

Effect of Spirulina (Spirulina platensis) And Riboflavin Against Arsenic Toxicity In Rat

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Abstract:

This study was undertaken to observe the effects of spirulina (*Spirulina platensis*) and riboflavin in hematological and biochemical parameters against arsenic toxicity in Long Evans rat. Sixty male rats were taken to perform the study. Experimental rats were divided into five equal groups. Each group consists of 12 rats. Animals of T0 group were given normal feed and water and kept as control. Rats of T1, were given arsenic trioxide @ 100 mg/L drinking water orally. Rats of group T2 were given arsenic trioxide @ 100 mg/L drinking water and with spirulina @ 1 gm/kg feed. Group T3 were given arsenic trioxide @ 100 mg/L with riboflavin @ 10mg /kg body weight. Group T4 were given arsenic trioxide and spirulina and riboflavin with same dose up to 45 days respectively. Four rats from each group (T0, T1, T2, T3 and T4) were sacrificed at 15 days interval to determine body weight, hematological and biochemical parameters. Result showed that in group T1, body weight gain was minimum, whereas in group T2, T3 and T4 the body weight gain in rats were better. Reduction of TEC, Hb values were significant ($P < 0.01$) in T1 group. Whereas in rest groups reduction of TEC, Hb were less than arsenic treated groups. The values of SGOT and SGPT were significantly ($P < 0.01$) decreased in all group but in it is more effective in combined group. In conclusion, spirulina and riboflavin has significant effect on hematological and biochemical parameters and increase body weight.



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1. INTRODUCTION

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). The safety limit of arsenic accepted by Bangladesh Government is 0.05 mg/liter for drinking water (WHO, 1999). The World Health Organization limit for drinking water 0.01 mg/liter and for foodstuffs is 2 mg/liter on a fresh weight basis (Robinson *et al.*, 2003). Chronic arsenic exposure is associated with many human health conditions, including skin lesions and cancers of the liver, lung, bladder and skin (Uddin and Huda, 2011). Arsenicosis presents with significant changes in the SGOT, SGPT, serum creatinine, urea, uric acid levels and various hematological parameters like TEC, TLC, Hb, blood sugar level in the Swiss albino rats (Yasmin *et al.*, 2011). Still there is no specific curative treatment against arsenic toxicity. Chelation therapy is recommended through intravenous line but the use of chelators in patients exposed to arsine gas is controversial (Anderson *et al.*, 2016).

Spirulina is a microscopic filamentous aquatic non-toxic blue-green algae belongs to the group cyanobacterium (Spolaore *et al.*, 2006) that is rich in proteins, lipids, carbohydrates, β -carotene, riboflavin, α -tocopherol and α -linoleic acid (El-Desoky *et al.*, 2013). It is not only a whole food, but it seems to be an ideal therapeutic supplement. It also exhibits antiviral (Hernández-Corona *et al.*, 2002), anti-bacterial (Ozdemir *et al.*, 2004), anti-platelet, anti-cardiotoxic, hypocholesterolemic and anti-nephrotoxic effects (Khan *et al.*, 2006). Phycocyanin of spirulina also prevents cancer and its growth (Peto *et al.*, 1981; Shekelle *et al.*, 1981). The bioremediation potential of spirulina against heavy metal ions in industrial effluents was studied by various researchers (Balaji *et al.*, 2014). It is reported that administration of spirulina provides a protective mechanism against arsenic induced toxicity in goats (Ghosh A., *et al.*, 2014). It is found that combined treatment of using spirulina and vitamin A is effective against chronic arsenicosis in rat (Hossain *et al.*, 2013). It has also protective effect against galactosamine-induced hepatotoxicity in mice (Vedi M., *et al.*, 2013). Spirulina is helpful on toxic signs, body weight and hematological parameters in arsenic induced toxicities in ducks (Islam *et al.*, 2009). Riboflavin, also known as vitamin B₂ is one of the B vitamins, which is water soluble. It is an important micronutrient that plays a key role in maintaining health in humans and animals. It is a precursor of FMN and FAD, which act as electron carriers, and therefore, plays an important role in a range of redox reactions, mitochondrial energy production, and cellular function (Depeint *et al.*, 2006). Riboflavin which causes plants to produce ROS when exposed to light, is an excellent photosensitizer for biocidal reactions. Riboflavin treatment reduces apoptosis and oxidative DNA damage in a rat spinal cord injury model (Sakarcan S. *et al.*, 2017).

In Bangladesh, data on the specific treatment for the prevention of arsenic toxicity in both human and animals is very little. Therefore, data on the effective prevention of arsenic toxicity with Spirulina and riboflavin and their combined efficacy will be the expected new findings especially for Bangladesh as well as for the world. So in the context of above, the present study was undertaken with the following objectives:

- I. To determine the efficacy of Spirulina and Riboflavin on arsenic induced toxicity in rats.
- II. To know the effect of arsenic, spirulina and riboflavin on body weight, hematological and biochemical parameters in arsenic fed rats.

CHAPTER 2

2. MATERIALS AND METHODS

This experiment was conducted during the period between 12th September to 10th November 2017 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.

2.1 Experimental animal

A total of 60 male Long Evan rats of 6 weeks age was purchased from International Centre for Diarrhoeal Disease Research Bangladesh (ICDDRDB). The animals were housed in compartmented rectangular metallic cage under standard laboratory conditions (12 h light: 12 h dark, 25 ± 2°C and humidity 60 ± 5%). Rats were acclimatized for 15 days in the laboratory before the experiment started.

2.2 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.

2.3 Test Chemicals

Arsenic trioxide was purchased from a scientific laboratory. Spirulina capsule (Navit®) was collected from Square Pharmaceuticals Limited and Riboflavin (Riboson®) from Jayson Pharmaceuticals Limited.

2.4 Preparation of treatment materials

2.4.1 Arsenic trioxide solution

On the basis of the total body weight of the rats, the required amount of arsenic trioxide for a day (100mg/L drinking water) was weighted separately for each group of rats. The respective pre-weighed arsenic trioxide was mixed with the drinking water daily for that 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*.

2.4.2 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 rats *ad libitum*.

2.4.3 Riboflavin mixed Water

Each Tablet of Riboflavin (Reboson®; Jayson Pharmaceuticals Limited; Bangladesh) containing 5mg of riboflavin. The tablet was made to a homogeneous powder with the help of

pestle and mortar. Then the powder was mixed with required amount of distilled water and simultaneously the water was stirred by a glass rod for homogenous mixing. After completion of proper mixing, the mixed water was provided to rat.

2.5 Experimental animal grouping

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

2.6 Experimental trial

The experimental trial was conducted for 45 days. Rats of Group T₀ were maintained with only normal pellet feed and water *ad libitum* as control, that of Group T₁ were treated with arsenic trioxide at a dose of 100mg/L drinking water. The rats of Group T₂ 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 rats of Group T₃ were treated with arsenic trioxide at 100 mg/L in drinking water daily and riboflavin Tablet (Riboson®; Jayson Pharmaceuticals Limited; Bangladesh) simultaneously at a dose of 10mg/kg bodyweight. The animals of Group T₄ were treated with arsenic trioxide at 100mg/L in drinking water daily and riboflavin at a dose of 10mg/kg body weight and Spirulina (*Spirulina platensis*) simultaneously at a dose of 1gm/kg feed. All treatments were given for 45 days.

2.7 Body weight (BW)

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

2.8 Clinical signs

Experimental rats were closely observed after feeding arsenic trioxide and spirulina daily for 3 times (morning, afternoon and evening) for the appearance of any toxic signs if in them, during the entire experimental period (from Day 1 to Day 45) and the findings were recorded.

2.9 Sampling

After starting treatment of 15 days 4 rats from each group were anesthetized using chloroform anesthesia and they were sacrificed and about six milliliters (ml) of blood samples were collected directly from cardiac puncture of each rat by using disposable plastic syringe. The blood from each rat was then transferred into two tubes for determination of biochemical parameters, hematological test. For the biochemical test 4ml of blood sample was taken into pre-marked centrifuge glass test tubes immediately after collection. Collected blood kept at room temperature to allow it to clot properly then stored in a refrigerator overnight. Serum was separated following centrifugation of the blood in the next morning and the supernatant serum was taken into pre-marked Eppendorf tubes. The harvested serum were kept at -20°C until used. For the hematological test and detection of arsenic concentration in blood 1 ml of blood for each was taken separately into EDTA coated tube. The total lung, liver, and kidney were collected aseptically, washed with physiologic saline and were kept in the pre-marked zipper polythene bag. Bloods samples for hematological

investigation were preserved at 4°C temperature. All blood were taken 1st on Day 15, 2nd on Day 30, and 3rd on Day 45.

2.10 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

The experiment was conducted to determine the efficacy of Spirulina and Riboflavin on arsenic toxicity in rats. It was also undertaken to observe the effects of Spirulina and Riboflavin on body weight, hematological and biochemical parameters in arsenic fed rats. Sixty rats were randomly divided into five equal groups to conduct the experiment. T₀ group served as negative control and fed with normal diet. Group T₁ were treated with arsenic trioxide at a dose of 100mg/L drinking water and this group were kept positive control. Group T₂ were treated with same dose of arsenic trioxide and Spirulina (*Spirulina platensis*) simultaneously at a dose of 1 gm/kg feed. Group T₃ were treated with same dose of arsenic trioxide and riboflavin tablet simultaneously at a dose of 10mg/kg bodyweight. Group T₄ were treated combine with arsenic, spirulina and riboflavin at a same dose. All the treatment were continued for 45 days and treated rats were closely observed through the entire period.

3.1 Clinical signs

There were no significant change in clinical signs of arsenic toxicity were observed in trial rats during the entire experimental period.

3.2 Body weight (BW) of the rats

Body weights (BWs) of experimental rats of all groups were taken fifteen days interval on day 0, day 15, day 30 and day 45. Table 1 showed that the body weight gain was highest (299.40 ± 3.70) in T₂ group rats at 45 days but the body weight gain was lowest (96.60 ± 2.62) in arsenic treated T₁ group at 45 days whereas body weight gain in T₀, T₃ and T₄ were 255.80 ± 5.12 , 284.80 ± 7.15 , and 275.80 ± 2.85 which were better than arsenic treated T₁ group. The body weight of initial groups were not significant ($p > 0.05$) but in 15 days, 30 days and 45 days mean value of body weight were significant ($p < 0.01$).

The body weight of treated group were increased with their age but in T₁ 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 T₂ group where spirulina were treated with arsenic. 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 arsenic, arsenic plus riboflavin, and arsenic plus riboflavin Plus spirulina on the body weight of rats

Treatment	T ₀	T ₁	T ₂	T ₃	T ₄	P. value
Initial	78.20 ± 3.28	82.60 ± 2.50	88.46 ± 3.32	84.20 ± 4.32	85.00 ± 2.78	NS
15 days	171.00 ^b ± 5.56	87.40 ^a ± 4.02	181.80 ^{bc} ± 5.21	188.40 ^c ± 4.97	195.00 ^c ± 5.17	**
30 days	220.20 ^b ± 4.32	94.80 ^a ± 2.63	261.20 ^d ± 2.92	255.40 ^d ± 2.04	239.40 ^c ± 4.74	**
45 days	255.80 ^b ± 5.12	96.60 ^a ± 2.62	299.40 ^d ± 3.70	284.80 ^c ± 7.15	275.80 ^c ± 2.85	**

In a row figures 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 Hematological parameter

3.3.1 Total Erythrocyte Count (TEC)

In Table 2, Total Erythrocyte Count (TEC) values were highest ($8.53 \pm .14$) found in T₄ group at 45 days where spirulina and riboflavin were treated against arsenic toxicity but lowest ($6.35 \pm .25$) value was found in T₁ group where only arsenic were given. TEC value found at 15 days ($7.10 \pm .04$) and 45 days were significant ($p < 0.01$) and values found at 30 days ($7.63 \pm .28$) were significant ($p < 0.05$).

Table 2: Effects of arsenic, arsenic plus riboflavin, and arsenic plus riboflavin Plus spirulina on Total Erythrocyte Count (TEC) values of rats

Treatment	T ₀	T ₁	T ₂	T ₃	T ₄	P. Value
15 Days	6.40 ^a ± .13	6.20 ^a ± .04	6.48 ^a ± .13	6.43 ^a ± .13	7.10 ^b ± .04	**
30 Days	6.48 ^{ab} ± .18	6.23 ^a ± .04	6.98 ^b ± .18	6.98 ^b ± .13	7.63 ^c ± .28	*
45 Days	6.71 ^{ab} ± .25	6.35 ^a ± .25	7.34 ^c ± .26	7.55 ^c ± .17	8.53 ^d ± .14	**

In a row figures 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

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3.3.2 Total Leucocyte Count (TLC):

In Table 3, Total leukocyte counts on Day 30 was found highest ($10.87 \pm .005$) in control group rats and lowest in T₄ group rats where spirulina and riboflavin were treated and the

difference were statistically significant among all group of rats ($p < 0.01$). So it can be recommended that spirulina and riboflavin decrease the TLC level.

Table 3: Effects of arsenic, arsenic plus riboflavin, and arsenic plus riboflavin Plus spirulina on Total Leukocyte Count (TLC) values of rats

Treatment	T ₀	T ₁	T ₂	T ₃	T ₄	P. Value
15 Days	9.56 ^d ± .003	9.09 ^a ± .005	9.41 ^b ± .005	9.37 ^b ± .030	9.51 ^c ± .006	**
30 Days	10.87 ^e ± .005	10.24 ^a ± .005	10.42 ^c ± .005	10.27 ^b ± .005	10.76 ^d ± .006	**
45 Days	10.82 ^c ± .003	9.79 ^a ± .008	9.87 ^{ab} ± .005	9.88 ^b ± .010	10.80 ^c ± .058	**

In a row figures 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

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3.3.3 Hemoglobin (Hb):

Highest (15.25 ± .78) Hb concentration was found in T₄ group at 30 days and lowest concentration was found in T₀ group (Table 4). Difference among values of 30 days of Hb concentration were statistically significant ($p < 0.01$) and the difference among values of 15 and 45 days of Hb concentration were statistically significant ($p < 0.05$). It might be concluded that Spirulina and riboflavin might slightly increase the values of Hb against arsenic toxicity in rats.

Table 4: Effects of arsenic, arsenic plus riboflavin, and arsenic plus riboflavin Plus spirulina on Hemoglobin concentration (Hb) (gm/dl) values of rats

Treatment	T ₀	T ₁	T ₂	T ₃	T ₄	P. Value
15 Days	13.50 ^b ± .65	11.13 ^a ± .43	14.00 ^b ± .41	13.13 ^b ± .31	12.88 ^b ± .43	*
30 Days	11.03 ^b ± .89	8.45 ^a ± .65	15.25 ^c ± .78	14.98 ^c ± .72	15.25 ^c ± .78	**
45 Days	15.13 ^b ± .97	7.75 ^a ± 1.78	15.28 ^b ± .83	15.05 ^b ± 1.05	17.20 ^b ± 1.54	*

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

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3.4 Biochemical parameters

3.4.1 Serum Glutamate Oxaloacetate Transaminase activity (SGOT)

The highest values of SGOT were observed in the control group (T₀) while the lowest values were observed in the T₄ group (Table 5). There were significant differences within the groups during the two days (30 and 45) of measurement (P<0.01) and in 15 days significant differences within the groups of measurement (P<0.05). It appears that while Spirulina alone has some effect in lowering the SGOT values in response to prolonged administration of arsenic, the combination of Spirulina and riboflavin produced a more significant reduction in SGOT level comparable to the control group (P<0.01). Although this finding disagreed with the previous findings that SGOT was reduced by As alone (Mahaffey *et al.*, 1981). It is similar with the findings of Yasmin *et al.* (2011) who indicated similar results. In Spirulina treated (T₂), riboflavin treated (T₃) and Spirulina plus riboflavin treated (T₄) experimental arsenicosis groups, there were significantly decreased values of arsenic recorded (P<0.01).

Table 5: Effects of arsenic, arsenic plus riboflavin, and arsenic plus riboflavin Plus on SGOT values of rats

Treatment	T ₀	T ₁	T ₂	T ₃	T ₄	P. value
15 Days	115.00 ^c ± 2.89	110.00 ^{bc} ± 2.89	100.00 ^a ± 2.52	106.67 ^{abc} ± 4.41	102.00 ^{ab} ± .58	*
30 Days	109.00 ^{bc} ± 3.79	117.00 ^c ± 8.50	97.33 ^{ab} ± 1.45	98.67 ^{ab} ± 1.86	88.00 ^a ± 1.53	**
45 Days	109.67 ^b ± 3.18	141.67 ^c ± 6.23	106.67 ^b ± 1.67	105.00 ^b ± 2.89	84.33 ^a ± 2.33	**

In a row figures 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

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3.4.2 Serum Glutamate Pyruvate Transaminase activity (SGPT)

Continuous administration of arsenic to Long-Evans rats caused a significant increase in the blood SGPT level. The highest values of SGPT were observed in the T₁ group where the rats were treated with only arsenic. There were insignificant differences within the groups during days 15 and 30 but this difference became statistically significant (p<0.01) by day of 45. The lowest values of SGPT were observed in the T₄ group where combine administration of spirulina and riboflavin against arsenic toxicity. In 15 days and 30 days blood SGPT level were increased however it is not statistically significant (p > 0.05). In 45 days the level of blood SGPT were decreased and it is statistically significant (p<0.01) (Table 6). Overall SGPT values have decreasing trend with the progress of time in all groups which was agreed with the findings of (Islam, 2008). It may be concluded that prolonged treatment with spirulina and riboflavin may reduce the blood SGPT level.

Table 6: Effects of arsenic, arsenic plus riboflavin, and arsenic plus riboflavin Plus on SGPT values of rats

Treatment	T ₀	T ₁	T ₂	T ₃	T ₄	P Value
15 days	71.67 ± .89	77.00 ± 1.53	64.33 ± 7.17	72.67 ± 1.45	79.00 ± 1.52	NS
30 Days	73.00 ± .58	76.00 ± 3.51	68.67 ± 8.95	64.33 ± 2.33	70.67 ± .67	NS
45 Days	74.00 ^b ± .00	89.33 ^c ± 4.84	68.67 ^b ± 7.36	42.33 ^a ± .89	45.67 ^a ± 2.33	**

In a row figures 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

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3.4.3 Serum creatinine

Serum creatinine value were highest found in T₃ group at 45 days and lowest values were observed in control groups. The differences were found significant ($P < 0.05$) on day 15. The differences between the mean values of 30 and 45 days groups were found significant ($p < 0.01$). On the day 30 lowest mean value were observed in control group and highest mean value were observed in T₃ group rats and the differences were statistically significant ($p < 0.01$). On the day 45 lowest mean value were observed in T₂ group and highest mean value were observed in T₃ group rats and the differences were statistically significant ($p < 0.01$). The differences of As content between T₂ and T₃ were statistically significant ($P < 0.01$). However the As contents increased in T₁, T₃ and T₄ group but decreased in T₂ groups on day 30 compared to day 45. On day 45, the values of serum creatinine was the highest in T₃ group rats and lowest in T₂ group. The differences were observed significant ($P < 0.01$) on day 45 (Table 7). There was significant difference in serum creatinine level observed between the control group and all other treatment group rats through the whole study period. Which disagree with the findings of Nabi *et al.* (2005) in human being who showed that the patients of arsenicosis had significantly lower level of serum creatinine compared to the control. There is a relationship between arsenic level and degree of chronic renal insufficiency in men. Islam *et al.* (2009) and Roger *et al.* (2000) which concluded that there were no significant rises in the serum creatinine levels of arsenic treated mice.

Table 7: Effects of arsenic, arsenic plus riboflavin, and arsenic plus riboflavin Plus on Serum creatinine values of rats

Treatment	T ₀	T ₁	T ₂	T ₃	T ₄	P. Value
15 Days	.51 ^a ± .005	.62 ^c ± .003	.52 ^a ± .005	.59 ^c ± .042	.53 ^a ± .003	*
30 Days	.52 ^a ± .003	.65 ^c ± .005	.54 ^b ± .008	.64 ^c ± .005	.52 ^a ± .006	**
45 days	.52 ^a ± .006	.67 ^c ± .003	.51 ^a ± .003	.69 ^c ± .012	.57 ^b ± .023	**

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 \pm SE (standard error); NS means not significant

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4. CONCLUSION

From this study it can be concluded that treatment with spirulina and riboflavin reduce arsenic toxicity. Spirulina and riboflavin has significant effect on hematological and biochemical parameters and increase the body weight in rat. This study suggested that spirulina and riboflavin can be used in arsenic toxicity in rats. Further investigation to determine arsenic level in blood and histopathology may make more clear evidence to use spirulina as a therapeutic treatment for arsenic toxicity

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