Effect of arsenic trioxide along with tannic acid, di-sodium hydrogen phosphate, alum and effects of sand-charcoal-iron-filter bed filtrated water along with alum on body weight and liver functions in rabbit


INTRODUCTION

Arsenic (As) is “The Silent killer” for Bangladesh at present. In world water day 1998 arsenic marked as the king of poisons. This poison created a major health concern affecting millions of people around the world, including Bangladesh. The arsenic disaster of Bangladesh has been called the most terrible environmental catastrophe of the twenty first century. WHO described the condition as “the largest mass poisoning of a population in history” [1]. About 85 million people are at risk of drinking arsenic contaminated water and foodstuffs [2, 3]. Together with the poor socioeconomic and nutritional status of the population, the chronic exposure to arsenic in drinking water is causing widespread health hazards in both man and animals in Bangladesh. Drinking water normally contains inorganic arsenic as arsenate (As (V)) and arsenite (As (III)). Inorganic arsenic is more dangerous than many other toxic substances. Arsenic remains one of the most important carcinogens and diabetogenics in human [4, 5, 6, 7]. Nowadays it is, after lead considered the most common toxic heavy metal affecting domestic animals [8, 9, 10]. Ingested inorganic arsenic is readily absorbed through the gastrointestinal tract and distributed in various tissues through blood circulation [11]. This arsenic eventually causes growth retardation, blood cells distortion and elevation of various serum enzymes like lactate dehydrogenase (LDH), alkaline phosphatase (ALP), serum glutamic pyruvic transaminase (SGPT) etc. in human and rodents. Chronic arsenic exposure is accompanied by its accumulation in liver, kidney, heart, lung, gastrointestinal tract and spleen [12, 13, 14, 15]. Hepatic damage has been reported as the most common complication of chronic arsenic exposures [16, 17, 18]. Although the toxic effects of arsenic are known for long time, the mechanism of its toxicity is still poorly understood. One of the known functions of arsenic is its high reactivity to sulphydryl groups of proteins/enzymes. Binding of arsenic to proteins or...
cross-linking of proteins involving sulfhydryl group may activate potential intracellular signaling pathways that ultimately lead to arsenic-mediated adverse effects [19, 20, 21]. To understand the arsenic-mediated health hazards in human experiments with human subjects is almost impossible for various legal reasons. Suitable animal model experiments, however, can help in this regard. These findings in Rabbit model might be useful for better understanding of the toxic effects of arsenic in order to develop effective remediation process against arsenic mediated effects on human health. The aim of the present study is to show the effect of arsenic alone and in combination with tannic acid, di-sodium hydrogen phosphate (DSHP) and alum on body growth & some biochemical parameters related to liver functions in rabbit.

MATERIALS AND METHODS

MATERIALS

Experimental Animals

One month old thirty apparently healthy adult Newzealand white rabbits (Oryctolagus cuniculus) weighing between 250-450 g were purchased from a local private farm of Muktigacha, Mymensingh, Bangladesh and brought to the Experimental Pharmacology and Toxicology laboratory at Bangladesh Agricultural University (BAU) for the present study. After two weeks of acclimatization animals were segregated on the basis of their age and body weight without significant differences. They were housed throughout the entire period of study in well ventilated animal house at a room temperature of 23 ±1°C and were supplied with standard ration formulated by ICDDRB, Dhaka and supplied fresh water ad libitum.

SCIF –bed filtered water

Sand-Charcoal-Iron-Filter (SCIF) bed was developed and used as arsenic purifying system. The artificially as contaminated water was passed sequentially four times through SCIF bed. The filtrated water was collected and examined by using Merck Arsen test kit and was used in the study.

Experimental chemicals

The alum, tannic acid, activated charcoal (Merck KGa, Darmstadt, Germany), wood charcoal (kat koila), sand were collected from local source and the Arsenic trioxide (As₂O₃,MW 197.84g/mol; product No. 37274, Loba chemic pvt. ltd, Mumbai, India), di-sodium hydrogen phosphate (Merck, India), iron oxide (BDH Lab., poole, England) were collected from Dhaka for this study.

Experimental design

The Rabbits were randomly divided in to 6 equal groups (A,B,C,D,E & F) at the ratio of three males and two females in each group, rats of group A was kept as control without giving any treatment, rabbits of group B received arsenic trioxide@100ppm, group C received arsenic trioxide@100ppm plus tannic acid@100ppm, group D received arsenic trioxide@100ppm plus di-sodium hydrogen phosphate@100ppm, group E received arsenic trioxide@100ppm plus alum @100ppm and group F received alum @100ppm in SCIF-bed filtrated water orally daily for 60 days in each cases. Prior to segregation, initial body weight of each rabbit was recorded and kept group wise in cages. After treatment all the rabbit were kept under close observation for a whole period of study and all the parameters (body weight gain or loss, some biochemical parameters related to liver functions) were recorded before and during treatment at specific time intervals.

Reagents for Liver function test

For determination of SGOT

1. Buffer substrate: (i) Tris buffer (84mmol/L pH 7.5) (ii) L-aspartate (260mmol/L)
2. Enzyme/ co-enzyme/α-oxoglutarate: (i) α-oxoglutarate (12 mmol/L) (ii) LD ≥1.2 U/ml (iii) NADH 0.18 mmol/L (iv) MDH ≥ 420U/L

For determination of SGPT

1. Buffer substrate: (i) Tris buffer (100mmol/L pH 7.5) (ii) L-alanine (0.6 mol/L)
2. Enzyme/ co-enzyme/α-oxoglutarate: (i) α-oxoglutarate (15 mmol/L) (ii) LD ≥1.2 U/ml (iii) NADH 0.18 mmol/L

METHODS

Measurement of body weight

The body weight of each rabbit was measured just before starting of treatment and body weight gain or loss was recorded in each 10 days interval up to sacrificing of the animals.

Procedures for the collection of blood sample for serum separation

Blood was collected just before treatment i.e. day 0 and during treatment on day 20, 40 and day 60 directly from marginal ear vein of rabbit. Immediately after collection blood was transferred to sterile tube containing anticoagulant (4% sodium citrate solution) at a ratio of 1:10 and used for determination of some biochemical parameters.

Biochemical study related to liver functions

Two widely used biochemical test such as SGOT and SGPT were determined by UV method using IFCC used Humalyzer 2000, Human type Germany

Determination of SGOT & SGPT

0.1 ml of serum was mixed with 1.0 ml kit solution 2 enzyme/coenzyme/α-oxoglutarate AL 1205 including buffer substrate with L-aspartate for SGOT and buffer substrate with L-alanine for SGPT determination. The wave length was set at 340nm Hg, 1 cm light path cuvette was used and analysis was done at 37°C. After mixing the cuvette was placed in the

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Humalyzer 2000. Initial absorbance was read after 1 minute. The final record was made at 1, 2, 3 minutes after initial reading. Absorbance was recorded each time (0.11 and 0.16 at 340nm/Hg 340nm). First two values for the first 2 minutes were used for the calculation.

Calculation: SGOT concentration: 1746 x absorbance U/1
Calculation: SGPT concentration: 1746 x absorbance U//1

Statistical Analysis
Collected data were statistically analyzed by the computer using statistical package programme MSTAT-C developed by [22]. A one way ANOVA was made by F variance test.

RESULTS AND DISCUSSIONS
Effect on body weight
The mean body weight of rabbits of group F (+9.15%) significantly increased as like control group (group-A) (+6.79%) which was statistically significant (P<0.01). In group B significant decreased of body weight was observed on 60 days of arsenic trioxide feeding which was statistically highly significant (P<0.01). The body weight of group C, D and E was slightly increased on 60 days of arsenic trioxide feeding in combination with tannic acid, DHSP and alum respectively but were statistically not significant (Table-1). The mean body weight of group B were decreased gradually and significant (P<0.05) decrease in body weight was observed at 40 days of feeding and at day 60 it was highly significant (P<0.01). The similar result on body weight was observed by many other workers [23, 24]. Rabbits of group C,D and E were apparently normal and mild body weight reduction were observed at the last part of the experiment which was statistically insignificant, this might be due to the interaction of arsenic oxide with other chemicals like tannic acid, DSHP and alum used in those groups respectively. No loss of body weight in rabbits of group F were observed during the whole experiment period might be due to supplied SCIF bed filtrated water with alum which indicates the successful use of SCIF bed to filtrate arsenic contaminated water as arsenic purifying system. The results of this study was in agreement with the findings of a research conducted by [25] on mice, ducklings and broiler chicken in Pakistan.

Table-1: Effect of Arsenic trioxide along with tannic acid, DSHP, alum and effects of SCIF-bed filtrated water along with alum respectively on body weight (gm) in rabbits

<table>
<thead>
<tr>
<th>Chemicals with dose &amp; route</th>
<th>Pre treatment</th>
<th>During treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 10</td>
</tr>
<tr>
<td>A</td>
<td>Control(untreated)</td>
<td>1620±1.7029</td>
</tr>
<tr>
<td>B</td>
<td>Arsenicosis control group AS$_2$O$_3$@100ppm orally</td>
<td>1650±1.7029</td>
</tr>
<tr>
<td>C</td>
<td>AS$_2$O$_3$@100ppm + Tannic acid @100ppm orally</td>
<td>1350±3.5355</td>
</tr>
<tr>
<td>D</td>
<td>AS$_2$O$_3$@100ppm + DSHP @100ppm orally</td>
<td>1715±3.5355</td>
</tr>
<tr>
<td>E</td>
<td>AS$_2$O$_3$@100ppm + Alum @100ppm orally</td>
<td>1430±1.7029</td>
</tr>
<tr>
<td>F</td>
<td>SCIF –bed filtrated water + Alum @100ppm orally</td>
<td>1420±1.7029</td>
</tr>
</tbody>
</table>

Values above represent the mean ± SE of 5 rabbits
a= Mean± SE of four rabbits
b= Mean± SE of two rabbits
* Indicates significant values
** Indicates highly significant values
+ indicates % increased – indicates % decreased.

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Effect on Biochemical parameters related to liver functions

Effect on SGOT/ AST and SGPT/ ALT

Table-2 represents the results of the effects of arsenic trioxide along with tannic acid, DSHP, alum and effects of SCIF bed filtrated water along with alum respectively on SGOT and SGPT in rabbit. The activities of SGOT and SGPT were elevated in all treated groups than control. But in group B, the elevation of SGOT and SGPT were higher at day 30(P<0.05) and much higher at day 60 which were statistically significant (P<0.01). The findings of the present study were in agreement with the findings observed by [26] in cattle at West Bengal, India, by [27] in sheep in Iran, by [28] in Swiss Albino Mice. The activities of two enzyme namely serum glutamate pyruvate transaminase (SGPT) recently called as alanine transaminase (ALT) and serum glutamate oxaloacetates transaminase (SGOT) have been widely used to assess the liver function. ALT was a cytoplasmic enzyme while AST was found in both cytoplasm and mitochondria. SGPT or ALT was found to increase in acute hepatitis (viral or toxic), jaundice, and liver cirrhosis. SGOT or AST was found to increase in myocardial infarction and different liver disorders. Activities of both SGOT and SGPT were significantly higher in arsenic treated mice indicated liver dysfunction. Arsenic was known to produce disturbance in liver function [29]. SGOT and SGPT were found as reliable determinants of liver parenchymal injury [30]. The increment of the activities of SGOT and SGPT in plasma might be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream [31], which gave an indication on the hepatotoxic effect of arsenic. The effects of arsenic on liver were found to depend completely on the nature of the arsenicals. Sodium arsenite was found to cause liver fibrosis in goat [32, 33], in rat [34, 35, 36, 37], liver necrosis in Duckling [38, 39, 40]. Sodium arsenite caused hepatocyte degeneration in mice [41]. Histopathological studies also revealed liver and spleen damage in arsenic induced toxicity [41]. These findings supported the results of the present study. Necrosis of hepatocytes and cytoplasmic blebbing in mice due to arsenic toxicity observed by [42] which was similar to the findings reported by [43, 44] in rat. Findings of Histopathological studies was also supported the findings of the present study. In the present study the values of SGOT and SGPT were significantly increased in all treated groups than in control group. In group B the elevation of SGOT and SGPT were much higher than other treated groups i.e. group C, D, E and F. But in other treated groups elevation of SGOT and SGPT were not statistically significant. This might be due to interaction of arsenic trioxide with others such as tannic acid DSHP, alum used in those group and use of SCIF bed filtrated water.

Table-2: Effect of Arsenic trioxide along with tannic acid, DSHP, alum and effects of SCIF-bed filtrated water along with alum respectively on SGOT/AST and SGPT/ALT (U/L at 37°C) in rabbits

<table>
<thead>
<tr>
<th>G r.</th>
<th>Chemicals with dose &amp; route</th>
<th>Pretreatment</th>
<th>During treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
<td>Day 30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SGOT/AST</td>
<td>SGPT/ALT</td>
</tr>
<tr>
<td>A</td>
<td>Control(untreated)</td>
<td>72.31 ± 0.7071</td>
<td>65.71 ± 0.7071</td>
</tr>
<tr>
<td>B</td>
<td>Arsenicosis control group</td>
<td>70.91 ± 0.7071</td>
<td>69.18 ± 0.8602</td>
</tr>
<tr>
<td></td>
<td>AS₂O₃@100ppm orally</td>
<td>70.06 ± 0.7071</td>
<td>68.01 ± 0.8602</td>
</tr>
<tr>
<td>C</td>
<td>AS₂O₃@100ppm + Tannic acid@100ppm orally</td>
<td>70.17 ± 0.7071</td>
<td>68.19 ± 0.8602</td>
</tr>
<tr>
<td>D</td>
<td>AS₂O₃@100ppm + DSHP @100ppm orally</td>
<td>70.07 ± 0.7071</td>
<td>68.23 ± 0.8602</td>
</tr>
<tr>
<td>E</td>
<td>AS₂O₃@100ppm + Alum @100ppm orally</td>
<td>71.98 ± 0.7071</td>
<td>67.31 ± 0.8602</td>
</tr>
<tr>
<td>F</td>
<td>SCIF-bed filtrated water + Alum @100ppm orally</td>
<td>71.98 ± 0.7071</td>
<td>67.31 ± 0.8602</td>
</tr>
</tbody>
</table>

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* Indicates significant values
** Indicates highly significant values

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CONCLUSION:
Treatment with arsenic oxide at low doses has harmful effects on experimental animals including disturbances of liver functions. Therefore, intake of alum treated SCIF bed filtered water might be helpful to reduce the body burden of arsenic toxicities.

REFERENCES
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