Original Research Article

Comparative Effects of Spirulina and Vitamin C Against Arsenicosis in Rat

ABSTRACT

An experiment was conducted with Long Evans rats to observe the comparative effects of Spirulina (*Spirulina platensis*) and Vitamin C to prevent Arsenic toxicity. Sixty male rats (age about 35 days; average body weight at day 0 was 75 g) were divided into five equal groups, namely control group (T_0), Arsenic treated group (T_1), Arsenic plus Spirulina treated group (T_2), Arsenic plus Vitamin C treated group (T_3) and Arsenic plus Spirulina plus Vitamin C treated group (T_4). Rats in the control group were given normal feed. Rats of T_1, T_2, T_3 and T_4 treated group were given Arsenic trioxide (As_2O_3) 100 mg/kg body weight daily for 45 days. In addition to As_2O_3, rats of group T_2 and T_4 were fed with Spirulina @ 1 g/kg of feed and T_3 and T_4 were fed with Vitamin C @ 250 mg/kg body weight simultaneously. Four rats from each group were sacrificed at 15 days interval to collect blood samples. The research result showed that higher body weight was found except T_1 group. It indicates administration of Spirulina tended to bring the body weight towards the normal. The values of SC increased significantly ($P<0.01$) in all the treated groups (T_1, T_2, T_3 and T_4) compared to the control group. Though the values of SGOT showed little significance ($P<0.05$) but SGPT showed highly significant value ($P<0.01$) among the treated groups. Spirulina with Vitamin C increased the significant values of TEC, TLC and Hb compared to control. The overall results of the study showed that the combined of Spirulina and Vitamin C were found more effective to decrease the level of arsenic and also to prevent the chronic arsenicosis in rats than the solitary use of Spirulina or Vitamin C.

Keywords: Arsenic; Spirulina; Vitamin C; Hematological and Biochemical parameters.

1. INTRODUCTION

As a natural element, Arsenic (As) is found in the earth's crust. In Bangladesh groundwater arsenic contamination is reported to be the biggest arsenic calamity in terms of the affected population in the world [1]. The Government of Bangladesh accepts the safety limit of arsenic is 0.05 mg/liter of drinking water [2]. The arsenic limit for drinking water is 0.01 mg/liter and far foodstuffs is 2 mg/liter on a fresh weight basis [3]. With many human health conditions, including skin lesions and cancers of the liver, lung, bladder and skin, chronic arsenic exposure is associated [4]. Acute, intermediate or chronic exposure to develop anemia and leucopeniain [5]. The SGOT, SGPT, serum creatinine, urea, uric acid levels and various hemato-logical parameters like TEC, TLC, Hb, blood sugar level changes due to the presence of arsenic in the Swiss albino rats [6]. About half of the total population (more than 50 millions) of Bangladesh is consuming arsenic (As) through drinking and cooking. Among them, more than 40,000 people have already developed the signs and symptoms of chronic arsenic toxicity [7,8]. Nearly 61, out of 64 districts of the country`s tube wells contain dangerous levels of inorganic As, where the tube wells are serving as main sources of water for drinking and cooking purposes. Spirulina (*Spirulina platensis*), one kind of algae having a very high congregation of micronutrients and vitamins may have good effects on people suffering from heavy metal poisoning suggested by clinical study. It is a blue-green algae, is rich in protein, phytonutrients, antioxidants, and polysaccharides that might have mitigating effects against heavy metal poisoning [9]. It is recommended as a chemoprotective against arsenic-induced toxicity in humans [10]. Vitamin C is a water soluble vitamin and also act as the effective antioxidant. For numerous intrinsic processes Vitamin C is essential. The most well-known and understood process of vitamin C is that of healing.. The reduction of arsenic induced higher blood glucose level of folic acid and vitamin C demonstrates that that have significant effect in preventing arsenic induced diseases [11]. In Bangladesh, elaborate data are available for arsenic only on tube-well water; however, data on the specific treatment in prevention of arsenic toxicity in both humans and animals is very limited. Therefore, this investigation was carried out to determine the efficacy of Spirulina and Vitamin C and their comparative as well as combined efficacy against arsenic toxicity of rat on the basis of body weight with some hematological and biochemical parameters.

2. MATERIALS AND METHODS

2.1 Place of Experiment

The investigation was conducted 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 during the period of 12th September to 10th November 2017.

2.2 Experimental Material

Sixty male rats of about 35 days of age were used in this experiment. All the 60 rats were bred in the laboratory of the International Center for Diarrheal Disease Research Bangladesh (ICDDRB). The animals were housed in a compartmented rectangular metallic cage under standard laboratory conditions (12h light: 12h dark, 25 ± 2^0C and humidity 60 ± 5%). Rats were acclimatized for 15 days in the laboratory before the experiment started.

2.3 Preparation of House

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

2.4 Test Chemicals

Arsenic trioxide (As_2O_3) was purchased from a scientific laboratory. Spirulina capsule (Navit®) and Vit. C tablet Cevit® was collected from Square Pharmaceuticals Limited.

2.5 Preparation of Treatment Materials

2.5.1 Arsenic trioxide solution

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

2.5.2 Spirulina mixed feed

Each capsule of Spirulina containing 500mg of Spirulina platensis. After opening of the capsule the powder of Spirulina was kept in a cup. With the help of electric balance the required amount of Spirulina (1 g/kg feed) was measured and make it a suspension by adding a small amount of distilled water. The feed was stirred with 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 50^0C overnight and kept in air-tied plastic container then supplied to rats ad libitum.

2.5.3 Vitamin C mixed feed

Each tablet contained 250 mg of vitamin C. The tablet was made into a homogeneous powder with the help of pestle and mortar. Then the powder was mixed with required amount of distilled water and stirred with a glass rod for

homogenous mixing. After that the mixed solution was added into drinking water and provided to rats.

2.6 Experimental Animal Grouping

Sixty rats were used in this investigation. These rats were divided into five groups containing 12 rats in each group. For identification they were individually marked using different color on their tail tips.

2.7 Experimental Trial

The experimental trial was conducted for 45 days. Rats of control group (T_0) were maintained by providing normal pellet feed and water adlibitum, that of group T_1 were treated with arsenic trioxide at a dose of 100 mg/L drinking water. The rats of group T_2 were treated with arsenic trioxide at 100 mg/L in drinking water daily and Spirulina simultaneously at a dose of 1 g/kg feed. The rats of group T_3 were treated with arsenic trioxide at 100 mg/L in drinking water daily and Vit. C simultaneously at a dose of 250 mg/kg body weight. The rats of group T_4 were treated with arsenic trioxide at 100 mg/L in drinking water daily and Vit. C at a dose of 250 mg/kg body weight and Spirulina at a dose of 1 g/kg feed. All treatments were given for 45 days.

2.8 Body Weight (BW)

Firstly the rats were individually weighed on day 0 (day 0= immediate previous day of starting treatment) after grouping and marking. After that, day 15, day 30 and finally on day 45 the results were recorded.

2.9 Clinical Signs

After feeding arsenic trioxide, Spirulina and Vit. C the experimental rats were closely observed daily for 3 times (morning, afternoon and evening). If any toxic sign was found in the rats during the experimental period it was recorded.

2.10 Sampling

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

2.11 Statistical Analysis

The collected data were statistically analyzed as per Steel and Torrie (1980) using Completely Randomized Design (CRD). Analysis of variance (ANOVA) and

Duncan's Multiple Range Test (DMRT) were performed with the help of SPSS 20 software to find out the difference among the treatments.

3. RESULTS AND DISCUSSION

Arsenicosis in rats increased the concentrations of arsenic in lung, liver, kidney and blood. In the above tissues Spirulina and vitamin C treatment lowered arsenic contents where Spirulina found more effective in reducing arsenic content from tissues except blood where vitamin C found more effective. The combined amount of vitamin C and Spirulina was more effective compared to the sole treatment.

3.1 Clinical Signs

There were no significant clinical signs observed in rats during the entire period of the experiment. But highly increased in the body weight were observed in all groups except arsenic treated group (T_1).

3.2 Body Weight of Rats

Body weights (BWs) of rats of all groups were taken on 0, 15, 30 and 45 days. The BWs on day 45 were found highest in rats of control group (T_0) . BWs were slightly increased in arsenic plus spirulina treated rats (T_2), whereas it was lost by the rats of arsenic T_1 treated group. On the other hand, T_2, T_3 and T_4 treated rats gained higher body weight than control. On day 30, BWs were found slightly increased in all groups except in group T_1 and the highest BWs gain was found in T_2, T_3 and T_4 treated group. On day 45 BWs were found slightly increased in T_2, T_3, T_4 and T_0 except arsenic T_1 groups group (Table 1). [12] reported the body weight increased due to spiraling addition in feed stuff of rat. The decreased body weight was observed in an arsenic treated group of Swiss albino mice [13]. [14] reported arsenic significantly ($p<0.01$) decreases the body weight of rats.

Table 1: Effects of Arsenic, Spirulina and Vitamin C on the body weight of rats

	Body weight (g)					
Treatments	**Group T_0 (Control)**	**Group T_1 (Arsenic)**	**Group T_2 (Arsenic+ Spirulina)**	**Group T_3 (Arsenic + Vitamin C)**	**Group T_4 (Arsenic + Spirulina + Vitamin C)**	**Level of significance**
Initial	78.20 ±3.28	82.20 ± 2.63	87.67 ± 3.08	83.06 ± 2.96	83.14 ± 3.70	NS

Day 15	171.00^{b} ±5.56	87.40^{a} ± 4.01	181.80^{b} ± 5.21	179.60^{b} ± 4.71	187.60^{b} ± 6.98	**
Day 30	220.20^{b} ± 4.31	94.80^{a} ± 2.63	261.20^{c} ± 2.92	250.30^{c} ± 4.01	259.60^{c} ± 3.92	**
Day 45	255.80^{b} ± 5.12	97.00^{a} ± 2.43	299.40^{c} ± 3.70	297.80^{c} ± 4.68	303.40^{c} ± 2.06	**

In a row figurers with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99% level of significance ($p<0.01$).
Figures indicate the Mean ± SE (standard error); NS means not significant
*= Significant at $p<0.05$ level of probability

3.3 Hematological parameter

3.3.1 Total Erythrocyte Count (TEC)

On day 15, total erythrocyte counts were found highest in arsenic plus Spirulina (T_2) group and lowest in arsenic treated group (T_1) and arsenic plus Vitamin C treated rats (T_3) and this decrease was not statistically significant. In T_4 treated group, TEC of rats were slightly decreased and the value was not significant compared to control treatment. On day 30 and 45 highest TEC value were recorded maximum in T_4 treated group and minimum in arsenic treated group (T_1) and found significant at the 5% level of significance (Table 2). The effects observed in this study and outcomes of treatment using Spirulina plus vitamin C treatment had earlier been corroborated by [15,16]. They reported decreasing RBC level with increased concentration of arsenic due to arsenic metabolism and its methylating activity.

Table 2: Effects of Arsenic, Spirulina and Vitamin C on TEC of rats

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

In a row figurers with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99% level of significance ($p<0.01$).

Figures indicate the Mean ± SE (standard error); NS means not significant
= Significant at p<0.05 level of probability= Significant at p<0.05 level of probability

3.3.2 Total Leukocyte Count (TLC)

Total leukocyte count on day 15,30 and 45 in rats was found highest (10.87± 0.01) in the control group (T_0) and lowest (9.09 ± 0.00) in arsenic treated group (T_1) and this change was statistically significant (p<0.01). The value of TLC in the other group is gradually increased which are statistically significant (Table 3). The result is dissimilar to the findings of [6]; but contradicts that of [10,11,17] where they found the WBC level decreased when rat were given higher dose of arsenic and that might be due to the apoptotic effect of arsenic on plasma cells. Both the vitamin C and Spirulina increase the amount of granulocytes and agranulocytes (leukocytes content) those are responsible for providing immunity to the body. Arsenic content decreased total leukocyte count by altering phagocytic activity of the rat. Arsenic leds to malfunctioning of enzymatic system and denaturation of cell organelles. It alters plasma membrane permeability and/or increased mechanical fragility. That's why apoptasis occur on plasma cells of rats due to the adverse effect of arsenic. Besides this, vitamin C and Spirulina synthesize white blood cells in rats.

Table 3: Effects of Arsenic, Spirulina and Vitamin C on TLC of rats

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

In a row figurers with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99% level of significance (p<0.01).
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

3.3.3 Hemoglobin Concentration (Hb)

The Hemoglobin (Hb) concentrations were statistically significant ($p<0.05$) and the highest value was observed in T_4 treatment on day 15 and 45 (14.35± 0.90 and 16.75 ± 1.37 respectively) but on day 30 it was highest (15.25 ± 0.78) in T_2 treatment ($p<0.01$). The lowest value of Hemoglobin (Hb) was found in T_1 treated group for all the days of observation (Table 4). It might be said that the values of Hb against arsenic toxicity in rats might slightly increase due to Spirulina and Vitamin C application. Arsenic creates a barrier around the blood by making a thin layer. That's why haemoglobin contents decreases with the advancement of the arsenic treatment. Arsenic creates a barrier around the blood by making a thin layer. That's why haemoglobin contents decreases with the advancement of the arsenic treatment.

Table 4: Effects of Arsenic, Spirulina, Vitamin C on Hb

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

In a row figurers with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99% level of significance ($p<0.01$).
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

3.4 Biochemical parameters

3.4.1 Serum Glutamate Oxaloacetate Transaminase activity (SGOT)

The values of SGOT were decreased significantly ($P<0.01$) at day 45 where there was an insignificant difference on day 15 and day 30 in the blood samples of the treated groups of rats (T_1, T_2, T_3 and T_4) compared to control (Table 5). Although this finding agreed with the previous findings that SGOT was reduced as alone [18]

disagreed with the findings of [8, 11,18, 19]. It concurred with the findings of [6] who indicated similar results.

Table 5: Effects of Arsenic, Spirulina and Vitamin C on SGOT of rats

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

In a row figurers with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99% level of significance ($p<0.01$).
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

3.4.2 Serum Glutamate Pyruvate Transaminase activity (SGPT)

Although the levels of SGPT in serum differ significantly at day 45 and day ($P<0.05$), it normalized in all groups and there was no significant difference on day 15 and day 30 except for the arsenic group (Table 6). It is possible that individual treatment using Vitamin C or Spirulina or a combination of these two will produce a significantly different result in long term arsenicosis. Interestingly, the level of SGPT did not change drastically in arsenic treated group (T_1) between days 15 and 30. It may be that once a peak level of arsenicosis is reached, the SGPT level will adjust to it and maintain a peak value. [20] had earlier found that no change was observed in SGPT level associated with arsenicosis over a 90-day period. However, the result of this finding was not corroborated with the finding of [11,14,18] who observed a highly significant ($P <0.001$) elevation in ALT activity of arsenic-intoxicated mice with respect to control animals for 30 days treatment.

Table 6: Effects of Arsenic, Spirulina and Vitamin C on SGPT of rats

Treatments	SGPT(U/L)					
	Group T_0 (Control)	Group T_1 (Arsenic)	Group T_2 (Arsenic+ Spirulina)	Group T_3 (Arsenic + Vitamin C)	Group T_4 (Arsenic + Spirulina	Level of significance

					+ Vitamin C)	
15 Days	71.67± 0.89	77.00 ± 1.53	64.33 ± 7.17	73.00 ± 2.52	68.00 ± 4.93	NS
30 Days	73.00 ± 0.58	76.00 ± 3.51	68.67 ± 8.95	68.33 ± 2.67	69.00 ± 2.08	NS
45 Days	74.00 ± 0.00[a]	89.33 ± 4.84[b]	68.67 ± 7.36[a]	63.67 ± 5.24[a]	64.00 ± 2.08[a]	*

In a row figurers with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99% level of significance ($p<0.01$).
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

3.4.3 Serum Creatinine

There were significant differences found in serum creatinine levels among the control group and all other treated groups throughout the study period. The values of serum creatinine were found lowest in the control group at day 15. The values in T_4 group are very similar than that of control group (T_0). The differences between the mean values of different groups were found significant ($p<0.01$). On day 30 the mean values of the T_0, T_2 and T_4 were found somewhat similar and found highest in the arsenic treated group (T_1). The differences between the mean values of different groups were found significant ($p<0.01$). Again, On day 45 lowest mean values was found in T_2 group and the highest mean value was found in arsenic (T_1) and others are near about similar to control group or arsenic treated group. The differences between the mean values of different groups were found significant ($p<0.01$) (Table 7). The findings disagreed with the results of [21] in the human being which showed that patients of arsenicosis had significantly lower levels of serum creatinine compared to the control. It is possible that the differences observed in these two studies are related to the differences in species used for the study. [22] had also observed that there is a relationship between arsenic level and degree of chronic renal insufficiency in men. The result of this study was dissimilar with the findings of [6,23,24] who concluded that there were no significant rises in the serum creatinine levels of arsenic treated mice. Although this finding agreed with the previous findings that serum creatinine was reduced by As alone [11,18] and slight reduction in serum creatinine was, however, observed with the time progression in our study for the Spirulina and Vitamin C treated group (T_4), suggesting that Spirulina and Vitamin C may improve kedny function.

Table 7: Effects of Arsenic, Spirulina and Vitamin C on Serum Creatinine of rats

Treatments	Serum creatinine (mg/dl)					
	Group T_0 (Control)	Group T_1 (Arsenic)	Group T_2 (Arsenic+ Spirulina)	Group T_3 (Arsenic + Vitamin C)	Group T_4 (Arsenic + Spirulina + Vitamin C)	Level of significance
15 Days	0.51 ± 0.01[a]	0.62 ± 0.00[b]	0.52 ± 0.01[a]	0.53 ± 0.003[a]	0.52 ± 0.01[a]	**
30 Days	0.52 ± 0.00[a]	0.65 ± 0.01[d]	0.54 ± 0.01[b]	0.61 ± 0.003[c]	0.51 ± 0.003[a]	**
45 Days	0.52 ± 0.006[a]	0.67 ± 0.003[c]	0.51 ± 0.005[a]	0.62 ± 0.005[b]	0.52 ± 0.005[a]	**

In a row figurers with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99% level of significance ($p<0.01$).
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. CONCLUSION

From this investigation, it can be concluded that the treatment with Spirulina and Vit. C lowered the value of arsenic toxicity. Spirulina and Vit. C increase the body weight and also has a significant effect on hematological and biochemical parameters in rats. This study suggested that the combined of Spirulina and Vitamin C were found more effective to reduce the level of arsenic and also helps to prevent the chronic arsenicosis in rats than the solitary use of Spirulina or Vitamin C.

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