Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 5, 2012

Research Article

REVERSING EFFECT OF A -TOCOPHEROL IN ARSENIC INDUCED TOXICITY IN ALBINO RATS

SUDHA. K AND S.K. MATHANGHI*

Faculty of Food Sciences, College of Food and Dairy Technology, Koduvalli, Chn-52. Email: mathanghisoma@gmail.com

Received: 25 July 2012, Revised and Accepted: 11 Sep 2012

ABSTRACT

Arsenic is a typical heavy metal and enters the animals through ingestion of arsenic contaminated water and gets deposited in visceral organs and it affects the fertility but it can be mitigated by supplementing with antioxidants like tocopherol and ascorbic acid. It exists ubiquitously in our environment and various forms of arsenic circulate in air, water, soil and living organisms. Since arsenic compounds have shown to exert their toxicity chiefly by generating reactive oxygen species. In this study we evaluated the effect of alpha-tocopherol on lipid peroxidation, antioxidants and enzymes in testes of 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 and ascorbic acid reversed the effect induced by the oxidative stress in testis of rats.

Keywords: Arsenic toxicity, Tocopherol, Ascorbic acid, Antioxidant activity, Free radicals

INTRODUCTION

The environment in totality represents the Physical, chemical, biological, behavioral, and socio-economical factors and conditions surrounding human. A harmonious interaction between the human being and environment is indispensable for the physical and mental well being of the mankind. Mankind has been introducing noxious elements into the environment in the form of industrial waste and automobile exhaust, which pollute the environment with potential negative effects on all lives on earth[1]

Steady decline in human semen quality over the past several years considered to the result of deteriorating environmental conditions[2] which may be attributed to technological and industrial growth. This is growing evidence that heavy metals pollutants like lead, mercury, cadmium, chromium and arsenic are toxic to reproductive and developmental health of human[13]. Information on the exact mechanism underlying the reproductive toxicity of each of these metal toxicants is essential to protect the subjects being exposed to such toxicants at work spot or otherwise[4].

With this perspective to know the reproductive toxicity of arsenic mediated by an imbalance in oxidant-pro oxidant status of male reproductive organs and to evaluate the antioxidant effect of alpha-tocopherol was studied.

MATERIALS AND METHODS

Male albino rats of wistar strain (120-150 gm) were used and housed in large spacious cages and were given food and water *ad libitum*. The animal's rooms were kept in well ventilated place with a 12 hr light / dark cycle, throughout the experimental period.

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 used for the conduct of the study.

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, blood sample and testis were collected for biochemical analysis.

The animals were divided into four groups namely,

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 gavage once per day
Group IV	Rats received only α-tocopherol (400 mg/ kg body weight dissolved in mineral oil) by oral gavage once per day

Biochemical analysis:

Parameters such as protein [5], Acid Phosphatase [6] , Alkaline Phosphatase [6], γ-Glutamyl Transferase [7], Lipid Peroxidase (LPO) [8], Superoxide Dismutase (SOD) [9], Catalase (CAD) [10], Glutathione Peroxidase (GP_X), Vitamin C [11], Vitamin E [12] was analyzed.

Statistics

Descriptive statistical analysis was carried out. 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 [13].

RESULTS

Table 1 shows the mean concentration of biochemical parameters in testis in different groups.

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 ($P<0.001$).

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 ($P<0.001$).

Table 2 shows the mean concentration of non-antioxidants in testis in different groups. 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.

Table 1: Concentration of Enzymatic Antioxidants in Testis in different groups

Parameters	Group I (control)	Group II (arsenic induced)	Group III (arsenic + α-tocopherol)	Group IV (Drug Control)
LPO	3.72 ± 0.21	8.01 ± 0.296[a]	5.64 ± 0.296[b]	3.51 ±0.11[c]
SOD	6.18 ± 0.42	5.22 ± 0.29[a]	5.93 ± 0.31[b]	6.24 ±0.49[c]
CAT	12.08 ± 0.74	8.91 ± 0.62[a]	11.18 ± 0.81[b]	12.21 ±0.79[c]
GP_X	10.14 ± 0.82	7.88 ± 0.81[a]	8.51 ± 0.49[b]	10.30 ±0.44[c]
GST	4.77 ± 0.19	2.99 ± 0.09[a]	3.29 ± 0.14[b]	4.68 ±0.18[c]

Each value is expressed as mean ± SD for 6 rats in each group.

LPO - nmoles of malondialdehyde released/mg protein; SOD (units/min/mg protein); CAT

(μmoles of H_2O_2 decomposed /min/mg protein); GP_X (μmoles of GSH oxidized /min/mg protein) ; GST (units/min/mg protein)

[a, b] represent P<0.001

a As compared with group I

b As compared with group II

c Individual assessment value

Table 2: Non-Enzymatic Antioxidants in Testis in different groups.

Parameter	Group I (control)	Group II (arsenic induced)	Group III (arsenic + α- tocopherol)	Group –IV (Drug Control)
GSH	8.61 ± 0.52	5.89 ± 0.48[a]	7.45 ± 0.36[b]	8.74 ±0.56[c]
Vitamin C	32.17 ± 1.81	19.74 ± 1.29[a]	28.11 ± 1.44[b]	34.08 ±1.92[c]
Vitamin E	53.72 ± 1.98	28.66 ± 1.98[a]	47.78 ± 1.49[b]	54.11 ±1.26[c]

Each value is expressed as mean ± SD for 6 rats in each group.

GSH (μg/mg protein); ascorbic acid μg/mg protein, alpha -tocopherol μg/mg protein

[a, b] represent P<0.001

a As compared with group I

b As compared with group II

c Individual assessment value

DISCUSSION

In the present study the animals treated with arsenic showed decreased activities of antioxidant enzymes superoxide dismutase, catalase, and glutathione-s-transfrerase and glutathione peroxidase. Testis has been considered to be highly susceptible to the damage induced by reactive oxygen species (ROS) because of their high content of polyunsaturated fatty acids. To counteract the effects of (ROS), testis is equipped with antioxidant defense systems which prevent cellular damage [14, 15].

The production of ROS by spermatozoa is a normal physiological process, which serve as on important mediator in signal transduction mechanism, regulation or sperm capacitating and facilitation of acrosome reaction and spermatozoa-oocyte attachment. Mammalian spermatozoa are rich in polyunsaturated fatty acids and, thus are very susceptible to ROS attack which results in a decreased sperm motility, presumably by a rapid loss of intracellular ATP with deleterious effects on sperm capacitation and acrosome reaction. Lipid per oxidation may damage membrane integrity with increased cell membrane permeability, thus leading to enzyme inactivation, structural damage to DNA and cell death [16, 17].

Antioxidant defense mechanisms include three levels of protection: prevention interception and repair. Prevention of ROS formation is the first line of defense against an oxidative stress. An example is the binding of metal ions, iron and copper ions in particular [18]. When transition metals become loosely bound to ROS, they can produce more oxidants, particularly OH [19].

Antioxidants, in general, are compounds and reactions which dispose, scavenge, and suppress the formation of ROS, or oppose their actions [20]. A variety of biological and chemical antioxidants that attacks ROS and LPO are presently under investigation. 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 [21] in the activity of catalase converts H_2O_2 to O_2 and H_2O [22]. 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 [23]. The inhibition of SOD by H_2O_2 and CAT by superoxide has been reported by Freeman and Crapo [24]. SOD protects spermatozoa against spontaneous O_2 toxicity and LPO. SOD and catalase also remove (O_2) generated by NADPH-oxidase in netrophils and may play in important role in decreasing LPO and protecting spermatozoa during genitourinary inflammation. 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 [25]. GSH-peroxidase and GSH reductase may directly act as antioxidant enzymes involved in the inhibition of sperm LPO. GSH has a likely role in sperm nucleus decondensation and may alter spindle microtubule formation in the ovum, thus affecting the outcome of pregnancy. Gamaglutamyl transpeptidase (GGT), considered being present in the mid piece and acrosomal regions of spermatozoa of certain mammalian species may further affect GSH content of oocyte at the time of sperm penetration. Thus, in the view of the great number of mitochondria in spermatozoa, these antioxidant mechanisms are important in the main tenancy of sperm motility, the rate of hyper activation, and the ability of sperm to undergo acrosome reaction. An increase in the activites 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.

Maintenance of normal cell function in the presence of oxygen largely depends of the efficiency of the defense mechanisms against free radical mediated oxidative stress [26]. Vitamin C and vitamin E comprise the non-enzymatic antioxidants components which may protect spermatozoa against the deleterious effects of free radicals.

Excessive product of ROS under pathological conditions can be effectively scavenged by endogenous antioxidant defense system [27].

Vitamin E (α-tocopherol), an endogenous antioxidant, is known to protect cells from diverse actions of free oxygen radicals. The ability of α-tocopherol to maintain a steady – state rate of peroxy radical reduction in the plasma membrane depends on the recycling of α-tocopherol by external reducing agent such as ascorbate or thiols. In this way the α-tocopherol is able to function again as a free radical chain – breaking antioxidant, even though its concentration is low [28]. Vitamin E a lipophilic antioxidant has been proved to eliminate free oxygen radicals in the reproductive tissues of TCDD- induced toxicity in male rats [28]. This effect may be attributed to vitamin E protecting cells from diverse actions of free oxygen radicals [29]. Vitamin C along with vitamin E has been shown to improve rat embryonic antioxidant defense mechanism [30].

CONCLUSION

In the conclusion the present study 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. This makes us conclude that this could be used as a safe chemotherapeutical drug without much compilation.

REFERENCES

1. Skakkeback NE, Negro-villar A, Michal F and Fathalla M, 1991. Impact of the environment on reproduction health report and recommendation of a WHO. International workshop Danish Medical Bulletin, 38,425-426.
2. Cheek AO and Mc Lachlan A, 1998. Environmental hormones and male reproduction system. Journal of Andrology, 19:5-10.
3. Bonde JP, Hjollund NH, Kolstad HA, Abell A and Larsen SB, 1999. Environmental semen studies are infertility increased by a decline in sperm count Scandinavian. Journal of work, environmental and health, 25 ,(supplement) 12, 26, discussion 76-78.
4. Sugiyama M, 1994. Role of cellular antioxidants in metal induced damage cell biology and toxicology, 10, 1-22.
5. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, 1951. Protein measurement with Folin's Phenol reagent. J Biol Chem, 193,265-75.
6. King J, 1965. The dehydrogenases or oxidoreductases - lactate dehydrogenase. In:. Practical Clinical Enzymology Van DM, editor. London: Nostrand Company Ltd, 83–93.
7. Orlowski M and Meister A, 1965. Isolation of α-glutamyl transpapdidase from dog kidney. J Bio, Chem, 240,338-347.
8. Ohkawa H, Ohishr N and Yagi K, 1979. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Annl. Biochem, 95, 351-358.
9. Marklund S and Marklund G, 1974. Involvement of the superoxide radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem, 47,469-474.
10. Sinha AK, 1972. Colorimetric assay of catalase. Anal Bio Chem 47, 289-394.
11. Omaye ST, Turnball JB and Sauberlich HE, 1979. Selected methods for the determination of ascorbic acid in animals cells, tissues and fluids, Methods Enzymal, 62,1-11.
12. Desai ID, 1984. Vitamin-E analysis methods for animal tissues. Methods enzymol, 105,138-1
13. Snedecor GW, and Cochran WG, 1967. Statistical methods, 6th Edition, Oxford and IBP Publishing Co., Calcutta.
14. Zahin M , Aqil F and Ahmad I, 2009. The *in vitro* Antioxidant Activity and Total Phenolic Content of Four Indian Medicinal Plants. IJPPS, Vol (1) Supply 1, Nov.-Dec.
15. Brijesh K, Tiwari RL and Khosa, 2010. Evaluation of the hepatoprotective and antioxidant effect of B*erberis asiatica* against experimentally induced liver injury in rats. IJPPS, Vol (2), Suppl 1, 2010.
16. Pompella A, Romani A, Benditti A and Comporti M, 1991. Loss of membrane protein thiols and lipids peroxidation of allyl alcohol hepatoxicity. Biochem Pharmacol, 41, 1225-1229.
17. Nisha K and Deshwal RK. Antioxidants and their protective action against DNA damage. IJPPS, ISSN- 0975-1491 Vol (3), Suppl 4, 2011.
18. Sies H, 1993. Strategies of antiocidant defence. Eur .J Biochem, 215, 213-219.
19. Halliwell B, 1990. How to characterize a biological antioxidant. Free radic. Res, 9,1-32.
20. Sanja SD, Sheth NR, Patel NK, Patel D and Patel B. Characterization and Evaluation of Antioxidant Activity of *portulaca oleracea.* IJPPS, Vol.1, Issue1. July-Sep. 2009.
21. Nissen HP and Kreysel HW, 1983. Superoxide dismutase in human semen. Kilnische wochenschrift, 61,63-65.
22. Jeulin C. Soufie JC, Weber P, Martin DL and Calvayrae R, 1989. Catalase activity in human spermatozoa and seminal plasma. Gamete. Res, 24,185-196.
23. Searle AJ and Wilson R, 1980. Glutathione peroxide effect of superoxide, hydroxyl and bromine free radical on enzyme activity. Int .J. Radiat Bio, 137,213-217.
24. Freeman BA and Crapo JD, 1982. Biology of disease. Free radicals and tissue injury. Lab invest, 47,412-426.
25. Kirkman MN and Gaetamin GF, 1984. Catalase: A tetrameric enzyme with four tightly bound molecules of NADPH. Proc Natl Acid Sci USA, 81,4343-4327.
26. Foraman HJ and Fisher AB, 1982. Antioxidant defense. In: oxygen and living process. An inter disciplinary approach. Gilber DL, New York, 235-249.
27. Jormieson D, 1989. Oxygen toxicity and reactive oxygen metabolities in mammals. Fee Rad. Biol.Med, 7,87-108.
28. Buettnar GR, 1993. The pecking order of radicals and antioxidants, lipid per oxidation, α-tocopherol and ascorbate. Arch. Biochem. Biophys. 300, 535-543.
29. Johnson MH and Everitt BJ, 1995. Testicular function. In: Essential Reproduction. MH, Johnson, BJ, Evertti (eds) Alden press, Oxford, PP 45-60.
30. Blake CA and Boockfor FR, 1997. Chronic administration of the environmental pollutant 4-tertoctylpherol to adult male rats interfere with the secretion of luteinizing hormone, follicle – stimulating hormone, follicle – stimulating hormone, prolactin and testosterone. Biol. Reprot. 57, 255-265.