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***Research Article***

# EFFECT OF ARSENIC INDUCED TOXICITY IN TESTIS OF MALE RATS

***Sudha K.**
**College of Food and Dairy Technology, Koduvalli, Chennai—600 052.*
**Author for Correspondence*

## ABSTRACT
Arsenic is a typical heavy metal and enters the animals through ingestion of arsenic contaminated water and gets deposited in visceral organs. Arsenic accumulated in reproductive organs affect fertility. However, the effect of arsenic toxicity is substantially reduced by the supplementing the antioxidants. In the present investigation α-tocopherol was treated for induced arsenic toxicity in albino male rats. A significant increase in the level of lipid peroxidation and decrease in the levels of antioxidants and enzyme activities were observed in arsenic treated rats. Arsenic generates ROS by decreasing the activation of antioxidant enzymes thereby causing stress in the testis of rats. Co-administration of arsenic with α-tocopherol reversed the effect induced oxidative stress in testis of rats.

***Key Words:*** *Arsenic, Antioxidants, A-Tocopherol, Testis.*

## INTRODUCTION
Arsenic poisoning is second to lead as the most frequently reported heavy metal toxicant. Inorganic Arsenic is incorporated into pesticides, which are the most common sources of arsenic poisoning (Jagjeet Singh et *al*, 2006). The incidence of fluoride, arsenic and iron in water has been reported in isolated pockets of India (Central Ground Water Board Report, 2010). Arsenic contamination has been found in West Bengal, Bihar, Chattishar, Uttar Pradesh and Assam. Use of contaminated water is a serious public health issue as ground water is used without any kind of treatment (ATSDR, 1993). Arsenic exposure causes both acute and chronic toxicity in human. Human arsenic exposure is related to severe health problems such as skin cancer, diabetes, liver, kidney and CNS disorders (Neiger et al, 1985). It also causes many other toxic effects (Jolliffe *et al*., 1991); WHO (1981); Pershagen *et al.,* 1983). Arsenic is highly sensitive in the process of reproduction, leading to increased rates of abortion in women and infertility in man (Skakkeback *et al.*, 1991). Information on the exact mechanisms of reproductive toxicity of heavy metals are not known. Hence, an attempt was made on the effect of alpha tocopherol on arsenic induced toxicity in testis of male rats.

## MATERIALS AND METHODS
### *Experimental Animals*
Male albino rats of wistar strain (120-150 gm) were used in this study. The animals were obtained from King's Institute of Preventive Animals Medicine, Chennai. The animals were housed in large spacious cages and were given food and water *ad libitum*. The animal rooms were kept in well ventilated place with a 12 hr light / dark cycle, throughout the experimental period.

### *Source of chemicals*
Sodium arsenite, α-tocopherol, trishydroxy methyl amino methane, thiobarbituric acid, 1- chloro-2, 4- dinitrobenzene, reduced glutathione, oxidized glutathione,5,5'-dithiobis(2-nitrobenzoic acid), pyrogallol and bovine serum albumin were purchased from Sigma chemicals.

### *Experimental design*
The animals were divided into three groups each consisting of six animals,

Group I : Rats received vehicle alone, served as control
Group II : Rats received arsenic as sodium arsenite in drinking water at a concentration of 100ppm.

Group III : Rats given arsenic along with α-tocopherol (400 mg/ kg body weight dissolved in mineral oil) by oral dose once per day.

Food and water intake and body weight of the animals were monitored throughout 30 days of experimental period. After the experiment period the animals were sacrificed, testis was collected for biochemical analysis. The following parameters were studied using the standard methods as given below.

| **Parameters** | **Methods** |
|---|---|
| Protein | Lowry (1951) |
| Acid Phosphatase | King(1965 ) |
| Alkaline Phosphatase | King(1965) |
| γ-Glutamy Transferase | Orlowski and Moister (1965) |
| Lipid Peroxidase (LPO) | Ohkawa *et al.*, (1979) |
| Superoxide Dismutase (SOD) | Marklund and Marklund (1974) |
| Catalase (CAD) | Sinha (1972) |
| Glutathione Peroxidase ($GP_X$) | Rostruck *et al.*, (1973) |
| Vitamin C | Omaye *et al.*, (1979) |
| Vitamin E | Desai (1984) |

Values are mean ± SD for six rats in each group, and significance of the differences between mean value were determined by one-way analysis of variance (ANOVA) followed by the Duncan test for multiple comparison. Values of P <0.001 were considered to be significant (Snedecor and Cochran, 1967).

## RESULTS AND DISCUSSION

Mean concentration of biochemical parameters in testis tissues in different groups is given in Table:1.

The increased activity of lipid peroxidase was observed to be 8.01 ± 0.44( nmoles of MDA released/mg protein) in arsenic treated animals. The levels of lipid peroxides in testis of arsenic exposed (Group-II) animals were found to be significantly increased when compared to control (Group-I) animals (P<0.001). Drug treated (Group-III) animals showed a significant decrease in the levels of lipid peroxides when compared with Group-II animals (P<0.001).

**Table 1: Enzymatic antioxidants in testis in different groups.**

| Parameters | Group I (control) Mean ± SD | Group II (arsenic induced) Mean ± SD | Group III (arsenic + α-tocopherol) Mean ± SD |
|---|---|---|---|
| Lipid Peroxidase (nmoles of MDA released/mg protein) | 3.72 ± 0.21 | 8.01 ± 0.296[a] | 5.64 ± 0.296[b] |
| SOD (units/min/mg protein) | 6.18 ± 0.42 | 5.22 ± 0.29[a] | 5.93 ± 0.31[b] |
| CAT (µmoles of $H_20_2$decomposed /min/mg protein) | 12.08 ± 0.74 | 8.91 ± 0.62[a] | 11.18 ± 0.81[b] |
| $GP_X$ (µmoles of GSH oxidized /min/mg protein) | 10.14 ± 0.82 | 7.88 ± 0.81[a] | 8.51 ± 0.49[b] |
| GST (units/min/mg protein) | 4.77 ± 0.19 | 2.99 ± 0.09[a] | 3.29 ± 0.14[b] |

*[a, b] represent P<0.001*
*a As compared with group I*
*b As compared with group II*

The enzymatic antioxidants SOD, CAT, $GP_X$ and GST were found to be significantly decreased in arsenic exposed (Group-II) animals (5.22 ± 0.29), (8.91 ± 0.62), (7.88 ± 0.81) when compared to control (Group-I) animals (6.18 ± 0.42), (12.08 ± 0.74), (10.14 ± 0.82) respectively. On drug treatment (Group-III) (5.93 ± 0.31), (11.18 ± 0.81), (8.51 ± 0.49) respectively the activities of antioxidants, SOD, CAT and GPX and GST were significantly increased when compared to Group-II animals (P<0.001).

In the present study, the animals treated with arsenic show decreased activities of antioxidant enzymes superoxide dismutase, catalase, and glutathione-s-transfrerase and glutathione peroxidase. It is interesting to note that testis has been highly susceptible to the damage induced by reactive oxygen species (ROS) (Pompella *et al*, 1991).

Antioxidants, in general, are compounds and reactions which dispose, scavenge, and suppress the formation of ROS, or oppose their actions. Among the well known biological antioxidants, SOD and its two isozymes, and catalase have a significant role. SOD spontaneously dismutates ($O_2$) anion to form $O_2$ and $H_2O_2$ (Nissen and Kreysel, 1983), in the activity of catalase converts $H_2O_2$ to $O_2$ and $H_2O$ (Jeulin *et al*., 1989). The reduction in the activity of catalase reflects in the ability of testis to eliminate $H_2O_2$ generated after exposure to arsenic. Similarly a decrease in the activity of SOD and CAT in arsenic exposed rats can be owned to an enhanced production of reaction oxygen species during arsenic metabolism (Searle and Wilson, 1980). SOD protects spermatozoa against spontaneous $O_2$ toxicity and LPO. Glutathione peroxidase, a selenium containing antioxidant enzyme with glutathione as the electron donor removes peroxyl (ROO) radicals from its native form. The paucity of NADPH production during arsenic exposure inhibits the activity of catalase (Kirkman and Gaetamin,1984). GSH-peroxidase and GSH reductase may directly act as antioxidant enzymes involved in the inhibition of sperm LPO. The great number of mitochondria in spermatozoa, these antioxidant mechanisms are important in the maintenance of sperm motility, the rate of hyper activation, and the ability of sperm to undergo acrosome reaction (Mana *et al.*, 2008 and Mukherjee *et al.*,2009). An increase in the activities of SOD and CAT was observed in arsenic intoxicated rats after treatment with α-tocopherol. This may be due to the direct reaction of α-tocoperol with superoxide, hydroxyl, peroxyl and alkoxy radicals.
Mean concentration of non-antioxidants in testis in different groups is given in Table:2

**Table: 2. Non-enzymatic antioxidants in testis in different groups.**

| Parameter | Group I (control) Mean ± SD | Group II (arsenic induced) Mean ± SD | Group III (arsenic + α- tocopherol) Mean ± SD |
|---|---|---|---|
| GSH (µg/mg protein) | 8.61 ± 0.52 | 5.89 ± 0.48[a] | 7.45 ± 0.36[b] |
| Vitamin C (µg/mg protein) | 32.17 ± 1.81 | 19.74 ± 1.29[a] | 28.11 ± 1.44[b] |
| Vitamin E (µg/mg protein) | 53.72 ± 1.98 | 28.66 ± 1.98[a] | 47.78 ± 1.49[b] |

*[a, b] represent P<0.001*
*a As compared with group I*
*b As compared with group II*

The decreased level of vitamin C was 19.74 ± 1.29 µg/mg proteins in arsenic treated animals and the level was in the control animal 32.17 ± 1.81 µg/mg proteins. In the experimental animal treated with arsenic, the concentration of vitamin E was measured 28.66 ± 1.87 µg/mg protein. The level of vitamin E concentration was decreased in arsenic treated animals.This effect may be attributed to vitamin E protecting cells from diverse actions of free oxygen radicals (Buettner *et al*,1993). Vitamin C along with vitamin E has been shown to improve rat embryonic antioxidant defense mechanism (Blake and boockfor, 1997).

***Conclusion***

The present study indicates that arsenic generate ROS by decreasing the activation of antioxidant enzymes thereby causing stress in the testis of rats. Co-administration of arsenic with α-tocopherol reversed the effect induced oxidative stress in testis of rats.

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