015. Spirulina platensis, is a photosynthetic, filamentous, spiral-shaped, multicellular and blue- green micro alga. As it contains chlorophyll a, like higher plants, botanists classify it as a micro alga belonging to Cyanophyceae class; but according to bacteriologists it is a bacterium due to its prokaryotic structure. Mexicans (Aztecs) started using this microorganism as human food. Its chemical composition includes proteins (55%-70%), carbohydrates (15%-25%), essential fatty acids (18%), vitamins, minerals and pigments like carotenes, chlorophyll a and phycocyanin. Pigments are used in food and cosmetic industries. The extracts of Spirulina could prevent or inhibit cancer in humans and animals and has immunopromoting effects. It is the most important commercial micro alga for the production of biomass as health, food and animal. In the present study, the mineral profile of Spirulina platensis was analysed. The results conclude that among minerals, potassium has the maximum composition followed by phosphorous, calcium, magnesium, iron and sodium. Based on the results, Spirulina supplementations proved with a larger evidence and based on scientific validation studies has been accepted by global accreditation as a safe nutritional and dietary supplement.

2.6.6 Pigments

Park *et al.*, 2018. Arthrospira platensis is the widely available source of spirulina that contains distinctive natural pigments, including carotenoids and C-phycocyanin (C-PC). In this study, the major carotenoid and C-PC contents were determined in seven commercially available spirulina powder products and laboratory-prepared A. platensis trichomes (AP-1) by an LC-DAD method andUV-Visible spectrometry, respectively. The correlation of these two pigment content levels with Hunter color coordinates and antioxidant activity was also evaluated. The L value failed to show a significant correlation with pigment content, but a positive correlation was observed between values and the contents of total carotenoid and C-PC. As b* values decreased, the chlorophyll a and C-PC contents increased. AP-1 exhibited the highest content of total carotenoids, chlorophyll a and C-PC, and antioxidant activities among the samples. This observation could be related to degradation of these pigments during the mass production process. The carotenoid profiles suggested that the commercial spirulina powders originated from two different sources, A. platensis and A. maxima. Total carotenoid and C-PC content exhibited positive significant correlations with antioxidant activities measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,20-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays. These results provide a strong scientific foundation for the establishment of standards for the commercial distribution of quality spirulina products.

Diaa *et al.*, 2013. Spirulina platensis is one of the most important microalgae that have high content of valuable pigments. In order to select the best medium for the biomass production of S. platensis and consequently pigments content; BG-11, modified BG-11, Zarrouk´s and SHU media were investigated. The maximum dry weight (4.87g l-1) and filaments count (8.8 x107filament ml-1)were estimated when using Zarrouk´s medium, whereas modified BG-11 medium gave the maximum content of chlorophyll (147.43µg ml-1) and carotenoids (139.88µg ml-1). Regarding to phycobiliprotein pigments, S. platensis produced the highest amount of phycocyanin (55.37 µg ml-1) and allophycocyanin (51.73µg ml-1) in modified BG-11 medium, while the maximum content of phycoerythrin (44.13µg ml-1) was observed in SHU medium.

2.7 Treatment with Spirulina

Dolatabadi and Hosseini, 2015. In this study, we examine the impact of Spirulina platensis growth on water treatment and its softening. TDS (total dissolved solids) was tested by gravimetric method. BOD (biological oxygen demand) was performed to measure the organic compound in water by Wrinkler's method. COD (chemical oxygen demand) was performed using potassium dichromate as a strong oxidative agent. Results have shown 50% improvement in reducing TDS, 82% for BOD5, 50% in case of COD, and 72% in terms of total hardness. The process of biosorption of organic and inorganic compounds in hard water by blue–green algae Spirulina platensis is discussed in this paper. Spirulina sp. was found to be a very efficient biosorbent.

Nagori and Sharma, 2014. Our skin is our body's largest organ and barrier from the outside world. When our skin is constantly exposed to pollutants and UV damaging rays over an extended period of time, it becomes more vulnerable to free radical damage. Excessive free radical exposure through a toxic diet and environmental pollutants harm the skin by damaging DNA and cell membranes that keep the skin plump and firm. As we all know, some of our illnesses are caused by having insufficient nutrients in our body. These illnesses are just the symptoms to show us that we may be lacking in some nutrients. If we replenish these nutrients in time, the symptoms usually disappear With over 100 nutrients, Spirulina is often described as the most complete food source in the world. The American National Aeronautical and Space Agency includes it in their astronauts diet and plans to grow Spirulina in its space station. Japan has some good examples of some Japanese seniors who have only relied on Spirulina and water for more than 20 years showing how good is Spirulina for the human body. Spirulina is effective in treating dark circles dry eye symptoms, detoxifies skin, dandruff, aging skin, rinkle and hairfall.

Nuhu, 2013. Cyanobacteria are aquatic and photosynthetic organisms known for their rich pigments. They are extensively employed as food supplements due to their rich contents of proteins. While many species, such as Anabaena sp., produce hepatotoxins (e.g., microcystins and nodularins) and neurotoxins (such as anatoxin a), Spirulina (Arthrospira) displays anticancer and antimicrobial (antibacterial, antifungal, and antiviral) activities via the production of phycocyanin, phycocyanobilin, allophycocyanin, and other valuable products. This paper is an effort to collect these nutritional and medicinal applications of Arthrospira in an easily accessible essay from the vast literature on cyanobacteria.

Hoseini *et al.*, 2013. Spirulina spp. and its processing products are employed in agriculture, food industry, pharmaceutics, perfumery and medicine. Spirulina has several pharmacological activities such as antimicrobial (including antiviral and antibacterial), anticancer, metalloprotective (prevention of heavy-metal poisoning against Cd, Pb, Fe, Hg), as well as immunostimulant and antioxidant effects due to its rich content of protein, polysaccharide, lipid, essential amino and fatty acids, dietary minerals and vitamins. This article serves as an overview, introducing the basic biochemical composition of this algae and moves to its medical applications. For each application the basic description of disease, mechanism of damage, particular content of Spirulina spp. for treatment, in vivo and/or in vitro usage, factors associated with therapeutic role, problems encountered and advantages are given.

El-Baz *et al.*, 2013. Spirulina platensis are filamentous, undifferentiated, non-toxigenic cyanobacteria that have been used as food since ancient times. There have been numerous studies on its antioxidant and antimicrobial actions. In this study antibacterial and antiviral effect of ethanol extract of Spirulina platensis were tested. The reduction of infectious viral units after treatment with ethanol extract of Spirulina platensis was tested. Non toxic doses of Spirulina platensis revealed 53.3%, 66.7%, 76.7%, 56.7%, and 50% reductions in vitro for infectious units of adenovirus type 7, Coxsackievirus B4, astrovirus type 1, rotavirus Wa strain, and adenovirus type 40 respectively. Using disc diffusion method to show the antibacterial effect of ethanol extract of Spirulina platensis against different bacterial strains (Escherichia coli, Staphylococcus aureus, Salmonella typhi, and Enterococcus faecalis) in addition to Candida albicans, inhibition zones were observed with Enterococcus faecalis and Candida albicans.

2.8 Beneficial effect of Garlic

Mulrow *et al.*, 2017. A total of 131 studies were included in the review: 45 cardiovascular trials, 13 cancer survey, case-control or cohort studies, and 73 studies of adverse effects. The total number of participants included in the review was not given. Compared with placebo, garlic preparations reduced the total cholesterol at one and 3 months: the mean reductions ranged from 1.2 to 17.3 mg/dL, and from 12.4 to 25.4

mg/dL, respectively (37 trials). No trial with 6 month outcomes (6 RCTs) found a significant reduction. Twenty-seven small RCTs reported some beneficial effect of garlic on blood-pressure, but this was not always statistically significant. Twelve small RCTs found no clinically significant effect of garlic on blood glucose in diabetics or non-diabetics. Two further RCTs found no significant effect on insulin or C peptide levels. Ten RCTs reported promising findings for the effects of garlic on platelet aggregation, but these were limited by their small size and short duration. There was insufficient evidence to support or refute a beneficial effect of garlic on clinical outcomes such as myocardial infarction or claudication. The very limited data available, mainly from case-control studies, suggested that garlic may have beneficial effect on the odds of laryngeal, gastric, colorectal and endometrial cancer and adenomatous colorectal polyps.

Khan *et al.*, 2012. Poultry researchers and nutritionists are looking for viable alternative feed additives since conventional supplements have been criticised for their potential negative impact on the food chain. Among the currently available poultry feed additives, natural herbs and plants have been widely advocated due to their reported widespread beneficial effects. Garlic (Allium sativum) is one such potential feed supplement which has recently been reported as having a wide range of beneficial effects on the production performance and physiological biochemistry of broilers and laying hens. Notable beneficial effects have been seen on growth, feed efficiency, egg production and quality, as well as stimulation of immune system and lowering blood cholesterol levels in poultry birds. The results reported vary from author to author probably due to variations in the dose of the product fed, the duration of feeding and processing techniques employed.

Mannucci *et al.*, 2011. The use of non-conventional medicines, especially herbal medicine, is common in patients with cancers including haematologic malignancies. Diet components may also modify the risk of cancer through the influence on multiple processes, including DNA repair, cell proliferation and apoptosis. Garlic (Allium sativum), considered either food or herbal medicine, possesses antimutagenic and antiproliferative properties that can be used in anticancer interventions. We analyzed literature data on effects of garlic and garlic compounds which can serve as basic information to design clinical approach in oncohematology. Garlic contains water soluble and oil-soluble sulfur compounds. The latter are responsible for anticancer effects exerted through multiple mechanisms such as: inhibition of metabolic carcinogenic activation, arrest of cell cycle, antioxidant and pro-apoptotic action. Evidence about the effects of main sulfur compounds diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), ajoene and S-allylmercaptocysteine (SAMC) in oncohematology was described. Our research highlights that data on garlic in oncohematology are essentially represented by pre-clinical studies. Although these studies must be considered as preliminary, they provided insight into biological activities of garlic compounds and support a rationale for the use of substances such as DAS, DADS, DATS and ajoene as promising anticancer agents in oncohematology.

Corzo-Martínez *et al.*, 2007. Garlic (*Allium sativum*) and onion (*Allium cepa*) are two food ingredients widely used in our gastronomy. Moreover, garlic and onion extracts have been recently reported to be effective in cardiovascular disease, because of their hypocholesterolemic, hypolipidemic, anti-hypertensive, anti-diabetic, antithrombotic and anti-hyperhomocysteinemia effects, and to possess many other biological activities including antimicrobial, antioxidant, anticarcinogenic, antimutagenic, antiasthmatic, immunomodulatory and prebiotic activities. Given the importance of these vegetables and derived supplements as much in feeding as in therapeutic, in the present work, their main biological activities have been reviewed, indicating the compounds responsible for each one of them. In addition, the influence of the processing on the bioactivity and the adverse effects and interactions with different medications have also been considered.

Baluchnejadmojarad *et al.*, 2003. The present study evaluated the beneficial effect of aqueous extract of garlic (Allium sativum L.; 100 mg/kg/day) on the alterations in vascular reactivity of streptozotocin-diabetic quail. After 8 weeks of treatment, thoracic aortic rings of quail were mounted in organ baths and contractile responses to phenylephrine and relaxant responses to acetylcholine and isosorbide dinitrate were assessed. Induction of diabetes significantly increased contractile responses to phenylephrine and impaired endothelium-dependent relaxations to acetylcholine in aortic rings, but did not change endothelium-independent relaxation to isosorbide dinitrate. Garlic administration significantly improved the impaired endothelium-dependent relaxations and decreased the enhanced contractile response to phenylephrine in diabetic quail. It is concluded that intraperitoneal administration of aqueous garlic extract can improve endothelial dysfunction in insulin-dependent model of uncontrolled diabetes.

Harris *et al.*, 2001. Although garlic has been used for its medicinal properties for thousands of years, investigations into its mode of action are relatively recent. Garlic has a wide spectrum of actions; not only is it antibacterial, antiviral, antifungal and antiprotozoal, but it also has beneficial effects on the cardiovascular and immune systems. Resurgence in the use of natural herbal alternatives has brought the use of medicinal plants to the forefront of pharmacological investigations, and many new drugs are being discovered. This review aims to address the historical use of garlic and its sulfur chemistry, and to provide a basis for further research into its antimicrobial properties.

CHAPTER-III

MATERIALS AND METHODS

This experiment was conducted during the period between 1st Nonember to 30th December 2018 at the animal shed under the Department of Physiology and Pharmacology, Faculty of Veterinary and Animal Science, in Hajee Mohammad Danesh Science and Technology University, Dinajpur.

3.1 Experimental site

The laboratory animal house at the Department of Physiology and Pharmacology was the experimental site.

3.2 Layout of experiment

The layout of the experiment is presented below:

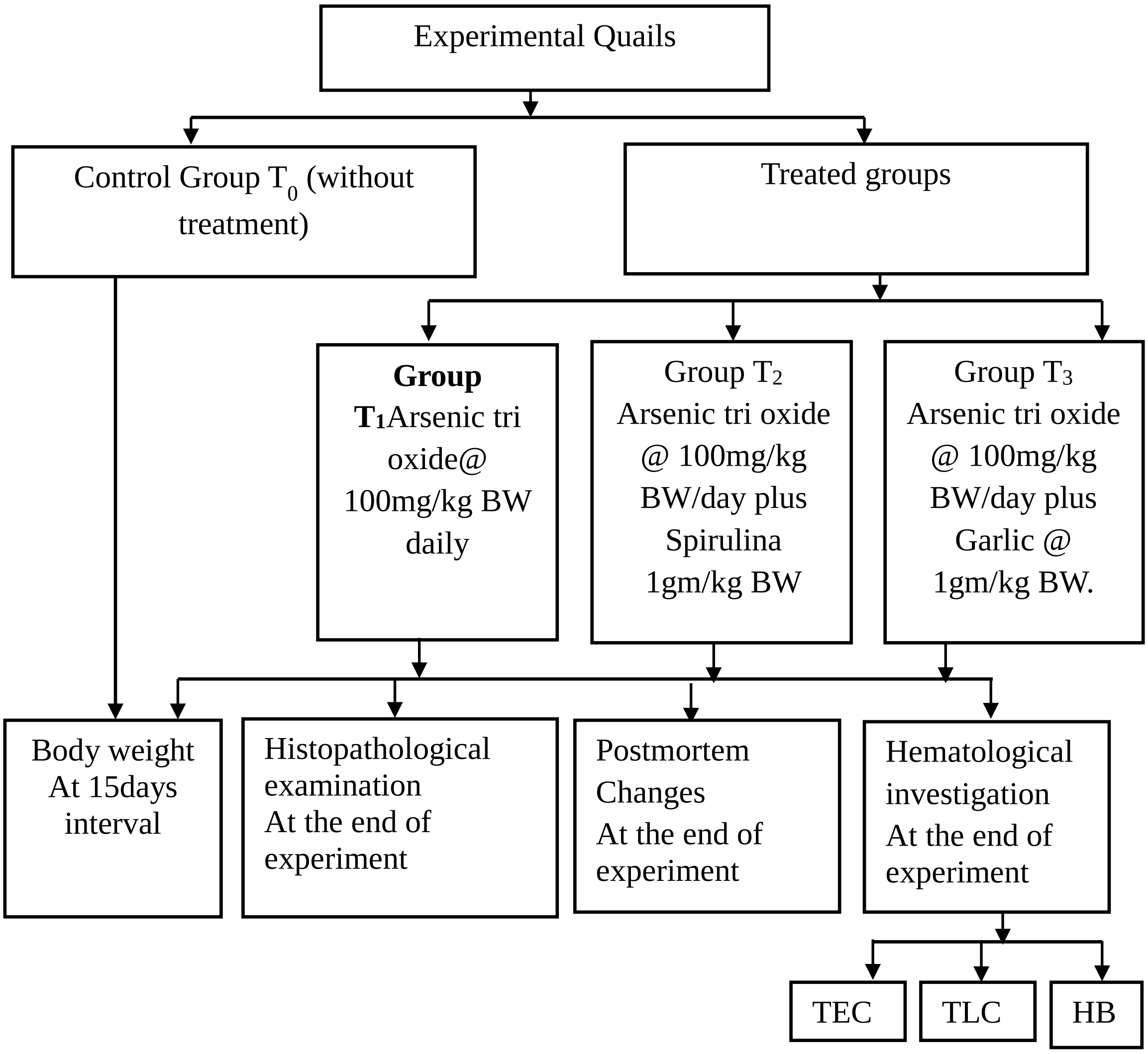

Figure 1. Layout of the experiment

3.3 Experimental quail

Sixty quails of same of age were used in this experiment which were randomly grouped into 4 groups and each group was consist of 15 quails.

Figure 2: Experimental quail

3.4 Preparation of house

At first the room as well as wire cages were washed by sweeping and washing with tap water using hose pipe connected with tap. The room was disinfected with a phenolic disinfectant and allowed to dry the room leaving unused with the electric fan and the bulb switched on. Proper ventilation was provided.

3.5 Test Chemicals

Arsenic trioxide (JKP Speciality Chemicals India Private Limitated, Navi Mumbai) was purchased from a scientific laboratory. Spirulina capsule (Navit®) was collected from Square Pharmaceuticals Limited Dhaka, Bangladesh and garlic was collected from local market.

3.6 Experimental animal grouping

Day old forty quails were collected for this investigation. These quails were divided into four groups containing 10 quails in each group. The groups were designed maintained as follows:

- Group T_0 (Control) was allowed to take normal feed and water.
- Group T_1 (Arsenic) treated with Arsenic tri oxide @ 100 mg / Kg body weight / day in drinking water.
- Group T_2 (Arsenic + Spirulina) treated with Arsenic tri oxide @ 100 mg / Kg body weight plus Spirulina @ 1gm / Kg BW in feed.

- Group T_3 (Arsenic + Garlic) treated with Arsenic tri oxide @ 100 mg / Kg body weight plus garlic 1gm/kg of feed ad-libitum.

Figure 3. Arsenic tri oxide solution

Figure 4. Feed with Spirulina.

Figure 5: Feed with garlic

3.7 Body Weight (BW)

The quails were individually weighed on Day 30 (Day 30= immediate previous day of starting treatment) after grouping and marking, Day 45 and finally on Day 60 and the results were recorded.

Figure 6: Measuring Body weight

3.8 Experimental trial

The experimental trial was conducted for 60 days. quails of Group T_0 were maintained with only normal feed and water *adlibitum* as control, that of Group T_1 were treated with arsenic trioxide at a dose of 100mg/L drinking water. The quails of Group T_2 were treated with arsenic trioxide at 100mg/L in drinking water daily and spirulina (*Spirulina platensis*) simultaneously at a dose of 1 gm/kg feed. The spirulina (Navit®) used in this experiment was collected from Square Pharmaceuticals Limited; as a capsule form. The quails of Group T_3 were treated with arsenic trioxide at 100mg/L in drinking water daily and garlic 1gm/kg of feed ad-libitum. All treatments were given for 60 days.

3.9 Preparation of treatment materials

3.9.1 Arsenic trioxide solution

On the basis of the total body weight of the quails, the required amount of arsenic trioxide for a day (100mg/L drinking water) was weighted separately for each group of quails. The respective pre-weighed arsenic trioxide was mixed with the drinking water daily for that particular group. Generally, 10ml drinking water per quail was allotted for mixing arsenic trioxide to make sure that the full amount of arsenic trioxide was taken by the quail. After finishing the drinking of the arsenic trioxide mixed water, normal drinking water was supplemented *ad libitum.*

3.9.2 Spirulina mixed feed

Each capsule of Spirulina (Navit®; Square Pharmaceuticals Limited, Bangladesh) containing 500mg of *Spirulina platensis*. The powder of spirulina was kept in a cup after opening from the capsule. The required amount of spirulina (1gm/kg feed) was measured with the help of electric balance. The powdered spirulina was kept in desiccators to prevent water absorption and change in quality of the powder. For proper homogenous mixing, small amount of distilled water was added to the pre-weighed spirulina powder to make it a suspension and then the suspension was added drop by drop to the feed and simultaneously the feed was stirred by a glass rod for homogenous mixing. As the feed was dried pellet, the spirulina was adhered on the pellets. After finishing the spirulina mixing, feed was dried in an electric oven at 50°C overnight and kept in air-tied plastic container then supplied to quails *ad libitum*.

Figure 7: Spirulina capsule

3.9.3 Garlic mixed feed

Garlic was cut into small pieces and mixed properly with feed. After completion of proper mixing, the mixed feed was provided to quail.

3.9.4 Collection and examination of blood

1ml blood from each group was collected from sacrificing of quail. The 1st blood sample was collected after the commencement of treatments (45days) then the end of the experiment (60days). The collected blood was sent to physiology and pharmacology laboratory, HSTU, Dinajpur, hematological parameters such as TLC (Total Leucocytes Count), TEC (Total Erythrocyte count), Hemoglobin percentage, PCV (Packed Cell Volume) and ESR (blood parameters were determined by semi automatic hematological analyzer machine (Cure inc. USA)

Figure 8: Collection of blood

3.10 Collection of viscera

At the end of the experiment, birds for each treatment group were randomly selected and slaughtered after 12-h of fasting to collect the viscera (liver, kidney, lung) and muscle samples and to find the gross changes of those organs.

3.11 Histopathology of sample

3.11.1 Collection

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

3.11.2 Fixation

Pieces of organs was 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 was 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 .9: Fixing in Formalin

3.11.3 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.

3.11.4 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.10 Block ready for microtomy

3.11.5 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 (Leica RM 2135, Germany). 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.

Figure.11: Sectioning by microtome

3.11.6 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. Steps are:

Clearing slide
↓
Soaking the slides with adhesive
↓
Attaching
↓
Drying

3.11.7 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.

Different steps followed in staining the tissue sections with H&E stain:

Placing slides containing paraffin sections on a slide holder.
↓
Deparaffinized with Xylene for 20 – 30 minutes and blot the excess xylene.
↓
Rehydrating the tissue successively with 100%, 90% and 80% isopropyl alcohol for 2 – 3 min. each and put it into water for 3 min.
↓
Blotting the excess water; putting the tissue into Hematoxylin stain for 1 – 2 min.
↓
Removing it from Hematoxylin stain and then again put it into tap water for 1 – 2 min.
↓
The slides containing tissue sections were dipping few times (3-10 times) into Lithium Carbonate.
↓
Immersing the tissue in Eosin stain for 30 secs.
↓
Dehydrating the tissue successively with 80%, 90%, 100% isopropyl alcohol and finally with Xylene for 20 – 30 min.

Figure.12: Different staining reagent

3.11.8 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.

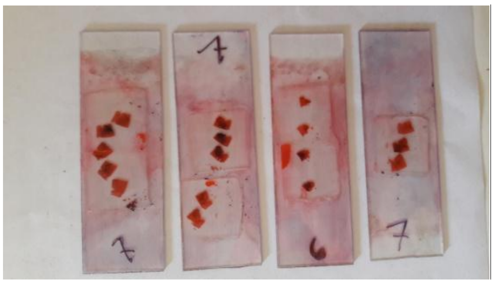

Figure.13: Cover Slipping

Figure.14: Observation under microscope

3.12 Statistical analysis.

Data were expressed as mean ± standard error (SE) and analyzed using one way analysis of variance (ANOVA) followed by Duncans 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 garlic on arsenic toxicity in quails. It was also undertaken to observe the effects of spirulina and garlic on body weight, hematological and biochemical parameters in arsenic fed quails. Forty 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 garlic simultaneously at a dose of 1gm/kg bodyweight. All the treatment were continued for 60 days and treated quails were closely observed through the entire period.

4.1 Body weight (BW) of the quails

Body weights (BWs) of experimental 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 (130.10 ± 1.03^d) in T_0 group quails at 60 days but the body weight gain was lowest (96.60 ± 0.62^a) in arsenic treated T_1 group at 60 days whereas body weight gain in T_2, T_3 were 116.50 ± 0.91^c, 107.50 ± 0.56^b which were better than arsenic treated T_1 group. The body weight of all groups were not significant initially ($p > 0.05$) but in 30 days, 45 days and 60 days mean value of body weight were significant ($p<0.01$).

The body weight of treated group were increased with their age but in T_1 group it decreased compared to other groups. In the present study arsenic reduced the body weight with their increasing age. The highest body weight gain was found in T_0 group where fed with normal feed and water. It recommends that spirulina act against arsenic in decreasing body weight. Sharma *et al.* (2007) reported that decreased body weight was observed in arsenic treated group of Swiss albino mice. Jun *et al.* (2008) who reported as significantly ($p<0.01$) decreases the body weight of rats.

Table 1: Effects of spirulina and garlic on the body weight of quails.

Days	T_o	T_1	T_2	T_3	P value
30	45.30˚± 0.76	43.50± 0.91	43.90 ±0.90	44.90± 0.82	NS
45	84.60 ± 0.76^c	64.50 ± 0.69^a	80.80 ± 0.74^b	79.70 ± 0.67^b	**
60	130.10 ± 1.03^d	96.60 ± 0.62^a	116.50 ± 0.91^c	107.50 ± 0.56^b	**

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

** = Significant at p<0.01 level of probability

*= Significant at p<0.05 level of probability

4.2 Hematological parameter

4.2.1 Total Erythrocyte Count (TEC)

In Table 2, Total Erythrocyte Count (TEC) values were highest ($3.00 \pm 0.006^d \times 10^{12}$) found in T_2 group at 60 days where spirulina was treated against arsenic toxicity but lowest ($2.87 \pm 0.003^a \times 10^{12}$) value was found in T_1 group where only arsenic were given. TEC value found at 45 days and 60 days were significant (p<0.05).

Table 2: Effects of Spirulina and garlic on Total Erythrocyte Count (TEC) values of quails.

Days	T_O	T_1	T_2	T_3	P value
45 days	$2.93 \pm 0.003^b \times 10^{12}$	$2.85 \pm 0.003^a \times 10^{12}$	$2.97 \pm 0.003^d \times 10^{12}$	$2.95 \pm 0.003^c \times 10^{12}$	**
60 days	$2.95 \pm 0.003^b \times 10^{12}$	$2.87 \pm 0.003^a \times 10^{12}$	$3.00 \pm 0.006^d \times 10^{12}$	$2.97 \pm 0.006^c \times 10^{12}$	**

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

** = Significant at p<0.01 level of probability

*= Significant at p<0.05 level of probability

4.2.2 Total Leukocyte Count (TLC):

In Table 3, Total leukocyte counts on Day 60 was found highest ($258.23 \pm 0.15^c \times 10^9$) in control group quails and lowest in T_2 group quails where sprulina was treated and the difference were statistically significant among all group of quails (p<0.01). So it can be recommended that sprulina decrease the TLC level.

Table 3: Effects of spirulina and garlic on Total Leukocyte Count (TLC) values of quails

Treatment	T_0	T_1	T_2	T_3	P. Value
45 Days	$22.73 \pm 0.15 \times 10^9$	$19.80 \pm 0.32 \times 10^9$	$23.20 \pm 0.21 \times 10^9$	$22.70 \pm 0.34 \times 10^9$	NS
60 Days	$24.71 \pm 0.24 \times 10^9$	$21.77 \pm 0.56 \times 10^9$	$25.04 \pm 0.15 \times 10^9$	$24.53 \pm 0.32 \times 10^9$	NS

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

** = Significant at $p<0.01$ level of probability

*= Significant at $p<0.05$ level of probability

4.2.3 Hemoglobin (Hb):

Highest (20.34 ± 0.17^c) Hb concentration was found in T_2 group at 60 days and lowest concentration was found in T_0 group (Table 4). Difference among values of 60 days of Hb concentration were statistically significant ($p<0.01$) and the difference among values of 45 and 60 days of Hb concentration were statistically significant ($p<0.05$). It might be concluded that Spirulina might slightly increase the values of Hb against arsenic toxicity in quails.

Table 4: Effects of spirulina and garlic on Hemoglobin concentration (Hb) (gm/dl) values of quails.

Treatment	T_0	T_1	T_2	T_3	P. Value
45 Days	11.73 ± 0.12^a	8.53 ± 0.15^b	12.70 ± 0.26^d	11.23 ± 0.15^c	*
60 Days	13.63 ± 0.09^a	10.90 ± 0.06^a	14.34 ± 0.17^c	13.50 ± 0.18^b	*

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

** = Significant at $p<0.01$ level of probability

*= Significant at $p<0.05$ level of probability

4.3 Postmortem examination

At the end of experiment, postmortem examination grossly showed diffuse congestion (Fig. 16), pale color, fragile and granular appearance showing possible arsenic deposition on liver. Kidney showed congestion (Fig. 18). In muscle (Fig.20) there was also haemorrhage. Gross examination of control group group (T_0) revealed normal liver (Fig. 15), kidney (Fig. 17) and muscles (Fig. 19).

Fig.15: Normal Liver (T_0)

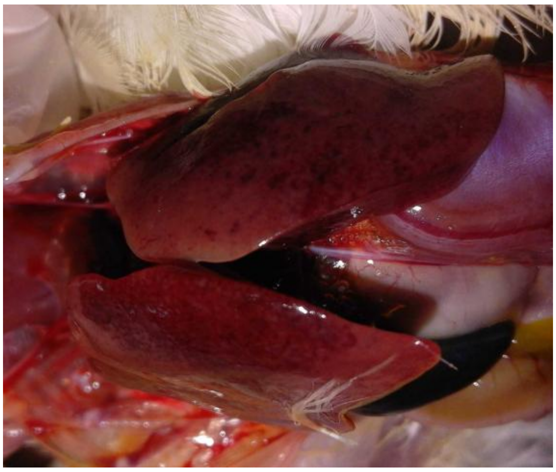

Fig. 16: Congestion of Liver (Arsenic treated group)

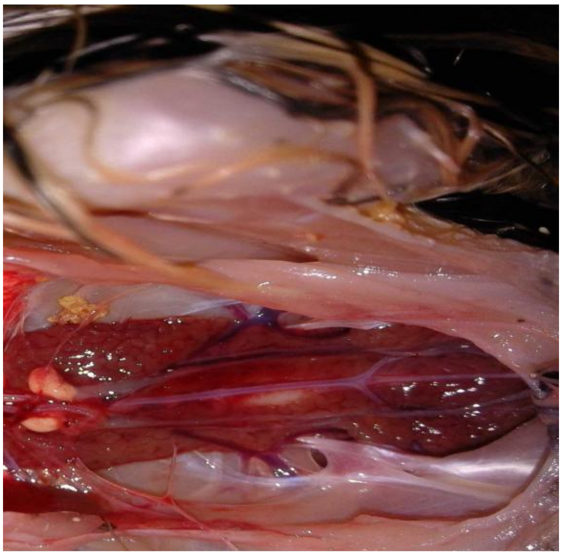

Fig.17: Normal Kidney (T_O)

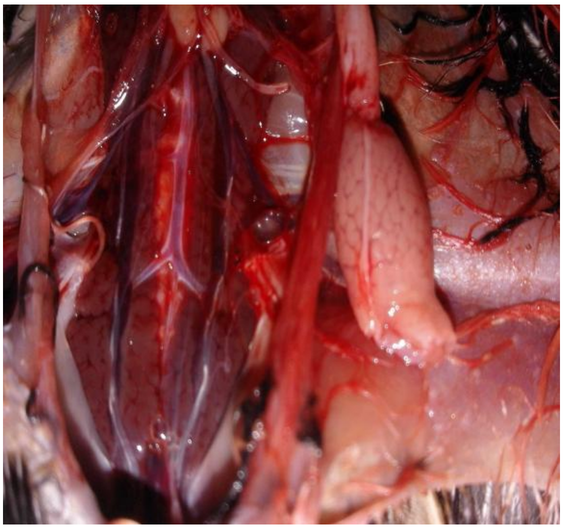

Fig.18: Congestion of Kidney (Arsenic treated group)

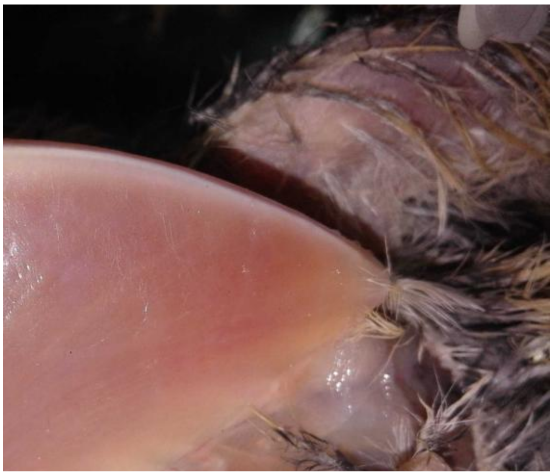

Fig.19: Normal Muscle (T_0)

Fig.20: Haemorrhage on Muscle (Arsenic treated group)

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

Microscopical examination of liver from control group revealed normal histological picture, however liver of arsenic treated group showed fatty changes, cirrsosis and congestion.

4.4.1 Kidney

Microscopically, kidneys from control (T_0), showed normal architecture with normal glomeruli. Fatty degeneration, cytoplasmic vacuoles were observed in the section of kidneys from arsenic treated group.

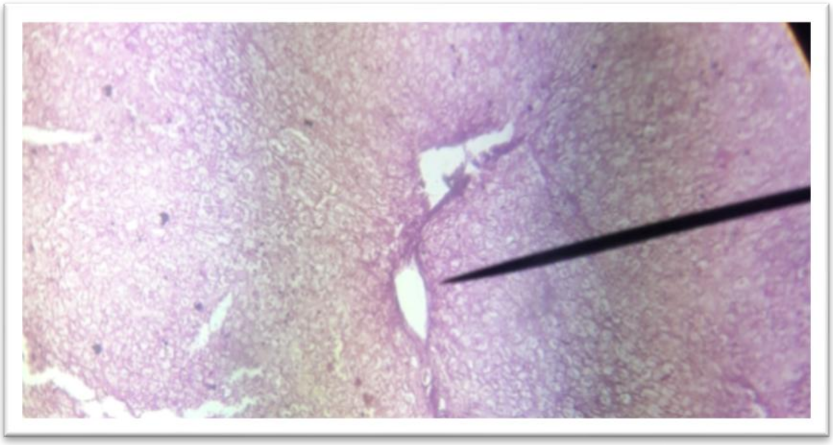

Fig. 21: Microscopic view of liver: showing regular pattern of hepatic cord in group T_0 (H and E, Dimension- 947×619)

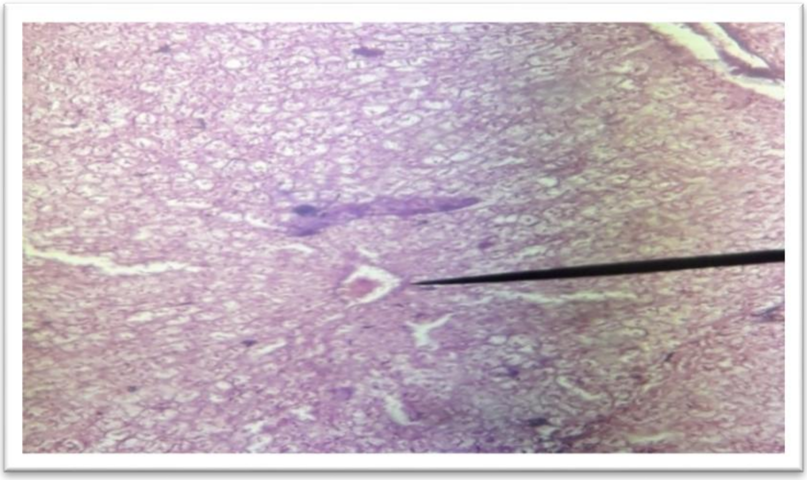

Fig.22: Microscopic view of liver: group T_1 showed congestion in liver (H and E, Dimension- 947×619)

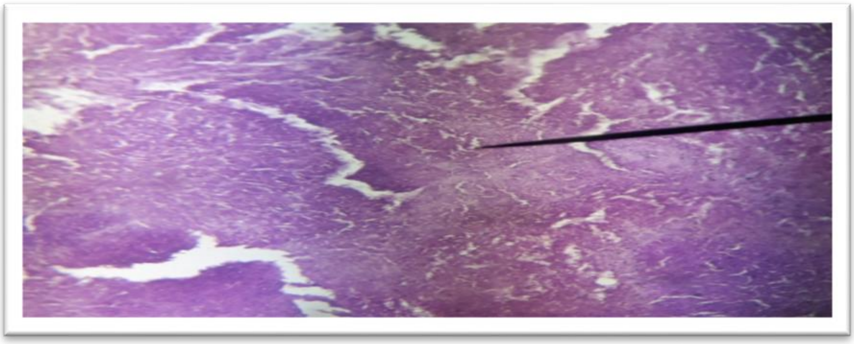

Fig.23: Microscopic view of liver: group T_1 showed cirrhosis in liver

(H and E, Dimension-947×619)

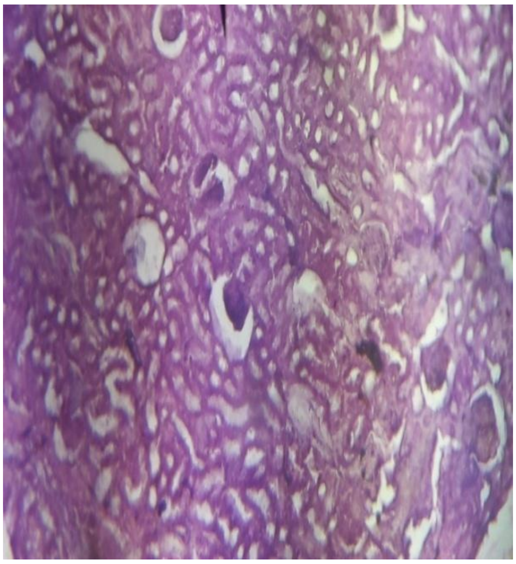

Fig.24: Microscopic view of kidney: group T0, showed no remarkable change in kidney tubules (H and E, Dimension- 947×619)

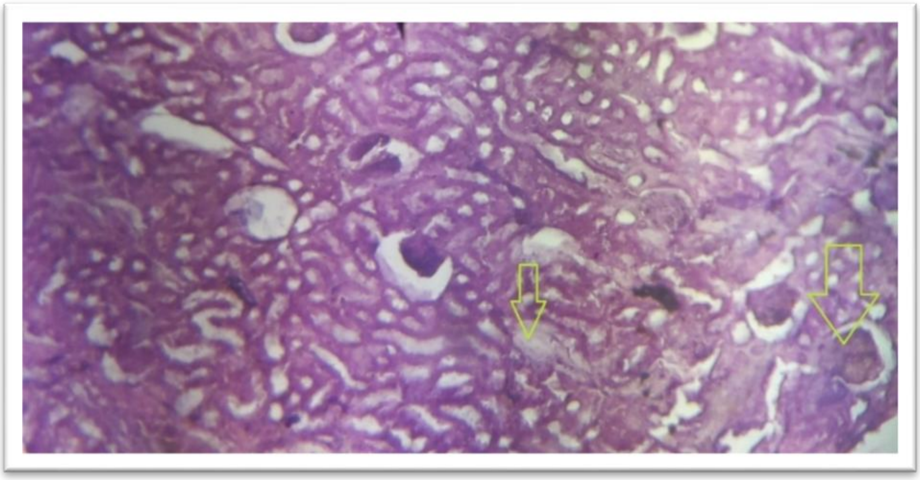

Fig.25: Microscopic view of kidney: group T_1 showed Fatty changes in kidney tubules (H and E, Dimension- 947×619)

CHAPTER V

DISCUSSION

Feeding of arsenic (100mg/kg Drinking water) toxicity in quails increased day by day. Spirulina and garlic treatment lowered arsenic toxicity where the Spirulina found more effective in reducing as content in the tissues. The treated quails did not show any clinical signs/lesion during the entire study period but slight increase in body weight in quails of most of the groups but the body weight of arsenic treated quails (T_1) was lower compared to quails of arsenic plus Spirulina (T_2), arsenic plus garlic (T_3) and control groups (T_0) (Table-1). The present findings are 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 arsenic treated groups. Moderate weakness was also observed in the quails of arsenic treated group compared to arsenic plus spirulina, arsenic plus garlic and control groups. Hence, it could be said that feeding of As2O3 caused chronic arsenic toxicity in quails, 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.

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 spirulina treated quails .The cause of change in hematological values might be due to the toxic effect of arsenic on haematopoietic system which is responsible for such alterations in hematological parameters. The value of TEC was highest in spirullina treated group T_2 and lowest in arsenic treated group T_1. However, the findings might suggest that chronic arsenic toxicity possibly decrease TEC in the quails and that could be recovered by spirulina (1 g kgG1 feed) or Garlic (1gm kgG1 feed) with feed within 60 days. Highest Hb concentration was found in T_2 group and lowest concentration was found in T_0 group. It might be concluded that Spirulina might slightly increase the values of Hb against arsenic toxicity in quails.

However, 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/garlic for a longer time might give a clear picture in this regard. Significantly increased ($p<0.01$) levels of As in the lung, liver and kidney following feeding of arsenic trioxide (100 mg kgG1 drinking water) to the quails of arsenic treated group compared to control 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 arsenic in quails for different periods induces a significant increase in arsenic accumulation.

Microscopical examination of liver from control group revealed normal histological picture, however liver of arsenic treated group showed fatty changes, cirrsosis and congestion.

Microscopically, kidneys from control (T_0), showed normal architecture with normal glomeruli. Fatty degeneration, cytoplasmic vacuoles were observed in the section of kidneys from arsenic treated group.

Spirulina reduced As level significantly from the tissues compared to only arsenic treated quails which was in agree with finding ofFariduddin *et al*. (2001), who stated that spirulina was effective in lowering arsenic level from the arsenic loaded tissues in quails and Ghosh *et al*. (2014), who stated that spirulina was effective in lowering of arsenic level from blood of induced arsenicosis in goats. Garlic against arsenic toxicity pertaining to its ability to eliminate arsenic from the blood and soft tissues and in reversal of arsenic-induced oxidative stress in affected tissues (Flora et al., 2009)..

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 garlic influences the body's enzyme functions and may help to stimulate the production of antibodies.

CHAPTER VI

CONCLUSION

The results of this study may be concluded as the following

- Arsenic toxicity reduce the body weight of quails.
- Treatment with spirulina and garlic might increase the body weight. But spirulina is more effective than garlic against arsenic induced toxicity in quails.
- This study suggested that spirulina and garlic has significantly reduced the arsenic concentration of inorganic arsenic toxicity in quails.
- Spirulina and garlic has significant effect on body weight, TEC, Hemoglobulin concentration of toxic quails. But the effect of spirulina was found better in recovering all parameters compare to garlic.
- Further investigation in this line may make more clear evidence to use spirulina as a therapeutic treatment for arsenic toxicity and arsenic determination may perform.

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