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Protective Effect of Spirulina Against Arsenic-induced Toxicities in Mice

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ABSTRACT

Background: Spirulina have been considered as a potential therapeutic supplement due to its ability to minimize several element induced toxicities in various species including man. The present study was designed to evaluate the potentiality of spirulina in arsenic induced toxic mice. **Materials and Methods:** Fifty mice were divided into 5 equal groups, including a control group (T_0). Arsenic trioxide (1 mg/2 mL drinking water) was administered in one group of mice (T_A), whereas the remaining three groups of mice (T_B, T_C and T_D) were given arsenic trioxide (1 mg) plus spirulina (at different doses) in drinking water daily for 90 days. Toxic signs, body weight, hematological and biochemical parameters were recorded at 15 day intervals. **Results:** Sudden onset of depression, restlessness, anorexia, ruffled hair coat and skin lesions in all parts of the body, especially on tail region of the arsenic trioxide administered mice were found that was mild in nature in other four groups. Biochemical and hematological studies explored that the mean body weight, the values of Total Erythrocyte Count (TEC), Hemoglobin (Hb) and Packed Cell Volume (PCV) were significantly reduced ($p<0.01$), whereas the values of Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT) and Alkaline Phosphatase (ALP) were significantly increased ($p<0.01$) in arsenic trioxide administered mice when compared with control group ($p>0.05$) and arsenic trioxide plus spirulina administered groups ($p>0.05$). **Conclusion:** The findings of this study illustrate that spirulina was found to be effective in the reduction of body burden of arsenic.

Key words: Arsenic, biochemical parameters, body weight, hematological parameters, spirulina, toxic sign

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INTRODUCTION

Arsenic is a naturally occurring omnipresent trace element available in both organic and inorganic forms in nature. The most important inorganic arsenic is arsenic trioxide, sodium arsenite, arsenic trichloride, arsenic acid and arsenites (trivalent forms), lead and calcium arsenates (pentavalent forms). The most common organic arsenic compounds are arsanilic acid, Monomethylarsinic Acid (MMA), Dimethylarsinic Acid (DMA) and arsenobetaine (Friberg *et al*., 1986; Lau *et al*., 1987). Inorganic arsenic is more toxic than organic form and the trivalent forms are more toxic than pentavalents. In nature, arsenic is also found to a small extent in the elemental form. For last 50 years, arsenic has been used in medicine, cosmetics industry and agriculture. It has been used in drugs and its main use today is as pesticides, veterinary drugs, herbicide, rodenticide and silvicides, desiccant, feed additives and as growth promoter of animal and poultry. Industrial uses of arsenic include doping of solid state devices, laser material; bronzing and smaller amounts in glass and ceramics industries (Friberg *et al*., 1986; WHO, 1981). Arsenic was used in malicious poisoning during nineteenth century and the first case of arsenic poisoning was identified in 1840 in France. Now 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

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in these countries (Rahman, 2006). Besides drinking water, general population is exposed to arsenic through dust, fumes and dietary sources like rice, milk, meat, seafood, mushrooms and poultry etc. The highest concentration of arsenic is reported in seafood, rice, mushrooms and poultry (Mandal and Suzuki, 2002); while fruit, vegetables and dairy products tend to have lower concentration (Yost *et al*., 1998).

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, vitamin 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 blue green algae is most effective in the treatment of different disease conditions in man and animal. It reduces mercury and other toxic metal accumulation in the tissue (Johnson and Shubert, 1986). Experimental studies found that spirulina alone or in combination with other vitamin or mineral is effective in the removal of arsenic from arsenic-loaded tissues in various species including man (Fariduddin *et al*., 2001; Misbahuddin *et al*., 2006; Awal, 2007). Spirulina extract plus zinc was found to be beneficial in patients of chronic arsenic poisoning (Misbahuddin *et al*., 2006). It also 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). Spirulina is not only a whole food but also an ideal therapeutic supplement. No other natural food is found with such a combination of nutrients like protein, amino acid, iron, betacarotene, phycocyanin, γ-lenolenic acid, vitamin B_1, B_2, B_3, B_6, B_{12} and essential fatty acid etc. In fact, it is the highest known source of protein, β-carotene which is a precursor of vitamin A and only vegetable source of vitamin B_{12} (Robert, 1989). Arsenic detection and remediation in food chain is still imperative for the reduction of arsenicosis in both man and animals. So, considering all the above factors, this study was carried out to observe the effects of spirulina and arsenic trioxide on toxic signs, body weight, some hematological (TEC, Hb and PCV) and biochemical parameters (SGPT, SGOT and ALP) in mice.

MATERIALS AND METHODS

Study design: This study was carried out in the Department of Biochemistry and Chemistry, Sylhet Agricultural University, Bangladesh, from March to September 2012. The study protocol was approved by the Ethical Committee of the Sylhet Agricultural University, Bangladesh. For the study purpose, 50 mice were divided into 5 equal groups which were maintained by feeding standard pellet feed and distilled water in the laboratory animal house. They were kept in different cages in a pre-disinfected and well ventilated room with controlled ambient temperature and natural relative humidity. One group (T_0) of mice was kept as control and one group (T_A) of mice were given arsenic trioxide at a dose of 1 mg/2 mL drinking water and the remaining three groups of mice (T_B, T_C and T_D) were given arsenic trioxide (1 mg) in addition with spirulina in three different doses i.e., 2, 4 and 8 mg/2 mL in drinking water daily for 90 days. Arsenic trioxide used in this study was collected from Loba Chemicals Company, Bombay, India and the spirulina was collected from Lifeline International Limited, Bangladesh, as powdered form. All treatments were given for 90 days.

Blood sample collection: Every 15 days, blood samples were collected from the coccygeal vein of mouse using disposable plastic syringe. The blood samples were then kept at room temperature for about 30 min to clot and centrifuged in centrifuge machine (Hettich, Universal 320, Germany) at 2218×g for 15 min to extract the serum. 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 the study day.

Clinical signs and body weight: After feeding arsenic trioxide and spirulina, all the control and treated mice were observed carefully for appearance of any toxic symptoms up to 90 days. Then body weights of the mice were measured at 15 days interval (0, 15, 30, 45, 60, 75 and 90).

Hematological parameters: In hematological parameters, the Total Erythrocyte Count (TEC), Hemoglobin content (Hb %) and Packed Cell Volume (PCV %) were determined as per method described by Lamberg and Rothstein (1977).

Biochemical examination: Blood sera biochemical parameters such as Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT) and Alkaline Phosphatase (ALP) were detected by auto analyzer using specific test kit. Kinetic methods for the determination of SGPT, SGOT and ALP were performed according to the recommendations of the expert panel of the International Federation of Clinical Chemistry (IFCC) without pyridoxalphosphate activation. The activity level of the supplied sample was expressed in IU L^{-1}.

Data analysis: All data were expressed as Mean±Standard Error (Mean±SE) with their corresponding p values. The experimental data were designed in CRD and analyzed statistically using one way analysis of variance (ANOVA) with the help of the SPSS software. The mean comparisons of the treatments were made by the Duncan's Multiple Range Test (DMRT).

RESULTS

Clinical signs: All the control mice (T_0) were quiet normal without any toxic signs during the whole experimental period. Mice of group T_A (only arsenic trioxide) were also apparently normal up to 45 days. After 45 days, mice of arsenic trioxide group showed sudden onset of depression, restlessness, anorexia, ruffled hair coat. After 75 days, skin lesions in all parts of the body especially on tail region were observed. Mice of remaining three groups T_B, T_C and T_D (1 mg of arsenic trioxide plus different doses of spirulina i.e., 2, 4 and 8 mg/2 mL in drinking water, respectively) were apparently normal up to 45 days. After 45 days and onwards, the signs of depression, restlessness, anorexia and ruffled hair coat were observed but were mild in nature. No skin lesion was found in the mice of group T_B, T_C and T_D throughout the experimental period.

Effect on body weight: The body weight gain was highest (26.25±1.30 g) in control group (T_0) after 90 days but in arsenic treated group (T_A) it was lowest (21.60±6.54 g), whereas, in arsenic plus spirulina treated groups (T_B, T_C and T_D) body weight gains (22.80±1.57, 24.25±1.77 and 25.25±1.30 g) were better than arsenic treated group but less than control group (Table 1). The body weight increased gradually but in case of T_A group mice the increasing trend of the body weight was significantly lower ($p<0.01$). The body weight decrease to a maximum level of 17.71% in arsenic treated group (T_A) while in other treated groups i.e., arsenic plus spirulina treated groups (T_B, T_C and T_D), the body weight decrease to a level of 13.14, 7.61 and 3.80%, respectively. The reduction of mean body weight were statistically significant ($p<0.01$) on 15, 30, 45, 60, 75 and 90 day of treatment in comparison to control group. In other treatment, the body weight was not statistically significant ($p<0.05$).

Hematological parameters

Total erythrocyte count (TEC): The TEC values were decreased significantly ($p<0.01$) to the extent of 18.65% in arsenic trioxide fed group (T_A) in relation to control group (T_0). The reduction of TEC values in other three groups (T_B, T_C and T_D) was 9.72, 8.71 and 7.70%, respectively which were less than arsenic treated group. The reduction of TEC values were statistically significant on 30 day of treatment ($p<0.05$) and on 45, 60, 75 and 90 day of treatment ($p<0.01$) in comparison to control group. In other treatments, the TEC values were not significant ($p<0.05$) (Table 2).

Hemoglobin (Hb): Table 3 shows that the Hb values were decreased significantly ($p<0.01$) to the extent of 37.73% in arsenic treated group (T_A) in relation to control group (T_0). However, in arsenic plus spirulina treated group (T_B), Hb value was reduced upto 14.25%.

Table 1: Effect of spirulina on body weight in arsenic treated mice

	Days (Mean±SE)							
Treatment	0	15	30	45	60	75	90	Decrease (%)
Control (T_0)	13.60±2.50	16.40±1.10^a	18.95±1.25^a	20.00±1.80^a	22.74±1.50^a	24.70±1.27^a	26.25±1.30^a	-
Arsenic trioxide 1 mg/2 mL drinking water (T_A)	12.22±2.60	13.50±2.28^e	15.00±1.29^e	16.30±3.31^e	18.00±5.33^e	19.80±4.93^e	21.60±6.54^e	17.71
Arsenic trioxide 1 mg+spirulina 2 mg/2 mL drinking water (T_B)	11.70±2.70	13.87±2.82^d	15.25±2.54^d	16.50±2.64^d	18.75±1.75^d	20.28±1.16^d	22.80±1.57^d	13.14
Arsenic trioxide 1 mg+spirulina 4 mg/2 mL drinking water (T_C)	12.60±3.10	14.25±1.36^c	16.00±1.30^c	18.12±1.79^c	20.25±1.29^c	22.25±1.53^c	24.25±1.77^c	7.61
Arsenic trioxide 1 mg+spirulina 8 mg/2 mL drinking water (T_D)	13.00±2.10	15.00±1.65^b	17.75±1.15^b	19.40±1.80^b	21.54±1.50^b	23.00±1.17^b	25.25±1.30^b	3.80
p-values	0.124	0.0025	0.0041	0.0002	0.0001	0.0004	0.0155	
Level of significance	ns	**	**	**	**	**	**	

All values were expressed in g, *Significant at 5% level of probability, **Significant at 1% level of probability, ns: Not significant, In a column figures with same or without superscript do not differ significantly as per DMRT

Table 2: Effect of spirulina on total erythrocyte count in arsenic treated mice

	Days (Mean±SE)							
Treatment	0	15	30	45	60	75	90	Decrease (%)
Control (T_0)	7.65±0.04	7.85±0.02	7.96±0.01^a	8.20±0.02^a	8.50±0.02^a	8.80±0.02^a	8.95±0.02^a	-
Arsenic trioxide 1 mg/2 mL drinking water (T_A)	7.50±0.03	7.76±0.03	7.64±0.03^b	7.56±0.03^d	7.46±0.04^e	7.36±0.04^d	7.28±0.03^d	18.65
Arsenic trioxide 1 mg+ spirulina 2 mg/2 mL drinking water (T_B)	7.60±0.02	7.84±0.05	7.75±0.02^b	7.82±0.04^c	7.91±0.04^{bc}	7.99±0.03^c	8.08±0.03^c	9.72
Arsenic trioxide 1 mg+ spirulina 4 mg/2 mL drinking water (T_C)	7.68±0.05	7.87±0.04	7.83±0.03^{ab}	7.92±0.03^b	8.01±0.03^b	8.09±0.04^c	8.17±0.03^{bc}	8.71
Arsenic trioxide 1 mg+ spirulina 8 mg/2 ml drinking water (T_D)	7.70±0.03	7.83±0.04	7.92±0.02^a	7.95±0.04^b	7.99±0.03^b	8.12±0.04^b	8.26±0.04^b	7.70
p-values	0.2162	0.254	0.0214	0.0047	0.0039	0.0005	0.0001	
Level of significance	ns	ns	*	**	**	**	**	

All values were expressed in million/μL, *Significant at 5% level of probability, **Significant at 1% level of probability, ns: Not significant, In a column figures with same or without superscript do not differ significantly as per DMRT

Table 3: Effect of spirulina on hemoglobin in arsenic treated mice

	Days (Mean±SE)							
Treatment	0	15	30	45	60	75	90	Decrease (%)
Control (T_0)	8.55±0.04	8.61±0.03	8.76±0.17^a	8.96±0.35^a	9.14±0.34^a	9.32±0.31^a	9.54±0.45^a	-
Arsenic trioxide 1 mg/2 mL drinking water (T_A)	8.60±0.05	8.62±0.04	7.06±0.34^d	6.74±0.24^e	6.50±0.39^e	6.26±0.30^e	5.94±0.23^e	37.73
Arsenic trioxide 1 mg+ spirulina 2 mg/2 mL drinking water (T_B)	8.62±0.06	8.64±0.02	7.36±0.23^c	7.5±0.26^d	7.7±0.37^d	7.86±0.32^d	8.18±0.33^d	14.25
Arsenic trioxide 1 mg+ spirulina 4 mg/2 mL drinking water (T_C)	8.50±0.03	8.59±0.03	7.86±0.21^b	8.12±0.32^c	8.36±0.26^c	8.28±0.36^c	8.62±0.46^c	9.64
Arsenic trioxide 1 mg+ spirulina 8 mg/2 mL drinking water (T_D)	8.63±0.03	8.63±0.03	8.4±0.16^a	8.66±0.21^b	8.82±0.24^b	8.72±0.35^b	8.96±0.57^b	6.70
p-values	0.2258	0.0954	0.0255	0.0033	0.0071	0.0002	0.0085	
Level of significance	ns	ns	*	**	**	**	**	

All values were expressed in %, *Significant at 5% level of probability, **Significant at 1% level of probability, ns: Not significant, In a column figures with same or without superscript do not differ significantly as per DMRT

Table 4: Effect of spirulina on packed cell volume in arsenic treated mice

	Days (Mean±SE)							
Treatment	0	15	30	45	60	75	90	Decrease (%)
Control (T_0)	30.00±0.04	30.22±0.03	30.44±1.22	30.7±1.21^a	31.32±1.18^a	31.96±1.79^a	32.42±2.41^a	-
Arsenic trioxide 1 mg/2 mL drinking water (T_A)	30.10±0.05	30.23±0.04	28.74±1.89	27.68±1.68^d	26.14±1.66^e	24.98±1.81^e	23.38±2.35^e	27.88
Arsenic trioxide 1 mg+ spirulina 2 mg/2 mL drinking water (T_B)	30.20±0.03	30.24±0.04	29.28±0.87	28.42±2.02^c	29.14±1.72^d	29.88±1.93^d	28.42±2.56^d	14.07
Arsenic trioxide 1 mg+ spirulina 4 mg/2 mL drinking water (T_C)	30.15±0.25	30.21±0.03	29.66±1.23	29.2±1.86^b	30.12±1.62^c	30.84±1.93^c	31.24±2.03^c	3.63
Arsenic trioxide 1 mg+ spirulina 8 mg/2 mL drinking water (T_D)	30.10±0.02	30.25±0.04	29.98±1.38	30.02±2.22^{ab}	30.94±1.99^b	31.48±2.23^b	32.04±2.74^b	1.17
p-values	0.0887	0.2144	0.0711	0.0477	0.0023	0.0004	0.0155	
Level of significance	ns	ns	ns	**	**	**	**	

All values were expressed in %, *Significant at 5% level of probability, **Significant at 1% level of probability, ns: Not significant, In a column figures with same or without superscript do not differ significantly as per DMRT

But in other two groups (T_C and T_D), the Hb values were reduced upto 9.64% and 6.07% respectively. The reduction of Hb values were significant on 30 day of treatment ($p<0.05$) and on 45, 60, 75 and 90 day of treatment ($p<0.01$) in comparison to control group. In other treatments, the Hb values were not statistically significant ($p<0.05$).

Packed cell volume (PCV): Similar to Hb, the PCV values were also decreased significantly ($p<0.01$) to the extent of 27.88% in arsenic treated group (T_A) in relation to control group (T_0). However, in arsenic plus spirulina treated group (T_B), PCV value was reduced upto 14.07%. But in other two groups (T_C and T_D), the PCV values were reduced upto 3.63 and 1.17%, respectively (Table 4).

Table 5: Effect of spirulina on serum glutamate pyruvate transaminase in arsenic treated mice

	Days (Mean±SE)							
Treatment	0	15	30	45	60	75	90	Increase (%)
Control (T_0)	35.05±0.07	35.13±0.07	35.39±3.32^{d}	35.80±0.45^{e}	35.96±3.63^{e}	36.10±0.81^{e}	36.50±3.67^{d}	-
Arsenic trioxide 1 mg/2 mL drinking water (T_A)	37.20±0.11	45.31±0.11	51.05±1.79^{a}	56.12±0.52^{a}	59.42±3.3l^{a}	63.46±0.93^{a}	65.46±6.93^{a}	75.96
Arsenic trioxide 1 mg+spirulina 2 mg/2 mL drinking water (T_B)	35.26±0.34	39.36±0.34	43.91±8.03^{b}	44.37±0.92^{b}	44.99±4.37^{b}	46.61±1.71^{b}	47.71±6.08^{b}	40.98
Arsenic trioxide 1 mg+spirulina 4 mg/2 mL drinking water (T_C)	35.61±0.17	37.12±0.17	38.19±0.78^{c}	38.97±1.88^{c}	43.12±0.84^{c}	45.50±0.79^{c}	47.65±1.76^{b}	27.77
Arsenic trioxide 1 mg+spirulina 8 mg/2 mL drinking water (T_D)	36.12±0.37	37.29±0.37	39.83±0.58^{cd}	41.46±1.55^{d}	41.64±1.05^{d}	42.98±0.89^{d}	43.61±1.62^{c}	21.76
p-values	0.5212	0.331	0.0541	0.0025	0.0049	0.0007	0.0020	
Level of significance	ns	ns	*	**	**	**	**	

All values were expressed in U L^{-1}, *Significant at 5% level of probability, **Significant at 1% level of probability, ns: Not significant, In a column figures with same or without superscript do not differ significantly as per DMRT

Table 6: Effect of spirulina on serum glutamate oxaloacetate transaminase in arsenic treated mice

	Days (Mean±SE)							
Treatment	0	15	30	45	60	75	90	Increase (%)
Control (T_0)	138.20±3.29	138.24±3.29	138.64±18.22	138.90±4.85^{b}	139.40±13.68^{b}	139.90±5.07^{b}	141.05±10.89^{b}	-
Arsenic trioxide 1 mg/2 mL drinking water (T_A)	139.50±6.91	149.08±6.91	157.98±28.15	163.80±5.41^{a}	177.35±29.51^{a}	187.30±8.55^{a}	195.85±33.38^{a}	40.39
Arsenic trioxide 1 mg+spirulina 2 mg/2 mL drinking water (T_B)	138.00±7.58	143.46±7.58	149.14±26.35	155.32±4.19^{c}	167.36±26.58^{c}	173.34±6.68	185.39±24.04^{c}	29.22
Arsenic trioxide 1 mg+spirulina 4 mg/2 mL drinking water (T_C)	140.80±7.51	141.94±7.51	145.16±4.07	152.10±3.77^{cd}	159.06±2.99^{cd}	163.56±3.74^{c}	167.38±4.71^{d}	17.99
Arsenic trioxide 1 mg+spirulina 8 mg/2 mL drinking water (T_D)	142.70±4.91	144.26±4.91	145.09±4.14	149.34±3.15^{d}	153.28±3.58^{d}	159.32±4.14^{d}	160.28±3.18^{e}	11.22
p-values	0.041	0.412	0.1140	0.0058	0.0091	0.0037	0.0002	
Level of significance	ns	ns	ns	**	**	**	**	

All values were expressed in U L^{-1}, *Significant at 5% level of probability, **Significant at 1% level of probability, ns: Not significant, In a column figures with same or without superscript do not differ significantly as per DMRT

The reduction of PCV values were statistically significant($p<0.01$) on 45, 60, 75 and 90 day of treatments in comparison to control group. In other treatments, the PCV values were not statistically significant ($p<0.05$).

Biochemical parameters

Serum glutamate pyruvate transaminase (SGPT): The SGPT values increased (75.96%) significantly ($p<0.01$) in arsenic trioxide fed group (T_A) in relation to control group (T_0). But the increasing percentage of SGPT values in other three groups (T_B, T_C and T_D) were 40.98, 27.77 and 21.76%, respectively which was less than T_A group. The increased SGPT values were statistically significant ($p<0.01$) on 45, 60, 75 and 90 day of treatment and on 15 day of treatment ($p<0.05$) in comparison to control group (Table 5).

Serum glutamate oxaloacetate transaminase (SGOT): The SGOT values were also increased significantly ($p<0.01$) to the extent of 40.39% in arsenic trioxide treated group (T_A) in comparison to control group (T_0). However, the values were increased to a level of 29.22, 17.99 and 11.22% in T_B, T_C and T_D groups, respectively (Table 6). The increase of SGOT values were statistically significant ($p<0.01$) on 45, 60, 75 and 90 day of treatment in comparison to control group. In other treatments, the SGOT values were not statistically significant ($p<0.05$).

Serum alkaline phosphatase (ALP): The ALP values were increased significantly ($p<0.01$) upto 19.01% in arsenic trioxide fed group (T_A) in relation to control group (T_0). But the increasing percent of ALP values in other three groups i.e., T_B, T_C and T_D were 14.97, 10.56 and 4.90%, respectively which were less than arsenic treated group (T_A) (Table 7). The increase of ALP values were statistically significant ($p<0.01$) on 45, 60, 75 and 90 days of treatment and on 30 days of treatment ($p<0.05$) in comparison to control group. In other treatments, the ALP values were not statistically significant ($p<0.05$).

Table 7: Effect of spirulina on serum alkaline phosphatase in arsenic treated mice

	Days (Mean±SE)							
Treatment	0	15	30	45	60	75	90	Increase (%)
Control (T_0)	260.70±3.55	261.94±3.55	264.32±3.15^e	266.28±4.92^e	269.38±3.16^e	271.32±3.83^e	273.98±3.28^e	-
Arsenic trioxide 1 mg/2 mL drinking water (T_A)	265.00±3.97	265.68±3.97	276.56±4.07^a	290.52±5.05^a	305.36±4.63^a	321.38±4.49^a	338.32±5.68^a	19.01
Arsenic trioxide 1 mg+ spirulina 2 mg/2 mL drinking water (T_B)	262.50±5.15	263.56±5.15	273.28±3.87^b	284.14±4.77^b	295.98±4.12^b	308.36±3.61^b	322.24±3.86^b	14.97
Arsenic trioxide 1 mg+ spirulina 4 mg/2 mL drinking water (T_C)	261.75±5.21	262.38±5.21	270.16±3.51^c	277.96±3.38^c	286.62±2.87^c	295.58±2.57^c	306.36±3.16^c	10.56
Arsenic trioxide 1 mg+ spirulina 8 mg/2 mL drinking water (T_D)	260.00±3.10	260.44±3.10	266.32±2.61^d	271.88±2.78^d	277.34±3.08^d	280.94±3.96^d	288.12±2.90^d	4.09
p-values	0.2247	0.1355	0.0247	0.0052	0.0007	0.0021	0.0003	
Level of significance	ns	ns	*	**	**	**	**	

All values were expressed in U L^{-1}, *Significant at 5% level of probability, **Significant at 1% level of probability, ns: Not significant, In a column figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly

DISCUSSION

Arsenic induced mice showed several clinical sign during the experimental period. Toxic signs observed in T_A group (only arsenic trioxide) is an agreement with the previous study (Islam *et al*., 2009) who reported that ducks of arsenic trioxide group showed depression, reduced feed intake, dullness and ruffled feathers which were in meek in nature in other groups i.e., arsenic plus spirulina. The present findings also support the report of previously conducted study which stated that arsenic treated rats showed severe symptoms of excitement, restlessness, anorexia, ruffled hair coat and skin lesions in all parts of the body, especially on tail region (Islam *et al*., 2005). Arsenic-induced skin lesions with definite skin pigmentation and keratosis on the palms and soles was observed in the population of Atacameno in Northern Chile with drinking water containing 750 to 800 μg L^{-1} of inorganic arsenic (Smith *et al*., 2000). Mitra *et al*. (2004) reported that low intake of calcium, animal protein, folate and fiber may increase susceptibility to arsenic-caused skin lesions. The inorganic forms of arsenic are classified as carcinogens, with chronic exposure (10-40 μg day^{-1}) associated with skin, respiratory and bladder cancers (Lasky *et al*., 2004).

In case of body weight, the present findings are in partial agreement with previously conducted study (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. This study showed that As-intoxication significantly reduced body weight gain of mice which comply with previous work (Akter *et al*., 2010; Islam *et al*., 2001; Sharma *et al*., 2007). Arsenic significantly ($p<0.01$) reduced the body weight in experimentally induced arsenic toxicities in mice (Islam *et al*., 2001). Sharma *et al*. (2007) reported that body weight was decreased in arsenic treated group of Swiss albino mice. Mahaffey *et al*. (1981) studied concurrent exposure to lead, cadmium and arsenic and effects on toxicity and tissue metal concentrations in the rat. Cadmium and As reduced weight gain even when differences in food intake were taken into account and administration of both Cadmium and As depressed weight gain more than did either metal alone. In accordance to the present findings significantly ($p<0.01$) reduced body weight was also observed in arsenic induced rats (Islam *et al*., 2005).

In present study hematological parameters were reduced by arsenic has been reported by many authors (Islam *et al*., 2005, 2009). However, in arsenic plus spirulina treated rest groups reduction of TEC, Hb and PCV were less than arsenic treated groups. The TEC, Hb and PCV values were significantly ($p<0.01$) reduced but ESR value was significantly ($p<0.01$) increased in arsenic treated rats (Islam *et al*., 2005). In arsenic treated rabbit, the TEC, Hb and PCV values reduced significantly ($p<0.01$) and ESR values increased significantly ($p<0.01$). In contrary, Mahaffey *et al*. (1981) observed the increased numbers of circulating RBCs in rats. However, they found that hemoglobin and hematocrit values were reduced in arsenic toxicities in rats as observed in the present study. The cause of change in hematological values might be due to the toxic effect of arsenic on haematotopoeitic system which is responsible for such alterations in hematological parameters. However, Islam *et al*. (2005) assumed that toxic effects of arsenic trioxide on bone marrow may be responsible for erythrocytopenia.

The serum levels of amino transferases 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 (SGPT, SGOT and ALP) were significantly ($p<0.01$) elevated in arsenic treated groups of mice. However, the elevation of these parameters was less in arsenic plus spirulina treated groups of mice which support the previous study (Islam *et al*., 2009). 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. Significantly higher arsenic effluxes ($p<0.01$) in blood and urine were associated with arsenic intoxication and the increment was continued with the elapse of exposure duration to goat. Previous study in layer birds observed that the SGPT, SGOT, ALP and LDH were increased due to dietary arsenic (Chiou *et al*., 1999). Similar to the present findings, elevation of biochemical parameters due to arsenic poisoning have been reported by many authors (Islam *et al*., 2005; Sharma *et al*., 2007; Olayemi *et al*., 2002). Islam *et al*. (2009) reported that SGPT and SGOT values were increased significantly ($P<0.01$) in arsenic treated rats. Similarly, Sharma *et al*. (2007) found that SGPT, SGOT, ALP and ACP were increased in arsenic treated group of Swiss albino mice. No change in SGPT was observed after arsenic supplementation (Kaur *et al*., 2005).

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_A group (only arsenic treated group). The increase of these biochemical parameters were less in remaining three groups (T_B, T_C and T_D) which were given spirulina in three different doses along with arsenic trioxide. It was also noticed that the rise of biochemical parameters were minimum with higher dose of spirulina (8 mg/2 mL) in drinking water indicating that spirulina has 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. So, these findings indicate that spirulina has the positive role in decreasing the increased biochemical parameters due to arsenic toxicities.

CONCLUSION

This study showed that arsenic is harmful to the body showing toxic signs, reduced body weight and alteration of some hematological and biochemical parameters. Spirulina was found to be effective in the reduction of body burden of arsenic. The present study is a preliminary work on the effects of spirulina in arsenic induced toxicities in Bangladesh. However, the result of this research work will certainly help the future researchers to provide guidance in carrying out further detail study in this aspect in Bangladesh and abroad.

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