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Prevention of arsenic toxicity in quail by using spirulina and garlic

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ABSTRACT

Chronic arsenic toxicity is a severe disease in men and animals which occurs severely in Bangladesh. Arsenic (As) contamination in ground water used for 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 study the comparative efficacy of Spirulina (*Spirulina platensis*) and Garlic (*Allium sativum*) for prevention of arsenic toxicity. Forty quails were used in this study and animals were divided into control group (T_0), Arsenic treated group (T_1), Arsenic plus Spirulina treated group (T_2) and Arsenic plus Garlic treated group (T_3). Each group consists of 10 quails. Quails of T_0 group were given normal feed and water and kept as control. Quails of T_1, T_2 and T_3 were given 100 mg Arsenic trioxide/L drinking water daily for 30 days. In addition to arsenic trioxide quails of group T_2 and T_3 were simultaneously fed with Spirulina @ 1 gm/kg feed and T_3 were simultaneously feed with Garlic @ 1gm /kg body weight up to 30 days respectively. Five quails from each group (T_0, T_1, T_2 and T_3) were sacrificed at 15 days interval in order to determine haematological parameters. Data showed that in group T_1, body weight gain was minimum, whereas in group T_2 and T_3 the body weight gain in quails were better. Reduction of TEC, Hb values were significant ($P<0.01$) in T_1 group. Whereas in rest groups reduction of TEC, Hb were less than arsenic treated groups. Noticeable change observed in liver and kidney of arsenic treated group in compare to control group. Spirulina and garlic have significant effect on body weight, hematological and postmortem changes.

INTRODUCTION

Access to safe drinking water is the basic human right. Now-a-days, one of the most serious worldwide environmental problems is drinking water polluted by arsenic. Arsenic poisoning from underground drinking water in Bangladesh was first identified in 1993 (Smith et al., 2000). Almost 57 of 140 million people (Mahmood, 2002) and 61 of total 64 districts of Bangladesh are reported to have dangerous levels of inorganic arsenic (>50 μg/L) in most of the tube wells (DPHE, BGS and MML, 1999; BAMWSP, 2001). Arsenic can enter into food chain (Ulman et al., 2004) causing wide spread distribution throughout the plant and animal kingdoms.

Arsenicosis presents with significant changes in the serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum creatinine, urea, uric acid levels and various hemato-logical parameters like TEC, TLC, Hb, blood sugar level in the quail.

The natural source of human exposure to arsenic occurs through consumption of drinking water sourced from groundwater that contains dissolved inorganic arsenic (Allan et al., 2000). Significantly elevated standard mortality from cancer of the bladder, lung, liver, kidney, skin and colon were found in the population living in an area of Taiwan, China and some part of Africa where arsenic contamination of the water supply was endemic (Azcue and Nriagu, 1995).

Chronic arsenic toxicity due to drinking of arsenic contaminated ground water is a major environmental health hazard throughout the world (Mazumder, 2008). Chronic arsenic poisoning results from drinking contaminated well water over a long period of time. This is due to arsenic contamination of aquifer water.

Spirulina (*Spirulina platensis)* is characterized by high nutritional value where it contains high protein content (60–70% by dry weight), plenty of vitamins, amino acids, gamma-linoleic acid, and

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minerals (Hoseini et al., 2013). In addition, spirulina has antidiabetic effect (Karkos et al., 2011). Spirulina provides protection against mercuric chloride-induced oxidative stress and alteration of antioxidant defense mechanism in the liver. These activities were largely related to phycocyanin, an active protein of Spirulina (Romay et al., 1998). Phycocyanin (Pc) is a biliprotein of the blue-green alga. This protein contains a tetrapyrrole phycocyanobilin, which is responsible for antioxidant properties of Pc (Bhat and Madyastha, 2000). It has been reported that Pc has significant antioxidant and radical scavenging properties, offering protection against oxidative stress (Lissi et al., 2000). Antioxidants can reduce arsenic toxicity through chelating it and scavenging free radicals (Rana, 2007). It was reported that Pc can bind with heavy metals (Gelagutashvili et al., 2003); hence, it can chelate and remove them. In view of the above concerns, the present study was designed to evaluate the antioxidant action of *S. platensis* enriched with phenolic compounds in ameliorating testicular dysfunction and oxidative stress induced by arsenic.

Garlic is well known as a folk remedy for a variety of ailments since ancient times, however, very few studies are available suggesting its beneficial role against arsenic toxicity pertaining to its ability to eliminate arsenic from the blood and soft tissues and in reversal of arsenic-induced oxidative stress in affected tissues. Further, an attempt to understand the mechanism of arsenic in inducing hepatic apoptosis was also studied. Results of the present study suggested that arsenic administration in quail caused generation of reactive oxygen species (ROS) causing apoptosis through mitochondria-mediated pathway. The ROS generation in hepatic tissue reverted to normal values after co-administration of garlic (Flora et a., 2009).

Rats given daily doses of arsenic in their water, in levels equivalent to those found in groundwater in Bangladesh and West Bengal. Research reported that rats fed garlic extracts had 40 per cent less arsenic in their blood and liver, and passed 45 per cent more arsenic in their urine (Chowdhury et al., 2008). The authors suggest garlic may provide some relief for millions of Bangladeshis and Indians whose drinking water is contaminated with arsenic.

Considering the above information the present study was conducted to delineate the effects of spirulina and garlic against arsenic in quail.

MATERIALS AND METHOD

This experiment 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.

Preparation of house

At first the room as well as wire cages were washed by sweeping and washing. 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.

Grouping of birds

Forty quails were collected for this investigation. These quails were randomly divided into four equal groups. Ten birds kept in each group were considered as an experimental unit.

Group T_0: The quails were fed normal diet and given water ad-libitum and their body weight was recorded at every 15 days interval. This group was served as “Negative control” group.

Group T_1: The quails were treated with arsenic tri oxide @ 100 gm/ kg body weight/ day in drinking water.

Group T_2: The quails were treated with arsenic tri oxide @ 100 gm/ kg body weight plus spirulina @ 1 gm/ kg body weight in feed.

Group T_3: The quails were treated with arsenic tri oxide @ 100 gm/ kg body weight plus garlic @ 1 gm/ kg body weight in feed.

Test chemicals

Arsenic trioxide was purchased from a scientific laboratory. Spirulina capsule (Navit®) was collected from Square Pharmaceuticals Limited and garlic was collected from local market.

Experimental trial

The experimental trial was conducted for 60 days. Quails of Group T_0 were maintained with only normal feed and water *adlibitum* as control and Group T_1 were treated with arsenic trioxide at a dose of 100mg/L drinking water. The quails of Group T_2 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 quails of Group T_3 were treated with arsenic trioxide at 100mg/L in drinking water daily and garlic 1gm/kg of feed ad-libitum.

Preparation of arsenic trioxide solution

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

Preparation of 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 quails *ad libitum*.

Preparation of garlic mixed feed

Garlic was cut into small pieces and mixed properly with feed. After completion of proper mixing, the mixed feed was provided to quail.

Recording of body weight

The quails were individually weighed firstly on Day 30 (Day 30= immediate previous day of starting treatment) after grouping and marking, Day 45 and finally on Day 60 and the results were recorded.

Observation of clinical signs

Experimental quails 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 30 to Day 60) and the findings were recorded.

Hematological parameters

1ml blood from each group was collected from sacrificing of quail. The 1st blood sample was collected after the commencement of treatments (45 days) then the end of the experiment (60 days). The collected blood was sent to physiology and pharmacology laboratory, HSTU, Dinajpur, such as TLC (Total Leucocytes Count), TEC (Total Erythrocyte count), Hemoglobin percentage, PCV (Packed Cell Volume) and ESR (blood parameters were determined by semi automatic hematological analyzer machine (Cure inc. USA).

Gross and histopathology

After killing, visceral organs (liver and kidneys) of each bird were weighed individually. Liver and kidneys were weighed and examined for gross lesions and tissues showing gross lesions were preserved in 10% buffered formalin. Specimens of 5 mm thickness from morbid organs were taken and processed for histopathological examination using the standard method of dehydration in ascending grades of ethanol, clearing in xylene and embedding in paraffin. Sections of 5µm thickness were cut and stained with Hematoxylin and Eosin (Bancroft and Gamble, 2007).

Statistical analysis

Data were expressed as mean ± 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).

RESULTS AND DISCUSSION

Clinical signs

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

Body weight (BW) of the quails

Body weights (BWs) 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^{d}) in T_0 group quails at 60 days but the body weight gain was lowest (96.60 ± 0.62^{a}) in arsenic treated T_1 group at 60 days whereas body weight gain in T_2, T_3 were 116.50 ± 0.91^{c}, 107.50 ± 0.56^{b} which were better than arsenic treated T_1 group. The body weight of initial groups were not significant ($p > 0.05$) but in 30 days, 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 T_1 group it decreased more 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 T_0 group where fed with normal feed and water. It indicates that spirulina acts 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.

Total Erythrocyte Count (TEC)

The Total Erythrocyte Count (TEC) values were found highest ($3.00 \pm 0.006^{d} \times 10^{12}$) in T_2 group at 60 days where spirulina was treated against arsenic toxicity but lowest ($2.87 \pm 0.003^{a} \times 10^{12}$) value was found in T_1 group where only arsenic were given. TEC value found at 45 days and 60 days were significant ($p<0.05$) in difference (Table 2).

Table 1: Effects of spirulina and garlic on the body weight of quails

Days	T_0	T_1	T_2	T_3	P value
30	$45.30^{\circ} \pm 0.76$	43.50 ± 0.91	43.90 ± 0.90	44.90 ± 0.82	NS
45	84.60 ± 0.76^{c}	64.50 ± 0.69^{a}	80.80 ± 0.74^{b}	79.70 ± 0.67^{b}	**
60	130.10 ± 1.03^{d}	96.60 ± 0.62^{a}	116.50 ± 0.91^{c}	107.50 ± 0.56^{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

Table 2: Effects of Spirulina and garlic on Total Erythrocyte Count (TEC) values of quails

Days	T_O	T_1	T_2	T_3	P value
45 days	$2.93 \pm 0.003^{b} \times 10^{12}$	$2.85 \pm 0.003^{a} \times 10^{12}$	$2.97 \pm 0.003^{d} \times 10^{12}$	$2.95 \pm 0.003^{c} \times 10^{12}$	**
60 days	$2.95 \pm 0.003^{b} \times 10^{12}$	$2.87 \pm 0.003^{a} \times 10^{12}$	$3.00 \pm 0.006^{d} \times 10^{12}$	$2.97 \pm 0.006^{c} \times 10^{12}$	**

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

Table 3: Effects of spirulina and garlic on Total Leukocyte Count (TLC) values of quails

Treatment	T_0	T_1	T_2	T_3	P. Value
45 Days	$256.20 \pm 0.21^c \times 10^9$	$254.80 \pm 0.15^b \times 10^9$	$253.00 \pm 0.32 \times 10^9$	$255.70 \pm 0.34^c \times 10^9$	**
60 Days	$258.23 \pm 0.15^c \times 10^9$	$256.77 \pm 0.24^b \times 10^9$	254.04 0.56a $\times 10^9$	$256.40 \pm 0.32^b \times 10^9$	**

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

Table 4: Effects of spirulina and garlic 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	18.23 ± 0.15^c	*
60 Days	15.63 ± 0.09^a	15.90 ± 0.16^a	20.34 ± 0.17^c	19.07 ± 0.18^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

Total Leukocyte count (TLC)

In Table 3, Total leukocyte counts on Day 60 was found highest (258.23 ± 0.15^c x 10^9) in control group quails and lowest in T_2 group quails where sprulina was treated and the difference were statistically significant among all group of quails ($p<0.01$) suggesting the decreased TLC level by effect of sprulina.

Hemoglobin

Highest (20.34 ± 0.17^c) Hb concentration was found in T_2 group at 60 days and lowest concentration was found in T_0 group (Table 4). Difference among values of 60 days of Hb concentration were statistically significant ($p<0.01$) and the difference among values of 45 and 60 days of Hb concentration were statistically significant ($p<0.05$). The results demonstrated that Spirulina might slightly increase the values of Hb against arsenic toxicity in quails.

Gross and histopathology

Grossly, kidneys were swollen and liver was pale and hemorrhagic in arsenic treated quails as compared to control quails (figure not shown). Microscopically, congestion, nuclei condensation of tubular epithelium, epithelial necrosis, increased urinary spaces with atrophy of glomeruli, along with sloughing of tubules from basement membrane and cast deposition in the lumen of renal tubules were observed in arsenic treated quails. Microscopically severe congestion and cytoplasmic vacuolation and bile duct hyperplasia was observed in liver (figure not shown).

CONCLUSIONS

Arsenic toxicity reduced the body weight of quails. Treatment with spirulina and garlic reduced by weight by preventing weight loss of quail. Spirulina is more effective than garlic against arsenic induced toxicity in quails.

Arsenic toxicity has adverse effect on hematological parameters, postmortem changes and histopathological changes in quail. Spirulina and garlic have protective effect in improving these parameters. However, the study suggests that spirulina and garlic has significantly reduced the inorganic arsenic toxicity in quails.

CONFLICT OF INTEREST

The authors do not have any conflict of interest.

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