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Research Article

Study on Prevention of Induced Arsenic Toxicity in Rats by using Spirulina and Thankuni

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Abstract: The prophyalactic contributions of spirulina and thankuni on rats experimentally induced with arsenic toxicity were tested and the comparative efficacies of both spirulina and thankuni were determined. Sixty apparently healthy rats were received, divided into 5 groups (T_0 for control, T_1 for arsenic treated group, T_2 for arsenic plus spirulina treated group, T_3 for arsenic plus thankuni treated group and T_4 for arsenic plus spirulina and thankuni treated group) and each group consisted of 12 rats for the experimentation. Rats of T_0 group were given normal feed and water and kept as control. Rats of $T_{1,}$ $T_{2,}$ T_3 and T_4 were treated with 4 mg Sodium arsenite ($NaAs_2O_2$)/kg body weight daily orally for 63 days. In addition to sodium arsenite rats of group $T_{2,}$ T_4 were simultaneously fed with Spirulina @ 1 gm/kg feed; and T_3 and T_4 were simultaneously feed with thankuni @ 400mg /kg body weight up to 63 days respectively. Four rats from each group ($T_{0,}$ $T_{1,}$ $T_{2,}$ T_3 and T_4) were sacrificed at 21 days interval in order to determine biochemical and haematological parameters. Result showed that no visible clinical signs were observed in any groups of experimental rats. Here, sodium arsenite feeding caused arsenic burden in rats but Spirulina and thankuni significantly ($p<0.01$) lowered the arsenic burden of rats whereas spirulina is more effective than thankuni against arsenic burden in rats. Finally it is concluded that the combination of spirulina and thankuni were found more effective in prevention of arsenic burden in rat.
Keywords: Induced Arsenocosis, Prophylactics, Spirulina, Thankuni treatments, Rats.

INTRODUCTION

Arsenic is a naturally occurring common health hazarding compound in Bangladesh and the toxicity is in people by drinking of arsenic contaminated water. The safety limit of arsenic accepted by Bangladesh Government is 0.05mg/ litre of drinking water (WHO) but World Health Organization (WHO) limits 0.01mg/litre in drinking water and 2mg/litre of foodstuffs on a fresh weight basis [1] Now arsenic creates a serious public health issue in different developing countries [2] but chronic arsenic toxicity is a global health issue at present [3]. It is also a major health problem of Bangladesh and surrounding regions [4].

The specific curative treatment against arsenicosis is not yet recommended. Stoppage of drinking arsenic contaminated water [5], improved diet [6], use of chelating agents [4] etc. are usually suggested to mitigate arsenocosis. Spirulina, blue green algae, reduces mercury and other toxic metal accumulation in the tissue [7]. Spirulina alone or in combination with other vitamin and/or mineral was found to be effective in the removal of arsenic from arsenic-loaded tissues in various species including man [8] in the treatment of chronic arsenic poisoning [9], in reducing arsenic toxicity induced skin manifestations of patients in Bangladesh [10]. Spirulina effectively reduces hepatic damage due to drug abuse and heavy metal exposure, inflammatory response [11], cellular degeneration [12], anaphylactic reaction [13].

Thankuni is essential for numerous intrinsic processes. The most well-known and understood process is that of healing. It is water soluble and also acts as the effective antioxidant. Epithelial cells are highly dependent on ascorbic acid and are commonly used to treat a variety of skin diseases. A developing fetus is also highly dependent on ascorbic acid, as it is essential to the growth of the eyes, lungs, ears and heart [14]. In Bangladesh, elaborate data is on arsenocosis is available, but data on specific treatment for the prevention of arsenic toxicity is very limited. However, the present study was undertaken to evaluate the effectiveness of Spirulina and vitamin C in rats experimentally induced with arsenic toxicity.

MATERIALS AND METHODS

Experimental areas and period

The experiment was conducted in the Arsenic Detection and Mitigation Laboratory (ADM Lab), Department of Pharmacology in collaboration with the Department of Physiology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. The course of the study was six months from July 2011 to December 2011.

Experimental animals, groupings and management

Sixty female Long Evans rats of about 6 months of age were used as the experimental animals. The rats were obtained from the laboratory animal section of the ADM Lab and randomly divided into 5 groups; marked using different colors on their tail tips. Each group consisted of 12 rats.

Groups T_0: Control group,
Group T_1: Arsenic treated group,
Groups T_2: Arsenic plus spirulina treated group,
Groups T_3: Arsenic plus thankuni treated group, and
Groups T_4: Arsenic plus spirulina and thankuni treated group

Rats of each group were kept in pre-disinfected separate steel wire cages in a well mechanical ventilated and controlled room temperature with natural relative humidity. Rats were maintained under normal pellet feed and drinking water with admissible levels of arsenic. The excreta of the animals were cleaned in the every morning and the animal room was managed with standard bio-security.

The rats were taken as the experimental animals. The rats were individually weighed on Day 0 (D_0 = immediate previous day of starting treatment), D_{21}, D_{42} and finally D_{63} post-treatment and marking and the results were recorded.

Treatment, sampling occasion and collection of samples

Sodium arsenite ($NaAsO_2$, MW 197.84 g/mol) (May & Baker Ltd, Dagenham, England) and Spirulina (*Spirulina platensis*) (Life Line International Limited, Bangladesh) were obtained as tablet form. The respective pre-weighed $NaAsO_2$ was mixed with the drinking water daily for the particular group. Generally, 10ml drinking water per rat was allotted for mixing $NaAsO_2$ to make sure that the full amount of $NaAsO_2$ was taken by the rats. The Spirulina tablet (500mg in each tablet) was made to a homogenous powder, mixed with feed as stated amount, finally dried with electric oven at 50^0C for 24 hours and stored in air tied plastic container. Dry thankuni leaves were made to homogenous powder and added to distilled water with required amount.

Table 1: Treatment and sampling occasions of the different experimental groups

Experimental groups	Rats/group	Treatment with and doses	Sampling occasions (Day=D)
Group T_0 (Control)	12	-	4 rats at $D_{21,}$ $D_{42,}$ D_{63} post-treatment
Group T_1 (Arsenic group)	12	$NaAsO_2$: 4mg/kg of body-weight (BW) in drinking water daily	As above
Group T_2 (Spirulina+ Arsenic)	12	Spirulina: 1 gm/kg of feed $NaAsO_2$: As above	As above
Group T_3 (Arsenic+ thankuni)	12	$NaAsO_2$: As above Thankuni : Rrequired amount	As above
Group T_4 (Arsenic + Spirulina + thankuni)	12	All three items as stated above	As above

Blood samples were collected directly from hearts of each rat by using disposable plastic syringe following midline injection after chloroform anesthesia for determination of biochemical as well as hematological parameters. The blood was allowed to clot to collect, prepare and store serum for biochemical tests.

Clinical findings

The animals were closely observed daily for 3 times (morning, afternoon and evening) for the clinical signs if any were recorded during the entire experimental period.

Determination of biochemical parameters

The sample sera were 4 fold diluted with phosphate buffer solution (PBS) for each test; and Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT) and serum creatinine were determined following a recommended procedure [15]. The findings were noted.

Determination of haematological parameters

The well mixed blood samples were prepared and processed for the haematological parameters such as total erythrocyte count (TEC), total leukocyte count (TLC) and haemoglobin (Hb) following a standard procedure [15]. The results of the tests were systematically recorded in a record book.

Statistical analysis

The experimental data were designed in CRD and analyzed statistically using one way ANOVA with the help of the SPSS 11.5 software. Mean comparisons of the treatments were made by the Duncan's Multiple Range Test (DMRT) [16].

RESULTS AND DISCUSSION

Arsenic toxicity following feeding of sodium arsenite (4 mg/kg BW) in rats increased day by day. Spirulina and thankuni treatment lowered arsenic toxicity where the Spirulina found more effective in reducing arsenic content in the tissues. Moreover,the combined dose of thankuni and spirulina was found most effective compared to other groups in rats.

Treatment effects on body weight and the clinical status

The treated rats did not show any clinical signs/lesion during the entire study period but slight increase in body weight in rats of most of the groups. The body weight was found in rats of arsenic treated group (T_1) was lower compared to rats of arsenic plus spirulina (T_2), arsenic plus thankuni (T_3) and arsenic plus spirulina and thankuni (T_4) and control groups (T_0) (Table 2). However, these changes were not remarkable. Hence, it could be said that feeding of $NaAsO_2$ (4mg/kg BW) could not cause chronic arsenic toxicity in rats, although for the manifestation of signs and symptoms of arsenic toxicity would take more time and/or higher dose of As.

Treatment effects on biochemical parameters

The values of SGOT (Table 2) were increased significantly ($p<0.01$) in all samples of treated group rats (T_1, T_2, T_3 and T_4) compared to control (T_0). Although this finding did not agree with the findings that SGOT was reduced by arsenic alone [26] but agreed with other findings [17]. The values were decreased significantly ($p<0.01$) in arsenic plus spirulina (T_2) and arsenic plus thankuni treated group (T_3) and arsenic plus spirulina plus thankuni treated group (T_4) compared to arsenic treated group rats (T_1). However, the arsenic toxicity caused hepatic insufficiency and spirulina and thankuni treatment improved the hepatic functions, which was in support of that spirulina reduced hepatic damage due to drug abuse and heavy metal exposure [11]

There was significant difference ($p<0.05$) with SGPT values of all groups at day 21 day 42 and day 63 (Table 2). Overall SGPT values have decreasing trend with the progress of time in all groups which was agreed with the findings of [17] No change in SGPT values was observed with As supplementation for 0, 45 and 90 days [18] But the results of this study did not agree with that finding. Detail physiological work on SGPT for arsenic, arsenic plus spirulina and arsenic plus thankuni could give correct information.

There was significant difference in serum creatinine level observe between the control group and all other treatment group rats throughout the study period (Table 2). Which disagree with the findings of [19] in human being who showed that the patients of arsenicosis had significantly lower level of serum creatinine compared to the control and [20] who observed that there is a relationship between arsenic level and degree of chronic renal insufficiency in men. However serum creatinine was decreasing with the time of Spirulina and vitamin C treatment in (T_4) group rats, which revealed that Spirulina and thankuni improved nephrotic function.

Treatmental effects on haematological parameters

Total erythrocyte counts were not significant on Day 21, Day 42 and Day 63. The values of TEC were gradually increased in all other treated groups (T_2, T_3 and T_4) compared to T_1 group of rats (Table 3). On day 21, day 42 and day 63 the values of TEC were increased in Arsenic plus Spirulina plus thankuni treated group (T_4) compared to arsenic treated group (T_1). However the findings might suggest that the chronic arsenic toxicity might cause decreased in TEC values and Spirulina plus thankuni treatment might be recovering it.

There was no significant difference in TLC and Hb values observed among all the groups during the entire study period but slight increase in TLC and Hb values (Table 3) in rats of all other treated groups (T_2, T_3 and T_4) compared to arsenic treated group (T_1). It might be concluding that Spirulina and thankuni might be slightly increasing the values of TLC and Hb against arsenic toxicity in rats but it does not fulfill the real picture of findings. More research is necessary to fulfill the proper findings.

From previous works it is well documented that micronutrients and antioxidants has significant role in the treatment of chronic arsenic poisoning. Ascorbic acid, [21] Vitamin A , zinc, iron, Spirulina [22], lipoic acid [23], ascorbic acid and α-tocopherol all have got ameliorating role against chronic arsenic poisoning [24],[25]. It has been observed in previous studies that alpha-lipoic acid, ascorbic acid and alphatocopherol have synergistic activity in decreasing tissue arsenic load when administered simultaneously than individually. As Spirulina is a rich source of vitamins, minerals and micronutrients, and vitamin C, all of which possess antioxidant properties. Thus, it can be suggested that a combination of vitamins, minerals, antioxidants and other micronutrients could be a novel therapeutic measure for the prevention of arsenicosis.

However, further studies with spirulina and thankuni need to be carried out both *in vivo* and *in vitro* to ascertain there therapeutic benefit as well as toxic effect if any in chronic arsenic poisoning.

Study of arsenic toxicity and its treatment with spirulina and thankuni in animals for a longer period could be able to establish unequivocal decisions about the impacts of arsenic, spirulina, thankuni on biochemical parameters, as well as about strong evidence of efficacy of spirulina treatment in arsenic toxicities.

Table 2: Effects on body weights, SGOT, SGPT and Serum creatinine of rats treated with arsenic, arsenic plus spirulina, arsenic plus thankuni and arsenic, thankuni plus spirulina

Experi-mental groups	Body weight (Day=D)				SGOT (Day=D)			SGPT (Day=D)			Serum creatinine (Day=D)		
	D_0	D_{21}	D_{42}	D_{63}	D_{21}	D_{42}	D_{63}	D_{21}	D_{42}	D_{63}	D_{21}	D_{42}	D_{63}
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Group T_0 (Control)	200.38 ±8.1	206.25 ±7.94	213.40 ±10.06	224.00 ±5.94	231.67 ±0.88c	235.67 ±2.40c	232.00 ±3.79d	42.30 ±1.14	41.97 ±1.45d	41.53 ±1.17e	0.52± 0.001	0.51 ±0.01	0.51 ±0.001
Group T_1 (Arsenic group)	212.67 ±5.5	212.33 ±5.49	212.83 ±4.75	202.50 ±1.18	241.00 ±1.15b	247.33 ±1.86b	259.67 ±2.03c	45.20 ±1.34	51.27 ±0.92a	58.90 ±2.29a	0.54± 0.01	0.56 ±0.001	0.51 ±0.00
Group T_2 (Spirulina+ Arsenic)	194.33 ±8.1	195.78 ±8.45	191.67 ±9.69	207.67 ±11.97	288.23 ±2.03a	293.67 ±2.91a	304.00 ±0.00a	44.57 ±1.07	42.70 ±1.89d	44.20 ±1.61d	0.61± 0.05	0.51 ±0.001	0.51 ±0.001
Group T_3 (Arsenic+ thankuni)	225.22 ±5.9	226.11 ±5.26	230.67 ±6.21	237.67 ±8.01	267.00 ±9.02d	272.33 ±6.06e	278.33 ±58.46b	31.73 ±2.07	47.25 ±5.53e	47.84 ±13.57c	0.82± 0.07	0.91 ±0.10	0.72 ±0.08
Group T_4 (Arsenic+ spirulina+ thankuni)	224.88 ±8.19	223.13 ±8.18	230.33 ±6.23	240.00 ±3.15	241.00 ±24.49b	198.33 ±20.95d	186.33 ±13.09e	35.66 ±6.20	46.42 ±11.88e	31.65 ±3.76d	0.83± 0.08	0.52 ±0.001	0.51 ±0.01
Level of significance	**	*	*	*	**	**	**	*	*	**	**	**	**
	* = Significant at 5% level of probability ** = Significant at 1% level of probability				** = Significant at 1% level of probability			* = Significant at 5% level of probability ** = Significant at 1% level of probability			** = Significant at 1% level of probability *= Significant at 5% level of probability		

a, b, c, d, e.- Values with different superscripts in the same column differ significantly ($p<0.05$/ $p<0.01$)

Table 3: Effects of different treatment on Total erythrocyte count (TEC), Total leukocyte count (TLC) and Haemoglobin concentration (Hb) values of rats

Experimental groups	TEC			TLC			Hb		
	D_{21}	D_{42}	D_{63}	D_{21}	D_{42}	D_{63}	D_{21}	D_{42}	D_{63}
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Group T_0 (Control)	7.05 ±0.35	7.57 ±0.36	7.57 ±0.36	10.70 ±0.34	10.76 ±0.86	10.82± 0.66	10.27 ±0.35	10.80 ±0.72	10.73 ±0.58
Group T_1 (Arsenic group)	7.29 ±0.29	7.23 ±0.40	7.23 ±0.40	9.56 ±0.76	10.19 ±0.35	9.65±0.63	10.17 ±0.39	10.01 ±0.77	9.32 ±0.50
Group T_2 (Spirulina+ Arsenic)	6.83 ±0.15	7.98 ±0.42	8.28 ±0.18	9.40 ±0.35	9.51 ±0.51	9.59±0.56	10.25 ±0.40	10.54 ±0.75	10.71 ±0.13
Group T_3 (Arsenic+ thankuni)	7.18 ±0.22	7.48 ±0.29	6.53 ±0.15	10.06 ±1.03	9.86 ±0.54	10.11± 0.35	9.93 ±0.37	10.22 ±0.42	9.66 ±0.18
Group T_4 (Arsenic+ spirulina+ thankuni)	6.25 ±0.48	6.69 ±0.26	7.53 ±0.45	10.13 ±0.24	10.29 ±0.28	10.43± 0.18	9.20 ±0.33	10.23 ±0.13	10.33 ±0.74
Level of significance	NS	NS	NS	NS	NS	NS	NS	NS	NS
	NS: Not significant								

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