Effects of Vitamin E on Hemato-Biochemical Constituents, Kidney Parenchyma and Tissue Accumulation in Sodium Arsenite Exposed Teddy Goat Bucks

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ABSTRACT

The objective of this study was to find out the toxicity of sodium arsenite on hematology, serum biochemical constituents and kidney of Teddy bucks. The accumulation of arsenic in blood as well as tissues along its excretion in feces and urine was also measured. Vitamin E was also used to ameliorate the toxicity on these parameters. Adult twelve Teddy bucks were equally divided into three groups; A, B and C. The animals in group A was kept as control, while in groups B and C were fed with sodium arsenite 5 mg/kg and sodium arsenite 5 mg/kg + Vit E 200 mg/kg body weights respectively for twelve weeks. Body weights, blood and serum biochemical parameters were evaluated fortnightly in all the groups. Similarly, total arsenic accumulation in blood and excretion in urine and feces was measured after every two weeks. At the end of experiment, animals were slaughtered and visceral organs were processed for arsenic level and histopathologically studies. On analysis of data, it was proved that there was significant P<0.05 decrease in body weight, total protein, albumin and creatinine in arsenic treated animals. Whereas, total level of arsenic in blood and tissues was increased significantly in arsenic exposed animals. The results also revealed that vitamin E alleviated toxic effects of sodium arsenite on body weight, blood and serum biochemical constituents in restoration of normal values of these parameters. It was also proved that this vitamin increased the arsenic concentration in urine and feces. It was concluded that arsenic causes the toxicity and vitamin E scavenges this toxicity.

INTRODUCTION

Arsenic (As) a metalloid toxicant is known as the major source of contamination of water. The contamination of groundwater and soil has become the global issue in many areas of world. This increase of arsenic in water accumulates in animal feed and fodders (Ghosh et al., 2013) with subsequent rise in animal blood and tissues (Mohanta et al., 2013). Exposure of this metal for certain period to goats results in alterations in hematopoietic biochemical constituents and antioxidant (Das et al., 2012). Kidneys are known as the primary target organs for the manifestation of toxicopathological effects and biochemical alterations of As in animals (Nandi et al., 2005). Arsenic binds with proteins containing the sulfhydryl groups and increases the level of free reactive oxygen species in body resulting in the oxidative stress (Mohanta et al., 2014).

The accumulated concentrations of arsenic are not uniform in the tissues, probably due to variations in the rate of accumulation and expulsion of residues from different tissues (Kumar et al., 2011). Urine concentration of arsenic can be used as a biomarker for the arsenic exposure (Bera et al., 2010) and higher level of As has been reported in the urine of goat (Akter et al., 2010). Cattle reared on increased level of arsenic have the higher level of As in feces (Datta et al., 2010).

In Sindh, Pakistan, concentration of As in drinking water of farms animals was present in the range of 461-1880 µg/L (Kazi et al., 2016). Antioxidants (Vitamin E) protect the animal bodies from the oxidative stress of metals (Mashkoor et al., 2013). This vitamin acts as the
defensive mechanism against the free radicals and restores the normal functions of tissues against the arsenic toxicity in goats (Mohanta et al., 2015).

Keeping in view the rising level of arsenic in drinking water under the climatic conditions of Pakistan, this study was designed to observe the toxic effects Teddy bucks. Vitamin E was also used along arsenic to see that toxicity and accumulation can be eliminated from blood and tissues.

MATERIALS AND METHODS

This study was performed at the department of Theriogenology, University of Agriculture Faisalabad, Pakistan. Adult twelve Teddy goat bucks were randomly divided into three groups; A, B and C. The animals in group A were maintained as control whereas the groups B and C were given (sodium arsenite 5 mg/kg BW) and (sodium arsenite 5 mg/kg BW + Vit E 200 mg/kg BW) respectively. The body weight of each animal was measured with weighing balance in standing position by using the method of Akpa et al. (1998).

Collection and evaluation of samples: Blood, feces and urine samples from each Teddy goat buck were collected fortnightly. Blood (5 ml) with and without anticoagulant was collected from jugular vein of each buck in sterilized vials. The blood with anticoagulant was stored at 4°C for arsenic analysis. Blood without anti-coagulant was used for separation of serum and stored at -20°C until further analysis. About 20 ml of first voided morning urine was collected and stored. Feces were collected from the rectum of each animal, dried and grinded for the estimation of arsenic. However, after the sacrificing of experimental animals, about 5.0 g (wet weight) of tissue from liver, kidney and testes, each was dissected out and immediately stored at -20°C for arsenic determination. At the same time pieces of kidneys were also taken and stored in 10% buffered formalin (Khan et al., 2013) for histopathological studies. Hematological parameters including total red blood cells (RBCs), white blood cells counts (WBCs), hemoglobin (Hb) and packed cell volume (PCV) values were evaluated by using the method of Sharaf et al. (2013). The serum biochemical constituents were measured through colorimetric method using total proteins kits (Cat No. 1405, Bio Rays), albumin using albumin kits (Cat No. 1405, Bio Rays). Serum creatinine was measured by the use of commercially available kits (Merck, France), Having catalog # 5.17551.0001.

Determination of arsenic in blood, urine and feces was measured after digestion of the samples following the procedures outlined by Akter et al. (2010). Briefly, 0.5 ml blood, 3 ml urine and 1 gram feces were kept separately in each digestion tube and then 5 ml with three acids mixture; HNO₃ - 10 parts, HClO₄ - 3 parts and H₂SO₄ - 1 part was mixed to each digestion tube. These tubes were heated in a block digester at 120°C until clear watery fluid appeared and then placed at room temperature for cooling. The tissue samples of testis, liver and kidney placed at -20°C storage were digested in triple acid mixture on a hot plate. After digestion, the samples were diluted individually with water in 50 ml flask and concentration of arsenic was determined in digested samples using double beam atomic absorption spectrophotometer (AAS).

AAS model PG-990 was equipped with computer atomic absorption AA Win 2 software (PG instruments Ltd., UK).

Data analysis: Mean±SD in this experiment were analyzed through two-way analysis of variance. Mean values of arsenic contents in tissues were analyzed through one-way analysis of variance. Value of P<0.05 was used to be significant.

RESULTS

Body weights and hematology: The overall mean body weight of bucks of groups B and C was significantly lower than that of group A (Table 1). Analysis of data after two weeks revealed that in group B, there was reduction in the body weight at day 42-84 as compared to control. There was non-significant decrease in body weight in group C due to vitamin E when compared to control. The overall mean values of blood parameters; RBCs count, hemoglobin, WBCs and PCV of groups B and C were significantly (P<0.05) lower (Table 1). Weekly analysis of data indicated that on day 42-84, the RBCs decreased significantly in arsenic treated as compared to control. However, there was non-significant reduction of this parameter in group C. On weekly analysis, there was no significant reduction in Hb concentration in three groups at 0 day. There was significant decrease on 56-84 day of experiment in arsenic treated as compared to group A. The concentration of Hb decreased non-significantly in group C. fortnightly analysis of data revealed that WBCs decreased on day 42-84 of experiment in group B. Supplementation of vitamin E in group c, there was non-significant reduction of WBCs. On day 0 of experiment, there was no significant difference in all the groups in PCV values. PCV concentration decreased significantly from 56-84 day of experiment in arsenic treated as compared to control. In group C, concentration of PCV slightly decreased.

Serum biochemical constituents: Mean level of total protein and albumin were lower in groups B and C when compared with control. Analysis of data after two weeks revealed that concentration of these two constituents reduced on day 24-84 in As group when compared with control. However, total protein and albumin were decreased non-significantly in group C. The mean level of creatinine was higher in group B and C as compared to control. Analysis of data after every two weeks revealed that there was non-significant difference in creatinine in 0 day of experiment. The level of creatinine on day 14-84 of experiment was decreased significantly in arsenic treated animals. In group C, there was non-significant decrease in creatinine was Table 1.

Arsenic level in blood, urine, feces and tissues; The overall higher total arsenic in blood, urine and feces were present in groups B and C as compared to control (Table 2). Analysis of data after every two weeks showed that As level was increased significantly in blood in arsenic treated animals as compared to control. Urine and feces have the higher level of As which decreases with the dose and duration of experiment. In group C, there was significant increase of As in urine and feces indicating its excretion due to vitamin E.
At a few places, urinary space was clear and dilated.

Kidneys of control bucks did not exhibit any histopathological change as seen in arsenic-exposed Teddy bucks. The latter displayed mild to severe necrosis and degenerative changes in kidneys. There were profuse hemorrhages in medulla, indicative of glomerular damage (Fig. 1B). These toxic lesions were ameliorated by feeding vitamin E (Fig. 1C). Severe congestion was present throughout the renal parenchyma. Acute tubular necrosis is noted by pyknotic nuclei was noted in proximal convoluted tubules as shown in Fig. 1E. At a few places, urinary space was clear and dilated.

Histopathological changes: Kidneys of control Teddy bucks did not exhibit any histopathological change as seen in arsenic-treated Teddy bucks (Fig. 1A & D). The latter displayed mild to severe necrosis and degenerative changes in kidneys. There were profuse hemorrhages in medulla, indicative of glomerular damage (Fig. 1B). These toxic lesions were ameliorated by feeding vitamin E (Fig. 1C). Severe congestion was present throughout the renal parenchyma. Acute tubular necrosis is noted by pyknotic nuclei was noted in proximal convoluted tubules as shown in Fig. 1E. At a few places, urinary space was clear and dilated.


discussion

Arsenic has pronounced toxicity in Teddy bucks in this study. In toxicological experiments, body weights have the pronounced toxic effects of metals. Body weights in arsenic-exposed Teddy bucks decreased significantly and these results are in close harmony with the findings in Black Bengal goats (Akter et al., 2010) and sheep (Keshavarzi et al., 2015). The reduction in body weights in goats can be due to intoxication of arsenic (Biswas et al., 2000) and this may vary due to dose, route and duration of sodium arsenite ( Chattopadhyay et al., 2001).
However, contrary to our studies, no effect of sodium arsenite on the body weights has been reported in mice by Sanghamitra et al. (2008). This difference may be due to species variations. Feeding of vitamin E with arsenic has non-significant effects on body weights. Similar kinds of results were present in birds (Mashkoor et al., 2013). This restoration in body weight may be due to antioxidant property of vitamin E (Yue et al., 2010).

Major hematologic alterations in terms of reduction in red blood cells (RBCs) count, hemoglobin (Hb) concentration and packed cell volume (PCV) were observed. These results are similar to previous reports in goats (Das et al., 2012) and cattle (Rana et al., 2010). The reduction in the erythrocytic indices might be due to severe hemolysis (Simonato et al., 2008), resulting in the reduction of PCV and hemoglobin. Increase in white blood cell counts (WBC) count in rats with arsenic trioxide has been reported by Campbell Charles (2014). However, contrary to our results, WBCs have been reported to decrease by Das et al. (2012). The increase in WBCs count observed in the present study might be due to increased level of thrombopoietin due to arsenic trioxide toxicity. Vitamin E neutralized the toxic effects of sodium arsenite in RBC count, hemoglobin content, PCV and WBCs count. The antioxidant effects of vitamin E might be due its scavenging properties through the increase in the production of glutathione and superoxide dismutase (Selvaraj et al., 2012; Mohanta et al., 2015).

**Fig. 1:** Histopathology of Kidney. A) Control Teddy bucks showing normal parenchyma. B) Arsenic treated kidney of Teddy buck showing atrophy of glomerulus (black arrow) congestion (arrow head) and pyknotic nuclei (red arrow). C) As+Vit E showing normal urinary space (black arrow) and pyknotic nuclei (red arrow). D) Teddy buck showing normal parenchyma E) given As, showing congestions and tubular necrosis (black arrow) and pyknotic nuclei (red arrow) F) given As+vit E, showing congestion (red arrow) and pyknotic nuclei (black arrow) H&E; A, B and C=200X; D) Teddy buck showing normal parenchyma E) given As, showing congestions and tubular necrosis (black arrow) and pyknotic nuclei (red arrow) F) given As+vit E, showing congestion (red arrow) and pyknotic nuclei (black arrow) H&E; D-F=200X.
Table 3: Mean values (±SE) of arsenic parts per million (ppm) in liver, kidneys and testes of Teddy bucks in three groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver (ppm)</th>
<th>Kidney (ppm)</th>
<th>Testes (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Control)</td>
<td>0.01±0.001A</td>
<td>0.015±0.003B</td>
<td>0.000±0.000C</td>
</tr>
<tr>
<td>B (Arsenic)</td>
<td>2.57±0.480A</td>
<td>1.75±0.111A</td>
<td>0.128±0.005A</td>
</tr>
<tr>
<td>C (As+vit E)</td>
<td>0.012±0.001A</td>
<td>0.013±0.003B</td>
<td>0.015±0.003B</td>
</tr>
</tbody>
</table>

Similar superscripts in a row or in a column are statistically nonsignificant (P>0.05). The capital letters represent the overall means and small letters represent the groups means.

The serum biochemical constituents are widely used by veterinarian to find the health and metabolic activities of ruminant. The reduction in the level of total protein is similar to the previous reports in goats (Das et al., 2012). Hypoproteinemina in arsenic treated goats may be due to damage of function of kidney parenchyma, resulting in excretion of protein through the urine (Das et al., 2012). Reduction of albumin in serum suggests the damage in the walls of glomerulos and mucous membranes, causing excretion of albumin in urine. The reduction in the level of serum albumin in this study is in line with previous findings in cattle (Rana et al., 2008) and broilers (Khan et al., 2013; 2014). The rise in serum level of proteins due to vitamin E may be attributed to reduce resistance of insulin in hepatic cells subsequently stimulation of amino acids into proteins (Manning et al., 2004).

Increase in the serum creatinine due to sodium arsenite is similar to other species like rats (Nandi et al., 2005) and goats (Akter et al., 2010; Das et al., 2012). This increase in serum creatinine level indicates the renal damages due to persistent exposure of arsenic (Das et al., 2012). Supplementation of vitamin E reduced raised creatinine level in serum due to antioxidant property of this vitamin.

The concentration of arsenic in urine can be considered as a biomarker for the arsenic exposure (Bera et al., 2010). The urine concentration of arsenic recorded in this experiment corroborates with the findings of Patra et al. (2012) and Gosh et al. (2013) who reported increased concentration of arsenic in urine of goats that consumed arsenic. The concentration of arsenic in urine in present the study was reduced at 56th day onwards, indicating its accumulation in various body tissues (Patra et al., 2012). Increased level of arsenic in feces of cattle given arsenic in the present study is similar to the findings of Datta et al. (2010), who reported higher concentration of arsenic in feces of cattle reared on arsenic contaminated zone. Vitamin E along sodium arsenite also increased the arsenic excretion in urine and feces. Increasing the thiamin, niacin, pyridoxine and vitamin B contents in the diet also increased the arsenic in urine (Argos et al., 2010). This increased level of arsenic in urine and feces of bucks given vitamin E might be due to better methylation process. Higher level of arsenic in blood in this study is similar findings of Akter et al. (2010). Teddy bucks treated with sodium arsenite along with vitamin E showed low level of arsenic in their blood. Similar reduction of arsenic was observed in blood of goats exposed to 50 ppm arsenic + 150 IU of vitamin E (Das, 2011).

Significantly higher amount of arsenic in tissues of Teddy goat bucks has been reported in arsenic exposed animals like pigs (Fouad et al., 2012). The residual concentrations of arsenic were not consistent in each organ due to accumulation and clearance of arsenic from tissues. Biswas et al. (2000) and Roy et al. (2008) observed that liver had the highest accumulation of arsenic. However, in other studies, the highest level of arsenic was observed in kidneys rather than liver (Mohanta et al., 2015). This seems to be due to rapid mobilization of arsenic in liver than other organs (Kumar et al., 2011). The reduced arsenic concentration in these tissues due to vitamin E can be attributed to excretion pattern of this vitamin. Reduction in the arsenic contents was observed due to vitamin E in liver, kidneys and testes of goats (Mohanta et al., 2015). Bowman’s capsular spaces were expanded due to the shrinkage of the glomerular cells. Similar findings have been observed in goats (Ghosh et al., 2014) and sheep (Ashrafihelan et al., 2013). Vitamin E supplemented reduced degenerative lesions due to antioxidant properties and production of glutathione and superoxide dismutase by reducing the cell injuries (Selvaraj et al., 2012).

Conclusions: It was concluded that exposure of Teddy bucks to sodium arsenite, results in the hemato-biochemical alterations as well as the accumulation and histopathological changes in tissues. Supplementation of vitamin E alleviated the toxic effects in blood and helps in the excretions from body.

Authors contribution: MZ, MA and HJ designed the experiment and analyzed the obtained data. MKS and ST Gull assisted during the histopathological studies of tissues. SU helped in the treatment and data collection.

REFERENCES


