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Protective Role of Spirulina and Vitamin E against Arsenic Toxicity in Rats

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ABSTRACT

Arsenic is one of the most sensitive environmental issues in Bangladesh; even it is a major health concern in Asia. Spirulina and vitamin E have been considered as a potential therapeutic supplement due to its ability to minimize several element induced toxicities in various species including man. The study was performed to evaluate the role of spirulina (*Spirulina platensis*) and vitamin E in prevention of arsenic toxicity in different groups ($T_{0\text{-}4}$, n = 60) of Long-Evans rats. T_0 was control group, T_1 was treated with sodium arsenite, T_2 was treated with sodium arsenite plus spirulina, T_3 was treated with sodium arsenite plus vitamin E and T_4 was treated with sodium arsenite plus vitamin E plus spirulina daily for 63 days. Sodium arsenite was at 4 mg kg^{-1} b.wt., spirulina was at 1 g kg^{-1} feed and vitamin E was at 200 mg kg^{-1} feed. Samples were collected on day 21, 42 and 63. Arsenic was detected from tissue samples by Hydride Generation Atomic Absorption Spectrophotometer (HGAAS). Sodium arsenite feeding in rats caused chronic arsenic toxicity and the arsenic content in tissues (blood, lung, liver and kidney) of the exposed rats were significantly higher than control rats. Spirulina and vitamin E treatments significantly lowered the arsenic content in tissues. Arsenic caused hepatic and renal dysfunction but spirulina and vitamin E improved the hepatic and renal functions. Spirulina feeding was more effective than vitamin E and their combined treatment was more effective compare to their single treatment. The study demonstrates the role of spirulina and vitamin E in the reduction of toxicity of arsenic.

Key words: Arsenic, HGAAS, spirulina, vitamin E, rat

INTRODUCTION

Now-a-days arsenic creates a serious public health problem in developing countries like Bangladesh, India, Argentina, Chile, China, Mexico, Nepal, Taiwan, Vietnam, Ghana, Thailand and in developed countries like USA, Romania and Hungary by contaminating the ground water which is the main source of drinking water in these countries (Rahman, 2006). Most of the districts of Bangladesh are recently reported to the presence of dangerous levels of inorganic arsenic in most of the tube wells, which are currently serving as water points mainly for drinking and cooking purpose (DPHE., BGS and MML., 1999; BAMWSP., 2001). Levels of arsenic in drinking water are so high that WHO describes arsenic contamination of Bangladesh's water supply as "the largest poisoning of a population in history" (FAO., 2006). The arsenic poisoning from the contamination of underground water is chronic in nature and most victims do not complain of the symptoms until

they are detected through screening survey. Arsenic poisoning occurs naturally in both organic and inorganic forms in water, food, soil, dust, wood and other materials. Inorganic arsenic is more toxic than organic form (Lau *et al*., 1987). From a half of century past, arsenic has been used in medicine, cosmetics industry and agriculture. It has been used as drugs and their main use today is as pesticides, veterinary drugs, herbicide, rodenticide and silvicides, desiccant, feed additives and as growth prompter in animals and poultry production (Friberg *et al*., 1986). Arsenic can enter into food chain (Ulman *et al*., 1998) causing wide spread distribution throughout the plant and animal kingdoms. The detection of arsenic in milk and meat is a new finding (Lasky *et al*., 2004; Awal, 2007). Poultry meat and feces may also contain arsenic because they also drink tube-well water especially in urban areas and at farm levels. Arsenic can cause acute, sub-acute and chronic poisoning. Gastrointestinal, cardiac, renal, bone marrow, central nervous system and hepatic damage may be noted at different stages of arsenic poisoning (Kurttio *et al*., 1999). Chronic arsenic exposure has also been associated with a greatly elevated risk of cancer; possibly cancers of lung, liver, bladder, kidney and colon (Smith *et al*., 2009).

Low intake of calcium, animal protein, folate and fiber may increase susceptibility to arsenic-caused skin lesions (Mitra *et al*., 2004). The potential of dietary antioxidants (vitamin C, E and β-carotene) may reduce the arsenic burden in human by increasing its metabolism (Dey, 2002). Clinical study suggests that algae having very high concentration of micronutrients and vitamins may have beneficial effects in heavy metal poisoning (Ciferri, 1983).

Spirulina a microscopic blue-green algae, has a property of reducing heavy metals and nephrotoxic substance from the body. Spirulina is not only a whole food but it seems to be an ideal therapeutic supplement (Robert, 1989). Spirulina effectively reduces hepatic damage due to drug abuse and heavy metal exposure, inflammatory response (Richmond, 1986; Romay *et al*., 1999), cells degeneration (Bulik, 1993) and anaphylactic reaction (Yang *et al*., 1997). Vitamin E is a lipid soluble free radical scavenger that protects the membrane from lipid peroxyl radicals (Wagner *et al*., 1996). It plays an important role in the body's enzyme function and may help to stimulate the production of antibodies. Vitamin E is also considered as an antioxidant (Ayaz *et al*., 2007) and it may work with other antioxidant to protect the cell from damage. Many scientists from different countries are working on the arsenic problem in Bangladesh, especially on ground water for human concern. But work on arsenic detection and remediation in food chain is still imperative for the reduction of arsenicosis in man and animal. So, considering all the above factors, the study was designed to detect arsenic concentration in different tissues (lung, liver, kidney and blood) of arsenic induced rats and to determine the protective efficacy of spirulina and vitamin E to prevent arsenicosis.

MATERIALS AND METHODS

Animals: Sixty female Long-Evans rats (*Rattus norvegicus*) (3 months aged) were randomly divided into 5 equal groups ($T_{0\text{-}4}$, n = 12 in each group). All the rats were maintained by feeding pellet poultry feed (Nourish Poultry Feed Limited, Bangladesh) and tape water.

Treatment materials: Sodium arsenite ($NaAsO_2$, MW 197.84 g mol^{-1}) from May and Baker Ltd., Dagenham, England; Spirulina from the Life Line International Company, Australia as spirilina tablet (500 mg of spirulina platensis/tab) and vitamin E from Square Pharmaceutical Limited, Bangladesh as vitamin E tablet (400 mg/tab).

Treatment: Rats of group T_0 were maintained with only normal feed and water as control group, rats of group T_1 were treated with sodium arsenite at 4 mg kg^{-1} b.wt. daily with drinking water and normal feed and rats of group T_2 were treated with sodium arsenite at 4 mg kg^{-1} b.wt. with drinking water and feed with spirulina at 1 g kg^{-1} feed, rats of group T_3 were treated with sodium arsenite at 4 mg kg^{-1} b.wt. in drinking water and vitamin E at 200 mg kg^{-1} feed and the rats of T_4 were treated with sodium arsenite at 4 mg kg^{-1} b.wt. in drinking water and vitamin E at 200 mg kg^{-1} feed plus spirulina at 1 g kg^{-1} feed. All treatments were given for 63 days.

Finding clinical signs: For clinical signs, rats were regularly observed, if any in them, during the entire experimental trial period (from day 1-63) and the findings were recorded.

Sampling: After every 21 days, 4 rats were sacrificed from each group and about 6 mL of blood samples were collected from hearts from each rats. For biochemical tests, blood sample was taken into pre-marked centrifuge glass test tubes immediately after collection. The blood sample was kept at room temperature for 1 h without agitation for clotting with a view to collect serum. For hematological tests and for the detection of arsenic concentration in blood 1 mL of blood for each was taken separately in EDTA coated tube. The total lung, liver and kidney were collected aseptically, washed with physiologic saline and were kept in the pre-marked gipper polythene bag. All the tissue samples were preserved in the deep freeze at -20°C. All blood and tissue samples were taken 1st on day 21, 2nd on day 42 and 3rd on day 63.

Preparation of samples (lung, liver, kidney and blood): Digestion and dilution of solid tissue samples were required for arsenic detection.

Digestion of samples: For the preparation of sample the digestion tubes were cleaned with detergent, then immersed in 1% hydrochloric acid (HCl; 35.4%, AnaloR®, BDH chemicals Ltd. Poole England, UK) overnight at room temperature and finally washed with deionized water and dried in a digital electric oven (memmert, Model 400, GmbH+Co. KG, D-91126, Schwabach FRG, Germany) at 70°C for 2-4 h. About 0.5 g of each sample (lung, liver and kidney) was kept in each digestion tube individually. Then 5 mL of acid mixture (69% nitric acid (HNO_3; Merck KGaA, Darmstadt, Germany and perchloric acid at 5:3 ratio) was added to each digestion tube containing sample and left overnight at room temperature. Following the similar procedure, 5 mL of acid mixture was taken in another empty digestion tube (without sample) for blank determination (as control). In the next day, the content in the digestion tubes was heated by placing these tubes in a block digester (M-24 plazas/samples, J.P. Selecta, Spain) at 120°C for 4 h and gradually the temperature was increased up to 250°C until nitrous oxide fume emission (brown colored fume) stopped. Brown fume generation would indicate oxidation of the sample by HNO_3 and then the samples were cooled to room temperature. During the heating period the digestion tubes were gently shaken every after one hour to make the contents homogeneous and to enhance fume release. The procedure followed in the digestion with slight modification of the procedure described by Wang *et al*. (2001). After completion of the digestion process the samples were then ready for dilution.

Dilution of digested samples: Completing the digestion, the samples were diluted individually with deionized water separately in 50 mL calibrated volumetric flask. After dilution, each sample

was filtered individually with filter paper (Whatman 42) into correspondingly marked sterile 50 mL screw capped sterile plastic vials and preserved at 4°C in a refrigerator until tested for arsenic.

Serum collection: After 1 h, the test tubes containing blood clot were centrifuged in centrifuge machine (Hettich, Universal II, Germany) at 2000 rpm (rotation per minute) for 15 min. The supernatant serum was collected gently in the correspondingly marked screw capped sterile test tubes with separate sterile Pasteur pipette and kept in deep freeze at -20°C until tested.

Detection of arsenic in tissues: Arsenic was detected in the Arsenic Detection and Mitigation Laboratory (ADM Lab), Department of Pharmacology, Bangladesh Agricultural University, Bangladesh with Hydride Generation Atomic Absorption Spectrophotometer (HG-AAS; PG-990, PG Instruments Ltd. UK). Arsenic was detected by forming AsH_3 at below pH 1.0 after the reaction of As with a solution of potassium borohydride (KBH_4 = 53.94 g mol^{-1}, BDH Chemicals Ltd., Poole England, UK.), sodium hydroxide (NaOH, M = 40.000 g mol^{-1}, Merck KGaA, Darmstadt, Germany) and 10% HCl.

Hemato-biochemical tests: The hematological and biochemical tests (using Reflotron® Plus, UK) were done in the Department of Physiology, Bangladesh Agricultural University, Bangladesh.

Statistical analysis: The experiments were designed in CRD and the data was analyzed using simple ANOVA for considering the significant differences among the mean values. Duncan's Multiple Range Test (DMRT) was performed to observe significant differences of protective effects in different groups of rats. Data was presented as Mean±SE. All analysis was performed using SPSS software version 20. The $p<0.05$ was considered as significant.

RESULTS

Arsenic toxicity in rats increased arsenic concentrations in lung, liver, kidney and blood. Spirulina and vitamin E treatment lowered arsenic contents in the above tissues where spirulina found more effective in reducing arsenic content from tissues except blood where vitamin E found more effective. The combined dose of vitamin E and spirulina was more effective compared to the single treatment.

Body weight gain and clinical signs: The treated rats did not show any clinical signs/lesion during the entire period of study but slightly increased body weight was observed in all groups because the rats were in growing stage. On the other hand, the body weight gain in rats of arsenic treated group was found lower compared to other treated groups. Moderate weakness was also observed in the rats of arsenic treated group compared to arsenic plus spirulina, arsenic plus vitamin E and control groups.

Effect on hematological parameters (Total Erythrocyte and Leucocyte Count; TEC and TLC): No significant difference on TEC and TLC was found among the groups though on day 63, the value of TLC and TLC was the highest in control rats and the lowest in arsenic treated rats and the values in group T_4 (Arsenic+spirulina+vitamin E) was near to the control group. However, the

findings might suggest that chronic arsenic toxicity possibly decrease TEC and TLC in the rats and that could not be recovered by spirulina (1 g kg^{-1} feed) or vitamin E (200 mg kg^{-1} feed) or both with feeding of within 63 days.

Effect on biochemical parameters

Serum Glutamic Oxaloacetic Transaminase (SGOT): The values of SGOT were significantly increased in all samples of arsenic treated rats compared to control group (Table 1). The arsenic toxicity caused hepatic insufficiency but vitamin E and spirulina improved the hepatic functions (decreased SGOT compared to arsenic treated rats).

Alanine aminotransferase (ALT): The values of ALT were the lowest in control group except day 63 and the highest was in arsenic treated group. On day 21, there was no significant difference on ALT values among groups but on day 42 and 63, the treatments decreased the ALT values than arsenic treated group significantly($p<0.05$ and 0.01, respectively). Among the treatments, combined spirulina+vitamin E treatment was more effective than others (Table 2).

Serum creatinine: Serum creatinine level was the lowest in control group but the levels on other groups fluctuated. Vitamin E treatment did not decreased the serum creatinine level significantly compared to arsenic treated group whereas both the spirulina and spirulina+vitamin E treatments decreased the creatinine levels significantly on day 42 and 63, even it was similar to control group (Table 3).

Effect on different tissues of rats

Arsenic content in blood: Arsenic level in blood was very low in control group. All the treatments decreased the arsenic level than arsenic treated group significantly. On day 21, vitamin E treatment decreased the arsenic level more than other groups compared to arsenic treated group but on day 63 combined spirulina+vitamin E treatment showed more efficacy to minimize the arsenicosis (arsenic level in tissue) than spirulina alone and vitamin E treatments (Table 4).

Table 1: Effects of treatments on serum glutamic oxaloacetic transaminase (U L^{-1}) of rats (Mean±SE)

Treatment groups	Day 21	Day 42	Day 63
T_0 (Control)	23.16±0.88^{d}	23.56±2.40^{d}	23.20±3.79^{e}
T_1 (Arsenic group)	35.66±2.96^{a}	35.93±1.86^{a}	36.20±1.43^{a}
T_2 (Spirulina+Arsenic)	32.23±2.03^{c}	29.60±3.61^{c}	27.76±2.03^{d}
T_3 (Arsenic+Vitamin E)	34.10±1.15^{b}	35.16±2.91^{b}	34.90±1.31^{b}
T_4 (Arsenic+spirulina+Vitamin E)	35.16±1.76^{a}	34.86±1.76^{b}	33.86±3.53^{c}
Level of significance	**	**	**

**p<0.01, In columns, same superscripts indicate insignificant whereas dissimilar superscripts indicate significant difference among the groups

Table 2: Effects of treatments on alanine aminotransferase (U L^{-1}) in rats (Mean±SE)

Treatment groups	Day 21	Day 42	Day 63
T_0 (Control)	42.30±1.14	41.97±1.45^{d}	41.53±1.17^{c}
T_1 (Arsenic group)	45.20±1.34	51.27±0.92^{a}	58.90±2.29^{a}
T_2 (Spirulina+Arsenic)	44.57±1.07	42.70±1.89^{dc}	44.20±1.61^{b}
T_3 (Arsenic+Vitamin E)	44.00±1.45	43.83±2.07^{b}	41.73±1.50^{c}
T_4 (Arsenic+spirulina+Vitamin E)	43.28±1.12	42.43±2.00^{d}	39.57±0.88^{d}
Level of significance	ns	*	**

*p<0.05 and **p<0.01, ns: Not significant. In columns, same or without superscripts indicate insignificant whereas dissimilar superscripts indicate significant difference among the groups

Arsenic content in lungs: Arsenic level in lungs was negligible in control group. Only spirulina+vitamin E treatment significantly lowered the arsenic level than arsenic treated group on day 21 observation. Though on day 42 and 63, all treatments decreased the arsenic level than arsenic treated group significantly, the combined spirulina+vitamin E treatment showed more efficacy to lower the arsenic level in lung tissue of rats (Table 5).

Arsenic content in liver: Arsenic remained as a trace element in liver of rats of control group and the arsenic level was always the highest in arsenic treated group. The treatments lowered the arsenic concentration in liver tissue compare to arsenic treated rats but not in significant level on day 21. The treatments significantly lowered the arsenic concentration in all treatments but spirulina alone treatment showed more efficacy on day 42 and 63 to lower arsenic concentration in liver tissue of rats (Table 6).

Table 3: Effects of treatments on serum creatinine (mg dL^{-1}) of rats (Mean±SE)

Treatments groups	Day 21	Day 42	Day 63
T_0 (Control)	0.52±0.001^c	0.51±0.01^c	0.51±0.001^c
T_1 (Arseni^c group)	0.54±0.01^b	0.56±0.001^b	0.60±0.01^a
T_2 (Spirulina+Arsenic)	0.57±0.05^a	0.51±0.001^c	0.51±0.001^c
T_3 (Arsenic+Vitamin E)	0.54±0.01^b	0.58±0.01^a	0.57±0.001^b
T_4 (Arsenic+spirulina+Vitamin E)	0.56±0.01^a	0.55±0.01^b	0.51±0.001^c
Level of significance	**	**	**

**p<0.01, In columns, same superscripts indicate insignificant whereas dissimilar superscripts indicate significant difference among the groups

Table 4: Arsenic content (ppm) in blood of rats (Mean±SE)

Treatment groups	Day 21	Day 42	Day 63
T_0 (Control)	4.60±0.40^e	4.29±0.46^e	4.13±0.64^d
T_1 (Arsenic group)	144.00±16.54^b	161.22±7.93^a	166.11±3.64^a
T_2 (Spirulina+Arsenic)	141.05±9.69^c	115.06±16.86^b	99.78±5.49^b
T_3 (Arsenic+Vitamin E)	120.11±10.72^d	110.10±14.87^c	97.75±8.94^b
T_4 (Arsenic+spirulina+Vitamin E)	147.26±1.73^a	100.79±3.83^d	91.60±5.10^c
Level of significance	**	**	**

** p<0.01, In columns, same superscripts indicate insignificant whereas dissimilar superscripts indicate significant difference among the groups

Table 5: Arsenic content in (ppm) lungs of rats (Mean±SE)

Treatments groups	Day 21	Day 42	Day 63
T_0 (Control)	0.41±0.05^c	0.40±0.12^d	0.46±0.03^d
T_1 (Arsenic group)	17.89±4.22^a	19.96±1.91^a	21.23±5.27^a
T_2 (Spirulina+Arsenic)	16.77±2.47^a	15.33±3.17^b	12.64±5.69^c
T_3 (Arsenic+Vitamin E)	16.73±0.81^a	15.04±3.29^b	15.56±4.47^b
T_4 (Arsenic+spirulina+Vitamin E)	12.59±5.00^b	13.86±5.05^c	10.35±0.44^c
Level of significance	**	**	**

**p<0.01, In columns, same superscripts indicate insignificant whereas dissimilar superscripts indicate significant difference among the groups.

Table 6: Arsenic content (ppm) in liver of rats (Mean±SE)

Treatment groups	Day 21	Day 42	Day 63
T_0 (Control)	0.15±0.02	0.04±0.01^c	0.09±0.02^c
T_1 (Arsenic group)	8.56±1.85	7.84±0.20^a	8.42±2.47^a
T_2 (Spirulina+Arsenic)	5.29±0.43	4.50±0.16^b	4.88±0.14^b
T_3 (Arsenic+Vitamin E)	5.37±2.17	4.94±0.19^b	5.04±0.15^b
T_4 (Arsenic+spirulina+Vitamin E)	5.60±0.67	5.21±0.66^a	5.20±0.42^a
Level of significance	ns	**	**

**p<0.01, ns: Not significant. In columns, same or without superscripts indicate insignificant whereas dissimilar superscripts indicate significant difference among the groups

Table 7: Arsenic content (ppm) in kidney of rats (Mean±SE)

Treatments groups	Day 21	Day 42	Day 63
T_0 (Control)	1.06±0.45	0.17±0.02	0.27±0.04^{d}
T_1 (Arsenic group)	26.82±3.37	34.48±8.91	35.23±3.95^{a}
T_2 (Spirulina+Arsenic)	20.71±3.03	19.40±11.08	17.64±1.04^{c}
T_3 (Arsenic+Vitamin E)	24.30±10.21	24.18±14.86	22.36±1.24^{b}
T_4 (Arsenic+spirulina+Vitamin E)	20.57±14.04	19.22±12.58	17.94±3.65^{bc}
Level of significance	ns	ns	*

*p<0.05, ns: Not significant. In columns, same or without superscripts indicate insignificant whereas dissimilar superscripts indicate significant difference among the groups

Arsenic content in kidney: Arsenic concentration also was very low level in kidney tissue of control rats in all observations. The spirulina and vitamin E treatments insignificantly lowered the arsenic concentration in kidney tissues on day 21 and 42 and significantly (p<0.05) lowered on day 63 compared to arsenic treated rats and both the spirulina alone or combined spirulina+vitamin E treatments showed more efficacy than vitamin E treatment (Table 7).

DISCUSSION

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

Arsenic induced mice showed several clinical sign during the experimental period but slightly increased body weight was observed in all groups because the rats were in growing stage. On the other hand, the body weight gain in rats of arsenic treated group was found lower compared to other treated groups. 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 rats of arsenic treated group compared to arsenic plus spirulina, arsenic plus vitamin E and control groups. Hence, it could be said that feeding of $NaAsO_2$ caused chronic arsenic toxicity in rats, although for the manifestation of more pronounced symptoms of arsenic toxicity would take more time.

There was no significant difference on TEC and TLC was found among the groups, the value of TLC and TLC was the highest in control rats and the lowest in arsenic treated rats and the values in group T_4 (Arsenic+spirulina+vitamin E) was near to the control group. 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 result did not strongly support the findings that spirulina decrease anemia (Puyfoulhoux *et al*., 2001). However, the findings might suggest that chronic arsenic toxicity possibly decrease TEC and TLC in the rats and that could not be recovered by spirulina (1 g kg^{-1} feed) or vitamin E (200 mg kg^{-1} feed) or both with feeding of within 63 days. 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/vitamin E for a longer time might give a clear picture in this regard.

Significantly increased (p<0.01) levels of As in the lung, liver, kidney and blood following feeding of $NaAsO_2$ (4 mg kg^{-1} b.wt.) to the rats 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 rats for different periods induces a significant increase in arsenic accumulation. Arsenic loads detected in lung, liver, kidney and blood of which highest accumulation was found in blood of all treated groups and the arsenic concentration in liver was the lowest and kidney was the medium. The data obtained in this study were not in favor of Marafante *et al*. (1982), who showed that the highest accumulation of arsenic in the spleen followed by lung, liver, kidney, skin and lowest accumulation was in intestine. The raised level in the blood is due to direct absorption of arsenic to the blood.

Spirulina reduced As level significantly from the tissues compared to only arsenic treated rats which was in agree with finding of Fariduddin *et al*. (2001), who stated that spirulina was effective in lowering arsenic level from the arsenic loaded tissues in rats and Ghosh *et al*. (2014), who stated that spirulina was effective in lowering of arsenic level from blood of induced arsenicosis in goats. Vitamin E reduced As level significantly from the tissues compared to only arsenic treated rats that agreed with the findings of Islam *et al*. (2005), who stated that supplementation of vitamin E, iron and zinc reduces the arsenic concentration in tissues like liver, kidney, spleen, heart, intestine, stomach, muscle and dermis of rats as well as lower the tissue damage caused by arsenic.

The levels of serum biochemical parameters have been reported to be markedly elevated in animals exposed to arsenicals, the exact mechanism involved in elevation of these enzymes have not been conclusively postulated. Several workers have suggested that such effect may be the result of cellular damage or increased plasma membrane permeability. Moreover, factors such as increased synthesis or decreased enzyme degradation may also be involved. We observed that the biochemical parameters (SGOT, ALT and serum creatinine) were significantly elevated in arsenic treated groups of mice.

The values of Serum Glutamic Oxaloacetic Transaminase (SGOT) were significantly increased in all samples of arsenic treated rats compared to control group. Although, this finding did not agree with the findings of Mahaffey *et al*. (1981), who stated that AST was reduced by As alone. However, the arsenic toxicity caused hepatic insufficiency and vitamin E and spirulina improved the hepatic functions (decreased SGOT compared to arsenic treated rats), which were supported the findings of Richmond (1986) and Romay *et al*. (1999), who stated that spirulina reduced hepatic damage due to drug abuse and heavy metal exposure. Previous study in layer birds observed that the SGPT, SGOT, ALP and LDH were increased due to dietary arsenic (Chiou *et al*., 1997). Akter *et al*. (2010) reported that serum activity of aspartate aminotransferase (AST) was increased by arsenic intoxication but serum creatinine values were fluctuating of goat.

The ALT was the lowest in control group except day 63 and the highest was in arsenic treated group. On day 21, there was no significant difference on ALT values among groups but on day 42 and 63, the treatments decreased the ALT values than arsenic treated group significantly($p<0.05$ and 0.01, respectively). The results conform to the finding of Mazumder (2003) and Saha (2003), who reported that the liver function test was disturbed in both man and animal after chronic exposure of inorganic arsenic. However, among the treatments, combined spirulina+vitamin E treatment was more effective than others. Serum creatinine level was the lowest in control group but the levels on other groups fluctuated. The findings did not agree with the findings of Zhang *et al*. (1995), who observed that there is a relationship between the As level and the degree of chronic renal insufficiency. Vitamin E treatment did not decreased the serum creatinine level significantly compared to arsenic treated group whereas both the spirulina and spirulina+vitamin E treatments decreased the creatinine levels significantly on day 42 and 63, even it was similar to control group.

In the present study, serum biochemical parameters were significantly elevated indicating some lesions or damages caused by arsenic trioxide. The rises of all parameters were maximum in T_1 group (only arsenic treated group). It was noticed that the rise of biochemical parameters were minimum in remaining three groups (T_2, T_3 and T_4) which were given spirulina, vitamin E and their combination along with arsenic indicating that spirulina and vitamin E have some extent of protective role against arsenic induced tissue injuries. The exact cause of this protective role in recovering tissue damages is not fully understood. However, it is known that spirulina is an enriched source of nutrients like protein, amino acid, iron, β-carotene, phycocyanin, γ-lenolenic acid, vitamin B_1, B_2, B_3, B_6, B_{12} and essential fatty acid which are very much helpful to maintain the normal health and vitamin E influences the body's enzyme functions and may help to stimulate the production of antibodies. So, these findings indicate that spirulina and vitamin E have the positive role in decreasing the increased biochemical parameters due to arsenic toxicities.

CONCLUSION

Arsenic toxicity/Arsenicosis (Arsenic in tissues) could be minimized by treating animals with spirulina and vitamin E and this speculation was established in this study. From these findings, we may conclude that spirulina was more efficacious than vitamin E in reducing arsenic load. It is needed to more study to optimize the dose of spirulina and vitamin E to minimize arsenicosis in animals.

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