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Effect of spirulina (*Spirulina platensis*) and vitamin E on arsenic induced toxicity in Quail

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Received: 26 February 2020/Accepted: 18 March 2020/ Published: 31 March 2020

Abstract: Chronic arsenic toxicity is a severe disease in men and animals which occurs severely in Bangladesh. Arsenic (As) contamination in ground water used in drinking is the major concern because arsenic is present in human and animal food chain. This work was done in quails with a view to observing the efficacy of spirulina (*Spirulina platensis*) and vitamin E for prevention of arsenic toxicity. 60 quails were used in this study and animals were divided into control group (T₀), arsenic treated group (T₁), arsenic plus spirulina treated group (T₂) and arsenic plus vitamin E treated group (T₃). Each group consists of 15 quails. Quails of T₀ group were given normal feed and water and kept as control. Quails of T₁, T₂ and T₃ were given 100 mg arsenic trioxide/L drinking water daily for 30 days. In addition to arsenic trioxide quails of group T₂ and T₃ were simultaneously fed with spirulina @ 1 gm/kg feed and vitamin E @ 400 mg /kg body weight up to 30 days respectively. Five quails from each group (T₀, T₁, T₂ and T₃) were sacrificed at 15 days interval in order to determine haematological parameters. Result showed that in group T₁, body weight gain was minimum, whereas in group T₂ and T₃ the body weight gain in quails were better. Reduction of TEC and Hb values were observed in arsenic treated group T₁. Whereas in rest groups the TEC and Hb values were comparatively higher than arsenic treated group. Noticeable change observed in liver and kidney of arsenic treated group in compare to the control group. Histopathological changes also observed in liver and kidney of arsenic treated group in compare to the control group. In conclusion, spirulina and vitamin E have significant effect on body weight, hematological and postmortem and histopathological changes.

Keywords: arsenic; spirulina; vitamin E

1. Introduction

Arsenic is one of the most sensitive environmental issue in Bangladesh even it is a major health concern in Asia. It creates a serious public health problem in developing countries. Arsenic can enter into food chain causing wide spread distribution throughout the plant and animal kingdoms. Arsenic is a shiny grayish non essential trace element that is widely distributed in nature. The inorganic forms of arsenic exhibit the highest toxicity level (FAO, 1983). Arsenic is a ubiquitous and one of the most potent toxic metalloids in environment. Globally millions of people are being exposed to inorganic arsenic through consumption of contaminated drinking water and food (Silbergeld *et al.*, 2008). Arsenic (As) is a metalloid that occurs in organic and inorganic forms in water and soil throughout the world especially in Bangladesh, India and several other countries of Southeast Asia (Bhattacharya *et al.*, 2009). The safety limit of arsenic accepted by Bangladesh government is 0.05 mg/liter for drinking water (WHO, 1999). The World Health Organization (WHO) limits for drinking water 0.01 mg/liter and for foodstuffs is 2 mg/liter on a fresh weight basis (Robinson *et al.*, 2003). Now

arsenic creates a serious public health issue in different developing countries (Rahman, 2006), where the drinking water contaminated with inorganic arsenic (As). Chronic arsenic toxicity is a global health issue at present (Yoshida *et al.*, 2004). It is also a major health problem of Bangladesh and surrounding regions (Kalia, 2005; Khalequzzaman *et al.*, 2005; Guha Mazumder *et al.*, 2001).

Clinical study suggests that algae having very high concentration of micronutrients and vitamins may have beneficial effects in heavy metal poisoning. 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. Spirulina is a microscopic filamentous aquatic non-toxic blue-green algae belongs to the group cyan bacterium (genus *Spirulina*, especially *S. platensis* synonym *Arthrospira platensis*) (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). Spirulina was found to be beneficial in goats of chronic arsenic poisoning (Halim, 2007) and spirulina extract plus zinc was found to be beneficial in patients of chronic arsenic poisoning (Misbahuddin *et al.*, 2006). Spirulina contains about 13.6% carbohydrates; (Shekharam *et al.*, 1987), vitamin B1, B2, B3, B6, folic acid, vitamin C, vitamin D, and vitamin E (Babadzhanov *et al.*, 2004). Spirulina have protective effect against arsenic toxicity.

It is reported that administration of spirulina provide a protective mechanism against arsenic induced toxicity in goats (Ghosh A, *et al.*, 2014). Spirulina is helpful on toxic signs, body weight and hematological parameters in arsenic induced toxicities in ducks (Islam *et al.*, 2009).

Vitamin E is a lipid soluble free radical scavenger that protects the membrane from lipid peroxy 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 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. Vitamin E (α -tocopherol) is considered as chain-breaking micronutrient antioxidant.

So considering all the above facts, this work has been undertaken with the following objectives:

- To know the effect of spirulina and vitamin E on the body weight of arsenic fed quails.
- To know the effect of spirulina and vitamin E on the hematological parameters in arsenic fed quails.
- To observed the effect of spirulina and vitamin E on the post-mortem changes and histopathological changes in arsenic induced quails.

2. Materials and Methods

2.1. Statement of the experiment

The experiment was accomplished under the Department of Physiology and Pharmacology, Faculty of Veterinary and Animal Science of Hajee Mohammed Danesh Science and Technology University, Dinajpur during the period of 1st November to 30th December, 2018.

2.2. Experimental animal

Day old sixty quails were used in this experiment. All the 60 quails were collected from the local hatchery. The quails were observed for 30 days for the adjustment with the environment. All the quails were maintained by feeding poultry feed (Nourish Poultry feed) and clean water *ad libitum*.

2.3. Preparation of house

They were kept in different cage in a pre-disinfected and well ventilated room with controlled ambient temperature and natural relative humidity. The room underwent disinfection with 5% phenol following detergent washing at everyday. The excreta of birds were cleaned properly in daily. The animal room was well ventilated.

2.4. Experimental animal grouping

60 quails were collected for this experiment. The birds were randomly divided into 4 equal groups and eventually each group comprised of 15 quails. Groups were identified as

T₀ = Control fed with normal feed and water

T₁ = Arsenic treated group

T₂ = Arsenic plus spirulina treated group

T₃ = Arsenic plus vitamin E.

2.5. Body Weight (BW)

Following grouping, all the quails were weighed individually firstly on (day 0 =immediate previous day of starting treatment) after grouping and marking, then on day 15 and finally on day 30 and the results of body weight were recorded.

2.6. Experimental trial

Each of group is treated by different parameter and the experiment was concluded by 60 days. Quails of group T₀ were maintained on only normal feed and water as control group, quails of group T₁ were treated with arsenic trioxide @ 100 mg/L of drinking water daily and normal feed and quails of group T₂ were treated with arsenic trioxide @ 100 mg/L of drinking water plus feed with spirulina (*Spirulina platensis*) @ 1 gm/kg feed. The spirulina (Navit) used in this experiment was collected from Square Pharmaceuticals Limited; as a capsule form, quails of group T₃ were treated with arsenic trioxide @ 100 mg/L of drinking water plus Vitamin-E Tablet (Square Pharmaceuticals Limited; Bangladesh) simultaneously at a dose of 400 mg/kg body weight. All treatments were given for 30 days.

2.7. Sampling procedure

After every 15 days from each group of quails blood samples were collected for haematological test and 1 ml of blood for each was taken separately in EDTA coated tube. Bloods samples for hematological investigation were preserved at refrigerator temperature. All blood were taken 1st on Day 15 and 2nd on Day 30.

2.8. Examination of blood for determination of hematological parameters

From each group, 5 birds were selected randomly and killed humanely on experimental days 15 and 30 and blood was collected for TEC, TLC and Hemoglobin concentration determination.

2.9. Statistical analysis

Data were expressed as mean \pm standard error (SE) and analyzed using one way analysis of variance (ANOVA) followed by Duncan's test as a post-hoc test using IBM SPSS Statistics 20.0 software package and the chart was created by Microsoft Excel 2007 software. Results were considered to be statistically significant when P values are less than 0.01 ($P < 0.01$).

3. Results

The experiment was conducted to determine the efficacy of spirulina and vitamin E on arsenic toxicity in quails. It was also undertaken to observe the effects of spirulina and vitamin E on body weight, hematological, postmortem and histopathological parameters in arsenic fed quails. Sixty quails were randomly divided into four 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 100 mg/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 vitamin E simultaneously at a dose of 400 mg/kg feed. All the treatment were continued for 30 days and treated quails were closely observed through the entire period.

3.1. Body weight (BW) of the quails

Body weights (BW) of experimental quails of all groups were taken fifteen days interval on day 30, day 45 and day 60. Table 1 showed that the body weight gain was highest (130.10 ± 1.03) gm in control group quails at 60 days but the body weight gain was lowest (96.60 ± 0.62) gm in arsenic treated T₁ group at 60 days whereas body weight gain in T₂ and T₃ were (116.50 ± 0.91) and (107.50 ± 0.65) gm which were better than arsenic treated T₁ group. The body weight of initial groups were not significant ($p > 0.05$) but in 45 days and 60 days mean value of body weight were significant ($p < 0.01$).

The body weight of treated group were increased with their age but in 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 control group where supplied normal feed and diet.

Table 1. Effects of spirulina and vitamin E on the body weight (gm) of quails.

Treatment	T ₀	T ₁	T ₂	T ₃	P value
Initial (30 days)	45.30 ± 0.75	43.50 ± 0.91	43.90 ± 0.90	44.90 ± 0.82	NS
45 days	84.60±0.76 ^c	64.50 ±0.69 ^a	80.80±0.74 ^b	79.70 ±0.67 ^b	**
60 days	130.10 ±1.03 ^d	96.60 ±0.62 ^a	116.50 ±0.91 ^c	107.50 ±0.65 ^b	**

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

** = Significant at p<0.01 level of probability

*= Significant at p<0.05 level of probability

3.2. Total erythrocyte count (TEC)

In Table 2, total erythrocyte count (TEC) values were highest ($3.00 \pm 0.006^d \times 10^{12}$) million/mm³ found in T₂ group at 60 days where spirulina were treated against arsenic toxicity but lowest ($2.87 \pm 0.003^a \times 10^{12}$) million/mm³ value was found in T₁ group where only arsenic were given.

Table 2. Effects of spirulina and vitamin E on total erythrocyte count (TEC) million/mm³ values of quails.

Treatment	T ₀	T ₁	T ₂	T ₃	P value
45 Days	$2.93 \pm 0.003^b \times 10^{12}$	$2.85 \pm 0.003^a \times 10^{12}$	$2.97 \pm 0.003^d \times 10^{12}$	$2.96 \pm 0.003^c \times 10^{12}$	**
60 Days	$2.95 \pm 0.003^b \times 10^{12}$	$2.87 \pm 0.003^a \times 10^{12}$	$3.00 \pm 0.006^d \times 10^{12}$	$2.98 \pm 0.003^c \times 10^{12}$	**

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

** = Significant at p<0.01 level of probability

*= Significant at p<0.05 level of probability

3.3. Total leukocyte count (TLC)

In Table 3, total leukocyte counts on Day 60 was found highest ($258.23 \pm 0.15^c \times 10^9$) Thousand in control group quails and lowest in T₂ group quails ($254.04 \pm 0.56^a \times 10^9$) Thousand where spirulina was treated and the difference were statistically significant among all group of quails (p<0.01). Spirulina decrease the TLC level.

Table 3. Effects of spirulina and vitamin E on total leukocyte count (TLC) thousand values of quails.

Treatment	T ₀	T ₁	T ₂	T ₃	P value
45 Days	$256.20 \pm 0.21^c \times 10^9$	$254.80 \pm 0.15^b \times 10^9$	$253.00 \pm 0.32^a \times 10^9$	$255.73 \pm 0.32^c \times 10^9$	**
60 Days	$258.23 \pm 0.15^c \times 10^9$	$256.77 \pm 0.24^b \times 10^9$	$254.04 \pm 0.56^a \times 10^9$	$256.71 \pm 0.33^c \times 10^9$	**

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

** = Significant at p<0.01 level of probability

*= Significance

3.4. Hemoglobin (Hb)

Highest ($20.34 \pm .17^c$) Hb concentration was found in T₂ group at 60 days and lowest concentration was found in T₀ group ($15.63 \pm .09^a$) (Table 4). Difference among values of 60 days of Hb concentration were statistically significant (p<0.01). It might be concluded that spirulina might slightly increase the values of Hb against arsenic toxicity in quails.

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

Treatment	T ₀	T ₁	T ₂	T ₃	P value
45 Days	14.73 ± 0.12^a	15.53 ± 0.15^b	19.70 ± 0.26^d	18.63 ± 0.09^c	**
60 Days	15.63 ± 0.09^a	15.90 ± 0.06^a	20.34 ± 0.17^c	19.37 ± 0.24^b	**

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

** = Significant at p<0.01 level of probability

*= Significant at p<0.05 level of probability

4. Discussion

Arsenic induced quails showed several clinical signs during the experimental period but slightly increased body weight was observed in all groups because the quails were in growing stage. On the other hand, the body weight

gain in quails of arsenic treated group was found lower compared to other treated groups. Moderate weakness was also observed in the quails of arsenic treated group compared to arsenic plus spirulina, arsenic plus vitamin E and control groups.

There was significant difference on TEC, TLC and Hb was found among the groups, the value of TLC was the highest in control group quails and the lowest in arsenic treated quails. The cause of change in hematological values might be due to the toxic effect of arsenic on haematopoietic system which is responsible for such alterations in hematological parameters. The value of TEC was highest in spirulina treated group T₂ and lowest in arsenic treated group T₁. The value of Hb concentration was highest in spirulina treated group T₂. However, the findings might suggest that chronic arsenic toxicity possibly decrease TEC level in the quails and that could be recovered by spirulina (1g/kg feed) with feed within 30 days.

Treatment of chronic As toxicity with spirulina/vitamin E for a longer time might give a clear picture in this regard. Increased levels of As found in the lung, liver and kidney following feeding of arsenic trioxide (100 mg/L drinking water) to the quails of arsenic treated group compared to control group during the whole study period and was increased with the length of exposure period. This finding agreed with the findings of Nabi *et al.* (2005) and Kamaluddin and Misbahuddin (2006). They showed that administration of arsenic in quails for different periods induced a significant increase in arsenic accumulation.

Microscopical examination of liver of arsenic treated groups (T₁) birds showed fatty changes and congestion. Cirrhosis (Fibrous tissue proliferation in the liver parenchyma), severe congestion of hepatic vessels and fatty changes were observed in the T₁ group (100 mg arsenic trioxide/L drinking water). Cirrhosis and congestion in the blood vessels of liver also reported by Singh *et al.* (2011) in albino rat treated by As containing ground water. However other group presented as normal image. Such histological changes in the liver of treatment group birds might be due to toxic effect of arsenic trioxide on liver because most of the toxicants (like arsenic) are metabolized in liver & thereby affect its morphology. Microscopically, kidneys from control (T₀), arsenic plus spirulina (T₂) & arsenic plus vitamin E (T₃) showed as normal architecture with normal glomeruli, proximal convoluted tubules (PCT) and distal convoluted tubules (DCT). Fatty degeneration, cytoplasmic vacuoles were observed in the section of kidneys from the birds receiving arsenic trioxide/L drinking water (T₁) group. These findings are in agreement with previous study of Dutta *et al.* (2007). The histopathological changes in arsenic treated group birds could be due to accumulation of arsenic in kidney & thereby exert harmful effect.

5. Conclusions

The exposure of the heavy metals like As may affect the physiology of poultry which in turn may be the issue of public health through feed chain. Moreover, the economic losses in the farming sector due to As exposure & its preventive strategies should also be undertaken. From this study it may be recommended that spirulina alone and vitamin E alone lowered arsenic toxicity in quails but spirulina was more efficacious than vitamin E in reducing arsenic load in quails. Arsenic toxicity has adverse effect in hematological and histopathological parameters in quails. This study suggested that spirulina and vitamin E has significantly reduced the arsenic concentration of inorganic arsenic toxicity in quails. Further investigation in this line may make more clear evidence to use spirulina as a therapeutic treatment for arsenic toxicity. More study is also needed to determine the level of arsenic in blood and to optimize the dose of spirulina and vitamin E to minimize arsenicosis in animals.

Conflict of interest

None to declare.

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