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RESEARCH ARTICLE

Dietary Vitamin E Supplementation: A Strategy to Combat Arsenic Induced Toxicity in Teddy Bucks

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ABSTRACT

The present study was designed to observe the toxic effects of sodium arsenite on serum biochemical constituents and liver parenchyma along with amelioration with vitamin E in teddy bucks. Adult sixteen bucks were purchased from the market and divided into four groups with specific treatments for 84 days; A (control), B (sodium arsenite 5mg/kg BW), C (Sodium arsenite 5mg/kg BW +Vit E 200mg/kg BW) and D (Vit E 200mg/kg BW). The serum biochemical parameters were evaluated fortnightly. Analysis of data revealed that there was significant $P<0.05$ rise in alanine aminotransferase (ALT), aspartate transaminase (AST), glucose and total cholesterol in arsenic treated animals. After slaughtering of animals, visceral organs were observed for gross lesions and processed for histopathologically studies. The major histopathological changes were congestions, pyknosis and cytoplasmic vacuolation in liver. Supplementation of vitamin E along with sodium arsenite alleviated the toxic effects on ALT, AST, glucose and total cholesterol level as well as histopathology of liver. It was concluded from the present study that sodium arsenite causes the toxicity in teddy bucks and vitamin E has the ameliorative effects on these toxic effects of arsenic.

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INTRODUCTION

Arsenic (As) is considered as one of the most potent environmental toxic metalloid and exist as organic and inorganic forms. Inorganic forms are more toxic and considered as potent carcinogen (Mashkoor *et al.*, 2013). Human beings and animals are being exposed to inorganic arsenic through consumption of contaminated drinking water and food. Now, the contamination of environment by inorganic arsenic has become life threatening issue around the world. The use of arsenic in herbicides, rodenticides and fungicides is considered as source of this metal in soil, air and ground water. Millions of population are exposed to higher level of arsenic compounds than the permissible level of <10µg/L due to increased levels of As in groundwater in many countries like United States, Thailand, Mexico and China (Mashkoor *et al.*, 2013).

The contamination of ground water with arsenic in many areas of Pakistan has also been identified (Anonymous, 2002). The increased level of arsenic in water leads to rise its level in fodder and feed (Ghosh *et al.*, 2013), resulting in its accumulations in the blood and tissues of animals (Mohanta *et al.*, 2013). The increases in the activities of liver enzymes like AST and ALT in the arsenic exposed animals are indications of arsenic toxicity (Rana *et al.*, 2010; Khan *et al.*, 2014). Arsenic toxicity results in damage of pancreas cells leading to raise the level of sugar (Pimpar and Bhave, 2010). Cholesterol rises in the serum in rats due to toxicity of arsenic (Muthumani and Prabu, 2013). The histopathological studies of liver revealed that long time oral exposure of arsenic caused the severe damage to liver (Bashir *et al.*, 2006; Zhang *et al.*, 2014).

Vitamins have the ability to reduce the toxic manifestations of heavy metals. The Vitamin E with dose of 50 IU/kg scavenged the heavy metals in mice (Atef, 2011). Total population of goat in world is 800 million out of which 66.6 million heads belong to Pakistan. Total teddy goat (*Capra hiricus*) population in Pakistan is 13.2 million (GOP, 2014). Teddy goats are one of the well-known goats of Pakistan, with Punjab being the home tract of this breed. Under the climatic conditions of Pakistan, goats can also be exposed to arsenic due to free grazing and drinking the contaminated water. Keeping in

view the contact of arsenic to goat along the rising level of arsenic in Pakistan, the present study was planned to investigate the toxic effects of sodium arsenite on Teddy bucks. Efforts were also made to see if these toxic effects can be ameliorated by vitamin E.

MATERIALS AND METHODS

Experimental animals: This study was performed on Teddy bucks at Semen Production Unit, Department of Theriogenology, University of Agriculture Faisalabad, Pakistan. A total of sixteen clinically healthy adult Teddy bucks, approximately 18 -24 months of age with 17-22 kg body weight, were purchased from the market. Animals were kept for 15 days for the acclimatization to experimental conditions. These animals were vaccinated against pleuoropnemonia and enterotoxaemia. The bucks were maintained under similar managemental conditions in well ventilated sheds with cemented floor and individual feeders. These bucks were tagged for identification and housed in separate sheds. They were treated for both ecto and endo-parasites before the start of the experiment. They were fed on 0.5kg concentrate ration of wheat bran, available green fodder with drinking water ad-libtum. The animals in each group were subjected to different treatments; A (Control group), B **(**Sodium arsenite BDH Laboratories Supplies, Poole, England with dose of 5 mg/kg BW /day), C (Sodium arsenite as in group B+Vit E (Lutavit E 50 BASF, Ludwigshafen Germany) at dose of 200mg/kg BW/day) and D (Vitamin E with dose of 200mg/kg BW/day. Sodium arsenite was packed in gelatin capsule and fed to animals orally. These treatments were continued for 84 days.

Collection of blood: Blood samples from all the animals in different groups were collected at day 0, 14, 28, 42, 56, 70 and 84 of the experiment. For this purpose, animals were properly restrained; area of blood collection i.e. jugular vein was cleaned, dried and disinfected by methylated alcohol swabs. About 5ml of blood with and without anticoagulant was collected from each experimental buck in sterilized vials. These blood samples were stored at 4ºC. Blood samples without anti-coagulant were used for separation of serum. Samples were allowed to clot at room temperature for 30-45min, and then centrifuged at 5000 g for 15min. Serum samples were stored at -20ºC and thawed at room temperature analyzed for serum biochemical constituents.

Serum activities of ALT and AST were measured utilizing commercially available colorimetric kits Fluitest company (Catalog # 1187) and (Catalog # 1626) respectively. Glucose and cholesterol was determined using the glucose kit (Breuer and Breuer Diagnostic Germany) and (Fluitest HDL-D Biocon Germany) respectively. Absorbance of the samples and standards was measured using chemistry analyzer (BTS-330, Biosystems, Spain).

Gross and histopathology: After euthanizing the animals, visceral organs were examined for gross lesions and tissues showing gross lesions were preserved in 10% buffered formalin. Specimens of 5 mm thickness from liver 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 made and stained with Hematoxylin and Eosin (Bancroft and Gamble, 2008).

Data analysis: Mean±SD were subjected by two-way analysis of variance (ANOVA) followed by the Tukey test for multiple comparison. $P \leq 0.05$ was considered to be significant.

RESULTS

Overall mean of ALT and AST of group B and C was higher as compared to group A. Fortnightly analysis of data revealed that at day 0, there was no significant difference in the mean values of these parameters. ALT and AST activities level increased significantly on day 14-84 in group B as compared to group A indicating the arsenic toxicity. However, there was less increase in the activity of these two parameters in C group when compared to group A. In group D, there was non-significant difference when compared to group A (Table 1).

Mean values of cholesterol in groups B and C were significantly higher as compared to group A. Whereas, mean value of cholesterol in group D was lower as compared to control. Fortnightly analysis of data indicated that in group B, on day 28-84, serum level of cholesterol increased significantly as compared to control indicating the toxicity of arsenic. There was no significant change in level of cholesterol in group C throughout experiment indicating the amelioration. There was significant reduction in level of cholesterol in group D during this experiment (Table1). Overall mean values of glucose in groups B and C were higher as compared to group A. Fortnightly analysis of data revealed that in group B, concentration of glucose increased on day 28-84 indicating toxic effect of arsenic. Co-administration of vitamin E and arsenic had the ameliorating pattern in group C. In group D, there was non-significant increase in the level of glucose (Table 1).

Gross and histopathology: Arsenic-treated bucks showed enlargement of the kidneys and abomasums. The liver suffered with greyish-white necrotic foci on the surfaces. The lungs exhibited severe congestion with adhesion of visceral organs. The abomasum, intestinal mucosa, kidneys and spleen were hemorrhagic in varying degrees, whereas the liver was necrosed. The intestine contained thick, viscid and slimy material. Supplementation of vitamin E along with sodium arsenite alleviated these lesions in visceral organs. The hepatic cells have nucleolus with chromatin material (Fig.1B). Arsenic treated teddy bucks showed hepatic parenchyma with pyknotic nuclei and congestions. Fibroblasts proliferation was increased. Mild to moderate degree of vacuolation was also present in hepatocytes (Fig. 1C). Arsenic+ vit E treated teddy bucks showed nucleoli of hepatocytes are normal in appearance. At few places, mild degree of congestion was present. Sinusoidal spaces were normal in appearance. Overall, ameliorative pattern has been observed (Fig. 1D).

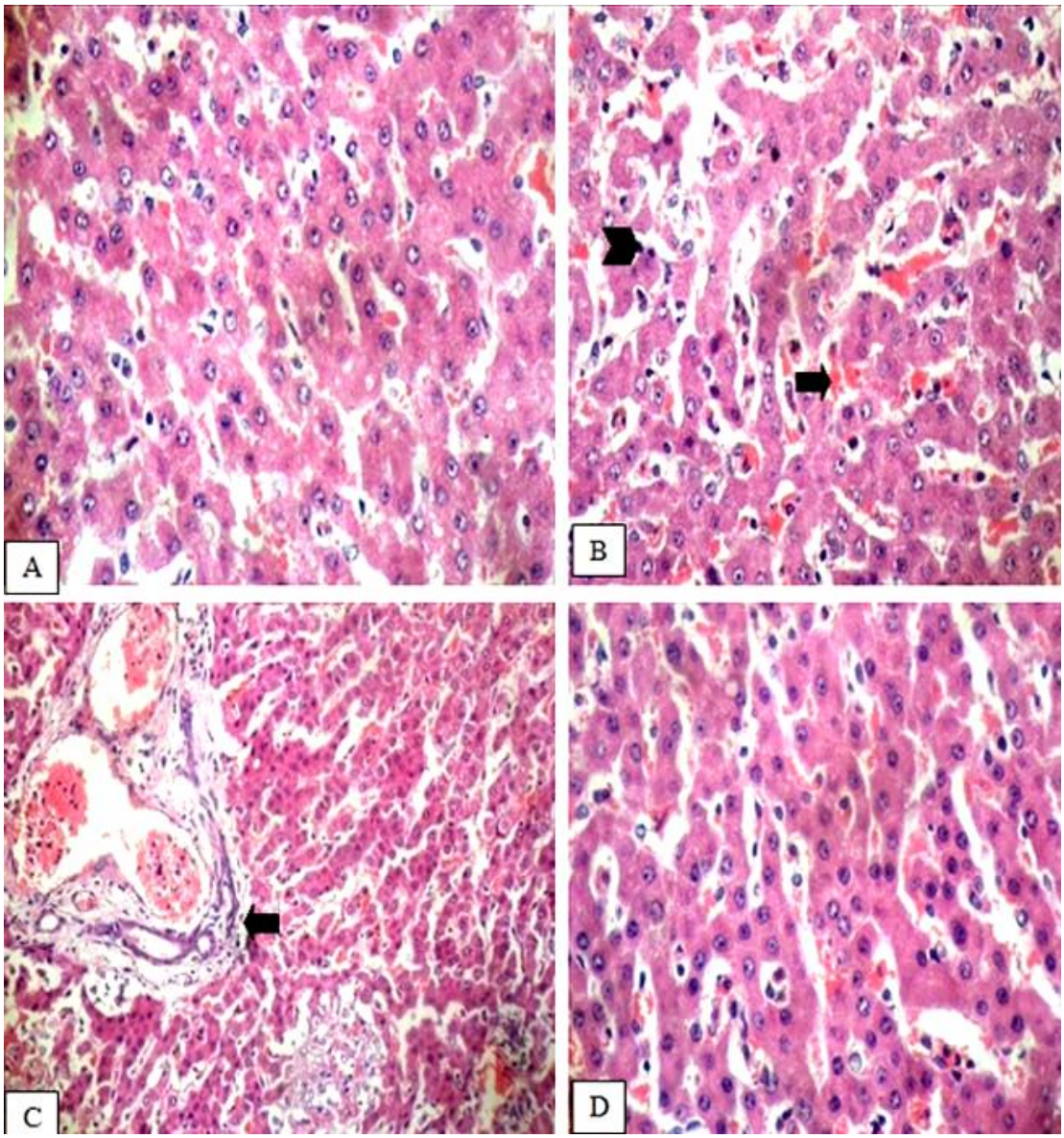

Fig. 1: Histopathology of liver. A) Control teddy bucks **s**howing normal hepatic parenchyma. B) Arsenic treated liver of teddy bucks showing pyknosis (Arrow head), and hemorrhages (Arrow).C) Arsenic treated bucks showing biliary hyperplasia (Arrow). D) Photomicrograph of liver (As+ vit E) of teddy bucks showing mild pyknosis and a few condensations of nuclei. H&E; A, B and D= 400X; C=200X.

Table 1: Toxic effects of arsenic on serum biochemical constituents and its alleviation with vitamin E

	Days after experimental feeding						
Groups	0	14	28	42	56	70	84
ALT (U/L)							
A	23.7±2.0efg	24.75±1.0efg	24.30±1.7efg	23.83±2.1efg	24.08±1.8efg	23.78±1.6efg	24.25±1.7efg
B	24.0±1.4efg	27.0±2.16d-g	30.50±3.7d	36.00±3.4c	43.75±2.7b	46.5±3.4ab	49.75±4.0a
C	22.75±0.9g	24.38±0.75efg	24.50±0.6efg	27.75±1.3d-g	27.5±1.73d-g	28.5±0.57de	28.25±0.95def
D	22.75±0.9g	23.25±0.9 efg	22.50±1.29g	23.0±1.6efg	24.75±2.2efg	22.75±1.70g	23.0±1.82fg
AST (U/L)							
A	130.75±1ef	130.85±0.87ef	129.05±0.9ef	129.25±2.7ef	127.75±1.5f	130.05±2.5ef	129.75±2.0ef
B	130±1.5ef	133.25±1.70ef	144.0±4.24cd	151.7±5.8bc	159.75±6.3b	170.0±8.1a	171.5±6.02a
C	129±0.8ef	130.0±0.81ef	132.75±1.70ef	134.0±0.81ef	135.2±1.25ef	136.7±2.5de	135.75±1.7def
D	131.5±2ef	130.42±1.84ef	129.5±1.73ef	130.4±2.07ef	129.65±1.8ef	128.65±1.5ef	127.88±1.32f
Cholesterol (mg/dL)							
A	62.72±3.62c-i	62.92±3.76c-i	64.17±2.77c-h	63.4±2.17c-i	64.8±3.0c-g	64.75±3.8c-g	66.25±4.2c-f
B	63.25±2.76c-i	66.5±2.4cde	81.5±3.2b	84.75±2.0ab	87.25±2.3ab	88.75±1.7a	89.87±1.6a
C	62.75±3.0c-i	63.25±2.2c-i	64.75±2.8c-g	67.0±2.6cd	67.50±1.2cd	68.25±1.7cd	68.5±1.73c
D	61.2±52.9d-i	59.7±2.21e-i	59.25±2.2f-i	58.0±2.5ghi	57.5±1.9hi	57.25±2.3hi	56.5±2.4i
Glucose (g/dL)							
A	47.43±0.42de	48.32±0.86cde	47.37±0.50de	48.10±1.4de	48.38±1.80cde	47.37±0.45de	46.92±1.30de
B	47.4±0.63de	49.65±0.95cde	52.68±1.67c	58.65±1.65b	61.60±1.79ab	65.77±2.70a	65.55±2.9a
C	48.52±1.30cde	48.23±1.24de	48.05±1.79de	49.50±0.58cde	50.10±2.3cde	50.75±2.22cd	50.55±3.24cde
D	47.25±1.3de	47.22±1.48de	48.18±1.36de	46.85±1.78de	47.22±1.30de	47.42±1.24de	46.32±0.68e

Values (Mean±SD) with different alphabets in a row (among experimental days) or in a column (under specific parameter) are differ significantly (P<0.05).

DISCUSSION

The serum biochemical constituents are used as extensively by veterinarian to check the health and metabolic status of ruminants (Hala *et al.,* 2014). In the present study, activities of serum enzymes AST and ALT were significantly increased in the arsenic exposed animals. Hepatocytes are adversely affected due to binding of arsenic to enzymes and protein containing thiol groups subsequently damaging the cell membranes and leading to elevate the activities of AST and ALT. Similar kind of reports are present in goats (Mohanta *et al.,* 2015) and sheep (Pattanaik *et al.,* 2012). The rise in the activities of ALT and AST was also reported by Rana *et al.* (2010) in cattle reared in the arsenic contaminated area. Liver is considered as the primary site for the biomethylation due to certain enzymes. The slow rise of ALT and AST in this study suggests the liver toxicity and damage (Rana *et al.,* 2010). Presence of these two enzymes in blood indicates the leakage from the cytosol. Supplementation of vitamin E @ 200mg/kg body weight in arsenic treated groups protected the rising level of ALT and AST in serum in present study. Similar kind of protection in transaminases due to antioxidants against arsenic has been reported in goats (Das *et al.,* 2012; Mohanta *et al.,* 2015) by supplementation of 250 IU vitamin E/kg diet.

Elevated level of serum glucose observed in present study was consistent with previous findings of Kumar*et al.* (2014). Increase in the level of glucose might be due to binding of arsenic with sulfhydryl groups of enzymes subsequently blocking the metabolism of glucose (Kumar*et al.,* 2014). Exposure of arsenic causes the toxicity of islets cells resulting in damage of pancreas leading to the rise of sugar (Izquierdo-Vega *et al.,* 2006). Supplementation of vitamin E restored the functions of pancreatic cells and maintained the normal level of glucose. This maintenance may be due to antioxidant property of vitamins (Acharya *et al.,* 2004). There was no significant effect of vitamin E alone on serum levels of glucose and similar kinds of results were present in mice (Dahdouh *et al.,* 2014). Cholesterol performs the important roles as precursor of hormones, storage of energy and regulators of inflammation. The significant rise in total serum cholesterols was noted in present study. This rise of cholesterol due to arsenic toxicity is in consistent with previous reports in rats reported earlier by Chattopadhyay *et al.* (2011) and Muthumani and Prabu, (2013). The higher level of cholesterol may be due to liver damage and obstruction of bilirubin due to insecticides. The supplementation of vitamin E may have suppressive effects on cholesterol biosynthesis and might increase the use of low-density lipoprotein (LDL) from body by liver Muthumani and Milton (2013). Reduction in total level of cholesterol in vitamin E group was similar to findings of Wojcicki *et al.* (1991) in rabbits. Contrary to present findings, higher level of total cholesterol was reported in buffalo heifer due to feeding of vitamin E (Nayyar *et al*., 2003).

In the present study, major toxicopathological changes were observed in liver parenchyma. The liver is considered as the major organs for histological changes due to accumulation of arsenic and unique vasculature along with the production of toxic metabolites (Ghosh *et al.,* 2014). Microscopically, hepatic parenchyma was showing normal appearance of hepatic cords in control animals. In the present study, liver parenchyma of arsenic treated teddy bucks exhibited necrotic and degenerative changes like intracytoplasmic vacoulation, alterations in nucleus like, pyknosis and cells without nucleus. Biliary hyperplasia was present due to infiltration of mononuclear cells near the portal vein. Mild to moderate degree of congestion was also present throughout the parenchyma. Lesions have been reported in various species like goat (Ghosh *et al.,* 2014), rats (Jadhav *et al*., 2007), cattle (Rana *et al*., 2010) and birds (Mashkoor *et al.,* 2013; Sharaf *et al.,* 2013; Kousar and Javed, 2015).

In addition to these changes, higher proliferation of connective tissue has been documented due to oral intoxication in mice. The mechanism of arsenic is assumed to bind the proteins/enzymes containing the sulfhydral groups which are more susceptible to reactive oxygen species (ROS) subsequently leading to inhibition their activities (Rana *et al.,* 2010). The production of ROS leads to oxidation of lipid could result into necrotic and degenerative deformities in hepatic cells. Histopathological observation supports the fact that arsenic badly affects the liver parenchyma due to long term exposure and it is supported by Bashir *et al.* (2006). Except mild degree of congestion in present study overall there was ameliorative pattern in sodium arsenite + vitamin E group. Similar findings have been reported in birds (Mashkoor *et al.,* 2013; Sharaf *et al*., 2013). It has been observed that feeding of arsenic attack the polyunsaturated fatty acids in the cell membrane and creates the chain reaction leading to deformities in the cell membrane function and integrity (Das *et al.,* 2012). This kind of chain reaction can be prevented by vitamin E by scavenging the free reactive oxygen species and production of α-tocopheroxyl radical which is not harmful to cell, thereby checking the lipid per oxidation. Reduction in lesions may be due to reduction of bowel transits stay time in intestine (Rana *et al.,* 2010).

Conclusions: It may be concluded from the present study that arsenic causes the toxicity in teddy bucks via the major alterations in serum biochemical parameters and severe toxic changes in liver parenchyma. The vitamin E alleviated the toxic effects of arsenic. This study reports a preliminary data of liver toxicity and envisages for further studies to find the toxic effects on kidney along accumulations of arsenic concentration in liver and kidney.

Authors' contribution: MZ and MA conceived the idea, designed the project, executed the experiment and analyzed the parameters. HJ and FD were actively involved in data analysis, interpretation, write up and revision of the manuscript.

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