



Effect of Vitamin E and Selenium Supplementation on Arsenic Induced Oxidative Stress in Goats

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ABSTRACT

Present study was designed to assess the effect of vitamin E+Se supplementation on growth performance as well as antioxidant status in arsenic toxicity in experimental goats. Eighteen cross breed goats of either sex, aged between 12 to 24 months of age were randomly selected and divided into three groups. Group I was considered as healthy control. Goats of group II and III received Sodium arsenite (NaAsO₂) @ 5 mg/kg orally daily for 45 days. The goats of group III also received the treatment of Vitamin E+Se. Increase growth performance was observed in animals supplemented with Vitamin E+Se treatment as compared to group II animals. Significantly (P<0.05) increased lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) enzyme activity in blood, liver and kidney tissue were observed in arsenic intoxicated goats. However, arsenic treatment caused depletion of reduced glutathione (GSH). Restoration of arsenic toxicity effects were observed in group III animals. (Kile *et al.*, 2007) Therefore, present study indicated the ameliorative potential of Vitamin E + Se against toxic effects of arsenic.

Keywords: Arsenic, Goats, Vitamin E, Oxidative stress, Antioxidant

Arsenic (As) is a metalloid found in water, soil, and air from natural and anthropogenic sources (Hughes, 2002). Arsenic occurs in both organic and inorganic forms in nature, but the inorganic form is more toxic and represents a potential threat to the environment, human and animal health. Various livestock animals are also the likely victims of such catastrophes arising from As pollution due to contamination of drinking water. Animals are mainly exposed to inorganic arsenic through drinking water; they are also exposed to some organic forms of arsenic through feed. The effects of arsenic accumulation after long-term intake of low doses to goats remain obscure and may possess a potential dietary risk to humans (Kile *et al.* 2007). Biotransformation of absorbed arsenic mostly takes place in liver and kidneys (Ford, 2002). Methylated metabolites formed during process of ion transformation are distributed throughout the body (Dopp *et al.*, 2004). Administration of As could affect animal performances and serum clinical parameters. Liver and kidneys

are considered as the primary targets for its toxicopathological manifestations and there are a few reports of biochemical alterations indicative of hepatic and renal system involvement in As toxicity in animals (Tandan *et al.*, 2012). Modi *et al.* (2007) have suggested that arsenic toxicity is associated with the induction of oxidative stress in vital organs through overproduction of reactive oxygen species (ROS). Therapeutic potential of various antioxidants against arsenic toxicity in laboratory animals have been reported by earlier studies (Tandan *et al.*, 2012; Munday *et al.*, 2013) but reports on this aspect in ruminants are very meager. The main biological function of vitamin E is its direct influence on cellular responses to oxidative stress through modulation of signal transduction pathways (Mohanta *et al.*, 2015). Vitamin E molecules can interrupt free radical chain reactions by capturing the free radical which imparts to them their antioxidant properties. The free hydroxyl group on the aromatic ring is responsible for its antioxidant properties. The hydrogen from this group is

donated to the free radical, resulting in a relatively stable free radical form of vitamin E (Sies and Murphy, 1991). In relation to the fortification with Se, it was observed that Se and Vitamin E is interrelated in antioxidant system by double way, first, the formation of glutathione peroxidase contribute decomposition of LPO and secondly as chain breaking antioxidant (Barcelia *et al.*, 2008). Thus the present investigation was undertaken to assess of vitamin E and selenium on growth performance and antioxidant oxidative/antioxidant status on arsenic induced alterations in goats.

MATERIALS AND METHODS

Chemicals

Sodium arsenite, Bovine serum albumin (BSA), Ammonium sulfate, 2,4-dinitrophenylhydrazine (DNPH), 5,5- dithiobis (2-nitrobenzoic acid) (DTNB), ethylene diamine tetra-acetic acid (EDTA), nitro blue tetrazolium (NBT), potassium dihydrogen phosphate, reduced glutathione (GSH), sodium arsenite (NaAsO₂), sodium azide, sodium pyrophosphate, trichloro acetic acid (TCA), thiobarbituric acid (TBA), were procured from Sisco Research Laboratory, India. All chemicals and reagents used in present experiment were of analytical grade.

Experimental animals and design

Eighteen clinically healthy, nonpregnant, jamunapari cross bred goats (*Capra hircus*) of approximately 12 to 24 months and 14-18 kg weight were used. They were housed in well-ventilated shed and were acclimatized to the environment for 7 days. Goats were dewormed and complete deworming was confirmed by evaluating fresh manure monthly by modified McMaster fecal egg counting test. Animals were kept on pasture daily between 0900 and 1600 hours along with ad libitum tube well water. The experiment was performed with approval from Institutional Animal Ethics Committee (IAEC-25).

The experimental animals were divided randomly into three groups of 6 goats each to study growth performance, arsenic burden in blood and tissue and oxidative/antioxidant status stress and ameliorative potential of vitamin E+Se during oral exposure of arsenic in goats. Goats of group I were kept as healthy control and they

do not received any treatment for the period of 45 days, whereas goats of groups II and III received arsenic as sodium arsenite salt @ 5mg/kg orally daily for 45 days. The animals of group III also received the treatment of Injection Repronol (Contained Tocopherol 50 mg and selenium 1.5 mg) of Cadila pharmaceuticals Ltd, India @ 1 ml/25 kg body weight, deep intra muscularly at weekly interval till the end of experimental trial.

Growth performance

To assess growth performance in experimental animals, initial and final body weights (BW) were measured and at the end of the study, body weight gain (BWG) was calculated by subtracting initial from final body weight.

Collection of sample

Blood samples collected aseptically from jugular vein of each animal using heparinized vacutainer at the end of the study and were divided into two parts. About 2 ml blood samples were preserved at 4°C for determination of elemental arsenic and other part was used to prepare hemolysate for oxidative stress markers determination.

After sacrificing two animals from each group, tissues like liver, kidney and brain (cerebral hemisphere) were excised, washed and homogenized in a mixture of 0.1M Tris HCl and 0.001M EDTA buffer (pH 7.4) and centrifuged at 12,000 g for 30 min at 4°C. The supernatant was collected and used for further analysis.

Arsenic residual effect

Arsenic concentration was determined in water, vegetation that animal consume during the experimental period. Briefly 0.5 ml blood was kept in the digestion tube and then 5 ml triple acid mixture (HNO₃ - 10 parts, HClO₄ - 3 parts and H₂SO₄ - 1 part) and 3 ml H₂O₂ was added to it. In each digestion batch one tube was kept as blank (without sample). Known amount of As standard was digested in a separate tube in each batch to validate the assay. The content in the digestion tubes was heated in a Teflon coated bomb at 120°C until clear watery fluid appears and then kept at room temperature for cooling. After digestion, the samples were diluted individually with deionized water in 50 ml calibrated volumetric flask. Samples were finally stored at -20°C till analysis. Arsenic was estimated using a Hydride

Vapour Generation System (Perkin Elmer model MHS-10) fitted with an atomic absorption spectrophotometer.

Lipid peroxidation was determined using the method of Placer *et al.* (1966). For the preparation of RBC hemolysate, blood samples were centrifuged at 200rpm for 10mts and supernatant plasma was separated out. The sedimentated cells were washed with 0.85% NaCl solution three times. Washed erythrocyte were hemolyzed with ninefold volume of distilled water to prepare 10% RBC hemolysate. Briefly, the reaction mixture consisted of 0.2 ml of RBC hemolysate, 1.3 ml of 0.2 M Tris-0.16M KCl buffer (pH-7.4) and 1.5 ml of TBA reagent. The mixture was heated in boiling water bath for 10 min using glass beads as condenser. After cooling, 3 ml of pyridine/n-butanol (3:1 v/v) and 1 ml of 1N NaOH were added to it and mixed by shaking vigorously. The absorbance was read at 548 nm. The nmol of malonaldehyde (MDA) per ml of RBC hemolysate was calculated using 1.56×10^5 as the extinction coefficient. Lipid peroxide level in the RBC hemolysate was expressed as nmol of MDA/mg of haemoglobin. In tissue homogenate lipid peroxide was expressed in nmol of MDA/ mg of protein using 1.56×10^5 as the extinction coefficient.

Non-enzyme antioxidant: Reduced glutathione

Reduced glutathione (GSH) was estimated in hemolysate and tissue homogenate by dithio-bis-2-nitro benzoic acid (DTNB) method as per the procedure outlined by Prins and Loos (1969). To 200 μ l of hemolysate, 4000 μ l of 0.08N H₂SO₄ was added and kept at room temperature. Sodium tungstate solution (500 μ l) was added to it and shaken vigorously for 5 min. The mixture was centrifuged at 2000 g for 20 min; 2000 μ l of supernatant was taken in a separate test tube and Tris buffer and DTNB reagents were added and mixed well. The absorbance was recorded spectrophotometrically at 412 nm against blank.

Antioxidant enzymes

Catalase activity was measured spectrophotometrically in hemolysate and tissue homogenate after appropriate dilution following the method of Cohen *et al.* (1970). The reaction commenced with the addition of 50 μ l of diluted sample to 3 ml of phosphate buffer-H₂O₂ solution. Initial absorbance was read after 20 seconds. Time required for initial absorbance to decrease by 0.05 unit was recorded

between 0.6 to 0.7unit at 240 nm. Catalase present in assay mixture was expressed in unit/ mg of haemoglobin and protein for blood and tissues, respectively.

Superoxide dismutase (SOD) activity was measured in hemolysate and tissue homogenate using nitrobluetertazolium as substrate as per the method of Marklund and Marklund (1974) with certain modifications suggested by Minami and Yoshikawa (1979). Briefly, the assay mixture consisted of 50 mM of triscacodylic acid buffer (pH - 8.2), and 0.2 nM of pryragallol in a total volume of 3.0 ml. The increase in absorbance due to auto-oxidation of pyragallol was recorded at 30 sec and 90 sec using 420 nm wavelength. One unit of SOD activity was defined as the amount of enzyme, which inhibited the auto-oxidation of pyragallol by 50% under the given laboratory condition and the value was expressed in unit/ mg of haemoglobin and protein for blood and tissues respectively.

Statistical analysis

Results were analyzed by one way analysis of variance (ANOVA) by SPSS (Ver. 10.0) followed by Duncan's Test at 5% (P<0.05) significance. Results were expressed as mean \pm standard error (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Growth performance

Data presented in Table 1 revealed that 45 days of oral arsenic exposure resulted in significantly decreased body weight in group II animals. However significant increase in body weight was observed after supplementation of Vitamin E+Se treatment. Our results are in agreement with the findings of Akther *et al.* (2012),

Residual concentration

As concentration was determined in the water and vegetation which was consumed by the animals and it was found to be 0.02 mg/L and 0.003 mg/kg respectively. The presence of arsenic in the tissues was determined by atomic absorption spectrophotometer and is presented in Table 2. Residual concentration of As in blood and tissues of arsenic intoxicated goats was increased significantly (p<0.05) with the elapse of exposure duration as compared

to control animals. Vitamin E supplementation significantly reverted arsenic residues close to the pretreatment state.

Arsenic contents of feed and tissues also had a strong positive correlation in animal models (Patra *et al.*, 2012). The significant elevation of blood and tissue arsenic level indicated an increase in arsenic accumulation upon arsenic exposure. Accumulation of high arsenic residues in tissues of arsenic-treated goats may be due to repeated doses. Different concentrations of arsenic in different tissues indicate different distributions. The residual concentrations were not uniform in the studied tissues, which might be due to difference in clearance from the tissues (Kumar *et al.*, 2011). Chronic administration of inorganic arsenic to experimental animals produced various liver lesions in a dose dependent manner, including inflammation and oxidative damage (Das *et al.*, 2005).

Present study revealed that liver, the main metabolizing organ had the highest arsenic concentration as compared to kidney and brain residue. However some reports (Cui and Okayasu, 2008; Mohanta *et al.*, 2015) recorded higher levels of arsenic in kidney than in liver. Similar to our finding, elevated residual arsenic concentration in tissues was observed in the arsenic exposed experimental animals (Mathews *et al.*, 2012; Munday *et al.*, 2013; Mohnata *et al.*, 2015). Vitamin E+Se treatment marginally reduced arsenic content in the tissues of experimental animals was also observed in the earlier study (Mohanta *et al.*, 2015). The findings of the present study suggest a therapeutic role of Vitamin E+Se to restores the normal arsenic values in blood and like liver, kidney and brain tissue.

Table 1: Effect of Vitamin E + Se supplementation on growth performance in Arsenic intoxicated goats

Parameters	Healthy control	Positive control As (5mg/kg)	As+ Vitamin E + Se treatment
Body weight (Kg)	15.12±3.26	16.26±4.29	15.71±4.83
Body weight after 45 days (Kg)	16.87±2.49*	16.81±2.79	18.52±2.37*
Body weight gain (Kg)	1.75±0.64 ^a	0.55±0.28 ^b	2.81±0.74 ^c

Values are Mean±SE; n = 6; * p < 0.05 compared to normal animals (Negative control)

^{abc} Means within a row without a common superscripts differ significantly (p<0.05).

Table 2: Effect of Vitamin E + Se supplementation on residual arsenic levels blood and tissues of Arsenic intoxicated goats

Groups	Blood µg/mL	Liver µg/mg	Kidney µg/mg	Brain µg/mg
Negative control	0.16 ± 0.12	0.13 ± 0.24	0.14 ± 0.17	0.12 ± 0.31
Positive control	2.34 ± 0.56*	5.38 ± 0.12*	3.75 ± 0.26*	1.87 ± 0.16
Arsenic + Vitamin E+ Se	2.28±0.48	4.72 ± 0.243	3.42 ± 0.35	1.32 ± 0.37

Values are Mean±SE; n = 6; * p < 0.05 compared to normal animals (Negative control)arsenic exposed animals (positive control) and arsenic exposed treated animals.

Oxidative stress

The extent of lipid peroxidation (LPO) was assessed by studying the level of formation of malondialdehyde, (Table 3). Arsenic exposure resulted in significantly (P<0.05) increased LPO levels in blood and tissue samples of positive control animals than in healthy control. Liver undergoes more oxidative damage as compared to kidney and brain. Vitamin E+Se treatment was found to be significantly (P<0.05) effective in the lowering of lipid peroxidation in the blood and studied tissues.

Present investigation revealed the therapeutic potential of Vitamin E+ Se to prevent the arsenic induced alterations in oxidative stress markers. These effects may be mediated via modulation of enzyme systems that generate free radicals.

Arsenic exposure produces free radicals which cause damage to lipid, protein and DNA of the body (Manna *et al.*, 2008). Present results do confirm that inorganic arsenic causes lipid peroxidation/oxidative stress in target organs viz. liver, kidney and brain. Oxidative stress occurs when the dynamic balance between oxidant and antioxidant mechanism is impaired (Flora *et al.*, 2009). As per study of Josephy *et al.* (1997) alternatively, the radical chain reaction may be broken by the action of antioxidants and thereby controls oxidative stress. Lowering in the levels of lipid peroxidation after administration of vitamin E+Se could be related to its ability to scavenge reactive oxygen species, thus preventing further damage to membrane phospholipids. Our results are in line with previous studies by Das *et al.* (2012) who observed that vitamin E treatment

can prevent formation of free radicals and protect the experimental goats from arsenic induced oxidative stress.

Effect on GSH

Arsenic administration significantly ($P < 0.05$) decreased GSH level in blood and studied tissue samples. Vitamin E and Se treatment was found to be significant ($P < 0.05$) (Kile *et al.*, 2007) effective in elevation of GSH level in blood, liver and kidney with a non significant increase in brain tissue (Table 4).

Glutathione is the main detoxifying antioxidant and the first line of defense against the oxidative stress. Arsenic binds with sulfhydryl groups of enzymes and proteins interfering GSH metabolism (Watanabe and Hirano, 2013). Arsenic exerts oxidative stress that results in depletion of the cellular endogenous antioxidant reserve, which leads to apoptosis (Gupta *et al.*, 2003). The decline in GSH level by arsenic in the positive control animals (Group I) in the present experiment confirms these facts. A significant decrease in GSH correlated well with an increase in arsenic concentration in the respective tissues. Animals treated with Vitamin E+Se along with arsenic exposure significantly ($P < 0.05$) increase GSH level near to normalcy. Adequate levels of the cellular GSH pool required not only for maintaining the cellular redox status by keeping sulfhydryl groups of cytosolic proteins in their reduced form but also because numerous toxic or potentially toxic compounds, including some metals, are either taken up by or removed from the cells by GSH-mediated pathways (Chouchane and Snow, 2001). A decrease in cellular GSH concentration has been inversely correlated with lipid peroxidation in the liver (Maiti and Chatterjee, 2001) therefore an increased GSH concentration by the Vitamin E+Se treatment given in group III could presumably protect the organ from arsenic induced lipid peroxidation.

Effect on antioxidant enzymes

The changes in CAT and SOD activity, which is indicative of oxidative stress following exposure to arsenic either alone or in combination with vitamin E+Se supplementation are presented in Tables 5 and 6, respectively. Significantly higher ($P < 0.05$) activity of CAT and SOD was observed in arsenic exposed positive control animals. Animals of vitamin E+Se treatment group responded and were found

effective to restore the normal values of CAT and SOD activity. Vitamin E+Se administration decreased SOD and CAT activities in all treatment groups compared to positive control group. Antioxidant enzymes CAT and SOD are considered to be the first line of cellular defense against oxidative damage. Increased activity of both the enzymes signified the presence of oxidative stress in order to combat an excess production of free radicals. SOD and catalase are important radical scavenging enzymes and constitute body's second defense system against oxygen metabolites produced by arsenic (Naujokas *et al.*, 2013).

The decrease in the erythrocytic components in goats indicated the presence of oxidative stress. Further, CAT is well known to catalyze the removal of H_2O_2 formed during the reaction catalyzed by SOD. Present study recorded significantly increased SOD and CAT enzyme activities in the blood and liver and non-significant increase was observed in kidney and brain tissues of experimental animals against arsenic intoxication. The sharp rise of SOD and CAT activity with respect to arsenic feeding might be attributed to up-regulation in the synthesis of SOD or CAT, as a self-protective response against oxidative stress (Pi *et al.*, 2002). Increased catalase activity showed activation of defense system and hence can be used effectively as marker of oxidative stress (Patra *et al.*, 2010) whereas Mohanta *et al.* (2015) found decreased levels of these enzymes in arsenic intoxication in goats. Rana *et al.* (2010) also reported significant decreased SOD and CAT activity in cattle of arsenic prone zone. In contrast to the present experiment, significant ($p < 0.05$) decreased SOD and a CAT activity was observed in arsenic treated groups (Das *et al.*, 2012).

Therefore it is concluded that the arsenic exposure led to changes in growth performance and varying degree of changes in antioxidant defense mechanism of body. Present investigation also revealed that the co administration of vitamin E and selenium was found effective in quenching singlet oxygen and scavenging free radicals. So, specific protection of vitamin E from oxidative damage may be helpful to combat arsenic-associated adverse effect in animal.

The present study further substantiate the physiological role of vitamin E on antioxidant defense systems and findings have significant implications in elucidating the therapeutic use of vitamin E as antioxidants drug and in management of Arsenic toxicity.

Table 3: Effect of Vitamin E + Se supplementation on Lipid Peroxidation (LPO) levels in tissues of Arsenic intoxicated goats.

Groups	Erythrocytes (nmol MDA/ mg of Hb)	Liver (nmol MDA/ mg of protein)	Kidney (nmol MDA/ mg of protein)	Brain (nmol MDA/ mg of protein)
Negative control	1.42 ± 0.23	0.32 ± 0.32	0.17 ± 0.15	0.04 ± 0.26
Positive control	6.87 ± 0.31*	4.13 ± 0.44*	2.65 ± 0.20*	1.75 ± 0.18*
Arsenic + Vitamin E	2.65 ± 0.24 ^a	1.52 ± 0.36 ^a	2.27 ± 0.38	1.26 ± 0.25

Values are Mean±SE; n = 6; * p < 0.05 compared to normal animals (Negative control) arsenic exposed animals (positive control) and arsenic exposed treated animals.

Table 4: Effect of Vitamin E + Se supplementation on Catalase levels in tissues of Arsenic intoxicated goats

Groups	Erythrocytes μmol/g of Hb	Liver μmol/g tissue	Kidney μmol/g tissue	Brain μmol/g tissue
Negative control	7.66 ± 0.31	5.12 ± 0.28	3.65 ± 0.36	2.13 ± 0.39
Positive control	2.87 ± 0.42*	1.51 ± 0.19*	1.13 ± 0.46*	1.83 ± 0.35
Arsenic + Vitamin E	5.16 ± 0.33 ^a	4.80 ± 0.25 ^a	3.28 ± 0.36 ^a	2.49 ± 0.21

Values are Mean±SE; n = 6; * p < 0.05 compared to normal animals (Negative control) and a P<0.05 compared with (positive control) arsenic exposed animals.

Table 5: Effect of Vitamin E + Se supplementation on Catalase levels in tissues of Arsenic intoxicated goats

Groups	Erythrocytes U/mg of Hb	Liver U/mg of protein	Kidney U/ mg of protein	Brain U/ mg of protein
Negative control	1.58 ± 0.26	9.25 ± 0.28	11.32 ± 0.45	7.65 ± 0.33
Positive control	4.16 ± 0.31*	12.44 ± 0.34*	15.39 ± 0.36*	12.43 ± 0.25*
Arsenic + Vitamin E	2.32 ± 0.73 ^a	9.81 ± 0.32 ^a	12.45 ± 0.28 ^a	9.12 ± 0.42 ^a

Values are Mean±SE; n = 6; * p < 0.05 compared to normal animals (Negative control) and a compared with (positive control) arsenic exposed.

Table 6: Effect of Vitamin E + Se supplementation on superoxide dismutase (SOD) levels in tissues of Arsenic intoxicated goats

Groups	Erythrocytes U/mg of Hb	Liver U/ mg of protein	Kidney U/ mg of protein	Brain U/mg of protein
Negative control	6.14 ± 0.25	5.37 ± 0.18	6.65 ± 0.28	4.32 ± 0.39
Positive control	10.32 ± 0.33*	9.32 ± 0.33*	10.22 ± 0.44*	7.76 ± 0.32*
Arsenic + Vitamin E	7.22 ± 0.44 ^a	5.76 ± 0.27 ^a	7.17 ± 0.41 ^a	5.17 ± 0.39

Values are Mean±SE; n = 6; * p < 0.05 compared to normal animals (Negative control) and a compared with (positive control) arsenic exposed.

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