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Original Article

PROTECTIVE EFFECT OF VITAMIN E ON SODIUM FLUORIDE INDUCED OXIDATIVE DAMAGE OF KIDNEY OF MALE WISTAR RATS

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Abstract: Fluorosis is a serious health problem in many part of the world including India. The present study was undertaken to investigate the propensity of protective effect of vitamin E on Sodium fluoride induced oxidative damage in rats. For this purpose, a total of 24 rats were randomly divided into four groups: group I used as control and groups II, III and IV were orally treated with sodium fluoride (8 mg/kg body weight), vitamin E (100 mg/kg body weight) and sodium fluoride plus Vitamin E, respectively for 45 days. Results obtained showed that mean values of SOD, GSH and catalase revealed significant decrease while there was significant increase in the mean values of LPO in kidney homogenate in sodium fluoride administration. Serum biochemical marker representing renal injury including creatinine, urea and uric acid in serum were significantly increased due to sodium fluoride administration. On the other hand, treatment with vitamin E alleviated the harmful effect of sodium fluoride induced oxidative damage and serum biochemical parameters in the rats of combination group (sodium fluoride plus vitamin E). In conclusion, supplementation of Vitamin E could diminish the sodium fluoride induced oxidative damage in male Wistar rats' kidney.

Key words: Sodium fluoride, Vitamin E, Rat kidney.

INTRODUCTION

Fluorine is a very reactive non-biodegradable and non metallic element. It exists in mostly as fluoride in a combined form with many minerals /element like calcium, aluminium, irons etc. Fluoride usually found in ground water and has affected many countries of the world [1]. The problem of fluorosis has been reported in various states of India, affecting more than 150,000 villages seriously [2]. It has been established that about 45% of drinking water sources in India are contaminated by fluoride [3]. Fluoride is toxic when consumed in excess but of benefit when consumed within permissible limit [4]. It is desirable in very limited quantities for healthy osteogenesis of bones and teeth preventing dental cavities, but in excess causes a disease known as fluorosis [5]. Vitamin E is a naturally occurring antioxidant nutrient, and a lipid-soluble vitamin present in lipid bilayer membranes that plays important role in animal health by inactivating harmful free radicals and inhibits free radical formation [6].

Vitamin E is known for its antioxidant property protecting the unsaturated bonds of phospholipids present in the cell membrane against free radical damage. The present study deals with the oxidative damage by sodium fluoride and also assessed the

protective effect of vitamin E in Wistar rats in sub chronic exposure of sodium fluoride.

MATERIAL AND METHODS

All the experimental procedures, housing and management of the rats were strictly carried out according to the recommendations and approval of the Institutional Animal Ethics Committee (IAEC) as per the guidelines set forth by committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Chemical: Analytical grade of sodium fluoride (NaF, Cas no 7681-49-4, purity 97%) was obtained from Hi Media (India) whereas, vitamin E acetate (C31H52O3, CAS no. 7695-91-2, purity- 95%) used in this study was procured from Central Drug House, New Delhi. All other chemicals were used standard analytical grade chemicals and test kits were procured from SRL (India), Merk (India), Hi Media (India), BDH, Qualigens, Span diagnostic Ltd. (India) and CDH (India).

Experimental animal: The study was conducted on 24 male Wistar rats (4 weeks old) weighing between 80-100 grams procured from Laboratory Animal Resources (LAR) Section of Indian Veterinary Research Institute (IVRI), Izatnagar (U.P.). After 15 days of acclimation, the rats were randomly divided in to four groups, each containing 6 male rats. In a single cage, three rats were kept together. Adequate lighting (12 hours light and 12 hour darkness), ventilation, temperature (21±2OC), relative humidity (50±10 %) and hygienic conditions were maintained throughout the experiment. The rats were provided with paddy husk as bedding material in the cages which was changed thrice a week to keep the surroundings dry. The animals were maintained under standard managemental conditions and were provided feed and water adlibitum. All the rats were given standard diet procured from Ashirwad Industries Limited, Punjab.

Treatment: Rat were divided in to four groups of six rats in each group.

Group I: Served as control (received normal water).
Group II: Sodium fluoride @ 8 mg/kg body weight in distilled water.
Group III: Vitamin E (100 mg/kg body weight) orally.
Group IV: Sodium fluoride @ 8 mg/kg body weight and vitamin E (100 mg/kg body weight) orally.

The experiment continued for 45 days. The parame ters of oxidative damage and Serum biochemical marker were carried out on day 45 of experiment.

Preparation of tissue homogenate: Rats were sacrifi ced by deep inhalation anaesthesia using chloroform and kidney were dissected out cleaned and stored at -80oC until assayed. Frozen kidney sample were thawed at room temperature and 200 mg of sample was weighed and taken in 2 ml of ice-cold saline for estimation of LPO, SOD, catalase and total protein. An amount of 200 mg of sample was weighed separately and taken in 2 ml of 0.02 M EDTA for GSH estimation. The homogenates prepared by using homogenizer, under cold conditions were centrifuged for 10 min at 3000 rpm. The supernatant was used for assay of reduced glutathione, lipid Peroxidation, superoxide dismutase, Catalase, and total protein.

Determination of total proteins: Total protein was estimated by the method of Lowery et al. [7] and 50 mg of bovine serum albumin was dissolved in 50 ml of distilled water and used as standard.

Malondialdehyde estimation: Malondialdehyde (MDA) was estimated by thiobarbituric acid (TBA) method [8]. One ml of tissue homogenate was incubated at 37 ± 0.5 0Cfor 2hrs. To each sample, one ml of 10% TCA was added. After thoroughly mixing, it was centrifuged at 2000 rpm for 10 mins. In one ml of supernatant liquid, an equal volume of 0.67% TBA was added and kept in boiling water bath for 10 mins. The samples were cooled and diluted with one ml distilled water. The absorbance was read at 535 nm.

Reduced glutathione estimation: Reduced glutathi one (GSH) was determined by estimating free – SH groups, using DTNB method of Sedlak and Lindsay [9]. 10% tissue homogenate were made in 0.02M EDTA. One ml of homogenate supernatant, 0.8 ml of distilled water and 0.2 ml of 50% TCA solutions were added and mixed well and kept at room temperature for 15 min. This solution was

centrifuged at 3000 rpm for 15 min. The supernatant measuring 0.4 ml of was added in 0.8 ml of 1M Tris buffer and 0.2 ml DTNB (0.01M). After mixing the absorbance was read at 412 nm within 5 mins.

Superoxide dismutase estimation: Superoxide dismutase (SOD) was estimated as per the method described by Madesh and Balasubramanian [10]. It involved generation of superoxide by pyrogallol autoxidation and the inhibition of superoxide dependent reduction of the tetrazolium dye MTT [3-(4-5 dimethyl thiazol 2-xl) 2,5 diphenyltetrazolium bromide] to its formazan. The absorbance was measured at 570 nm. The reaction was terminated by the addition of dimethyl sulfoxide (DMSO), which helps to solubilize the formazan formed. The colour evolved is stable for many hours and the activity was expressed as SOD Units (one unit of SOD is the amount in µg of protein required to inhibit the MTT reduction by 50%).

Catalase estimation: Catalase was assayed and calculated in tissue homogenate by the method of Bergmeyer [11]. A dilution rate of 1:10 of tissue homogenate was used for estimation of catalase. In a test tube, 2 ml Phosphate buffer and 10 µl homogenate were added and the contents were transferred to the cuvette. By adding one ml of H2O2 directly into the cuvette, the reaction immediately started. Optical density was recorded at every 30 seconds for 3 mins at 240 nm against blank (water).

Serum biochemical marker: The blood samples were collected from retro-orbital plexus on day 45 from the rats of all the groups using micro-capillary tubes in 5.0 ml vacutainer. Serum was separated and stored at -20oC for further analysis of biochemical parameters. From serum of different groups creatinine, urea, and uric acid were estimated using standard diagnostic kits (Span Diagnostic Ltd., Surat).

Statistical analysis: The quantitative data of biochemical observations as well as oxidative stress parameters were analyzed by Duncan's multiple range tests as per (Snedecor and Cochran [12].

RESULTS

Vitamin alone application to control animals (Group III) has a negligible effect in the level of in any of the biochemical parameters studied (compare group III with Group I). Table 1.

The mean values of malondialdehyde, an indicative of lipid peroxidation, in kidney of fluoride intoxicat

Table 1: Mean values of different oxidative stress parameters in kidney tissue in different experimental groups on day 45 in Wistar rats (mean ± SEM, N=6).

Oxidative Stress Parameters	Experimental Groups			
	Group I	Toxicated Group II	Only vitamin Group III	Toxicated + vitamin Group IV
LPO (nM MDA/g tissue)	58.09^{A}±0.34	80.15^{C}±.0.56	57.56^{A}±0.25	68.15^{B}±0.34
GSH (mMGSH/g tissue)	3.91^{A}±0.17	2.07^{C}±.0.21	4.01^{A}± 0.15	2.77^{BC}±0.11
SOD (U/ mg of protein)	28.12^{A}±0.56	19.16^{C}±0.58	28.11^{A}±0.36	20.24^{B}±0.59
CAT(mMH_2O_2 utilized/min/ mg of protein)	70.13^{A}±0.58	55.13^{C}±0.58	72.25^{A}±0.38	61.15^{B}±0.86
Mean with different superscript (A, B, C) differing significantly in between the groups, otherwise non-significant.				

Table 2: Changes in the mean values of different Serum biochemical markers on day 45 in Wistar rats. ((mean ± SEM, N=6)

Serum Biochemical markers	Experimental Groups			
	Group I	Toxicated Group II	Only vitamin Group III	Toxicated + vitamin Group IV
Creatinine (mg/dl)	0.68^{A}±0.01	2.13^{C}±0.02	0.68^{A}±0.002	1.61^{B}±0.05
Urea(mg/dl)	38.02^{A}±0.32	77.18^{C}±0.37	37.16^{A}±0.34	66.15^{B}±0.35
Uric acid (mg/dl)	7.12^{A}±0.33	12.25^{C}±0.34	7.03^{A}±0.33	11.53^{BC}±0.30
Mean with different superscript (A, B, C) differing significantly in between the groups, otherwise non-significant.				

ed rats (group II) revealed a significant ($P<0.01$) increase than the rats of control group (I), while vitamin E application to sodium fluoride intoxicated animals showed significantly improved level of malondialdehyde, (group IV).

All the three enzymes (GSH, SOD and catalase), related to glutathione metabolism are significantly ($P<0.01$) decreased in fluoride intoxicated rats (Table 1). While vitamin application to intoxicated animals (Group IV) significantly recover their levels. Nevertheless, an absolute control level is not achieved similar to Group 1.

Table 2 reveals the mean values of creatinine, urea and uric acid, which is significantly ($P<0.01$) increased in intoxicated rats of groups-II. Only vitamin application to normal animals (groups-III) has no affect and the level of all components is almost unchanged. to these animals (group-IV). Vitamin E application to intoxicated animals (group-IV) revered the level of creatinine, urea and uric acid up to some extent. As for example level of creatinine became 1.61 from 2.13, urea 66.15 from 77.18 and uric acid 11.53 from 12.25. All these changes are not much significant with in 45 days.

DISCUSSION

Kidney is the prime organ responsible for removal of fluoride from body as there is a higher concentration of fluoride in the filtrate [13]. An increase of malondialdehyde, which is an indicative of lipid peroxidation, certainly show an actue toxic effect of fluoride. Present study show a reduction of reduced glutathione level and inhibition of two important oxidative enzymes (SOD and catalase) during fluoride intoxication. This suggest disturbance in glutathione metabolism. Fluoride induces excessive production of free radicals [5,14]. The enhancement of lipid peroxidation in erythrocytes, liver, kidneys, brain, and other tissues of experimental animals has also been reported [15,16].

In present study, low level of GSH, SOD and catalase in kidney homogenate of group (II) show significant increase of fluoride toxicity. These results are in agreement with earlier studies in Sodium fluoride toxicity in the rat [17,18] and mice [19,20]. GSH is an important naturally occurring antioxidant, which prevents free radical damage and helps detoxification by conjugating with chemicals. Under oxidative stress, GSH is consumed by GSH related enzymes to detoxify the agents that increased lipid peroxidation [21]. Decreased SOD levels indicate increased utilization of this enzyme for dismutation of excessive superoxide radicals produced due to fluoride toxicity. Thus decreased SOD activity suggests that the accumulation of superoxide anion radicals might be responsible for increased lipid peroxidation following sodium fluoride treatment. Catalase is responsible for breakdown of hydrogen peroxide, an important reactive oxygen species produced during metabolism. Stress conditions in which free radical generation occurs result in the depletion in catalase activity [22].

The present study revealed significant ($P<0.05$) increase in plasma creatinine, urea and uric acid level in the fluoride intoxicated group (II) at the days 45. Similar observations have been reported in fluoride toxicity in rats [23,24] and mice [25]. Their elevated levels might be considered as an indicator of impaired kidney function [26]. Urea is the major end product of protein metabolism. It constitute largest fraction of the non-protein nitrogenous component of the blood urea which is produced in the liver excreted through the kidneys. Consequently, the circulating levels of urea depend upon protein intake, protein catabolism and renal function. Elevated plasma urea concentration is observed in impaired kidney function [27]. This may account for the increase of creatinine, urea and uric acid concentration in the animals receiving sodium fluoride. Creatinine, urea and uric acid that might indicate a nephrotoxic potential of sodium fluoride in the rats that may be due to kidney damage caused by the enhanced generation of ROS as observed in the of this study.

The vitamin application in group (IV) revealed significant increase in the mean value of SOD, GSH and catalase and also decrease mean value of LPO, creatinine, urea and uric acid as compared to control. Since vitamin E is an important free radical scavenger [28,29] and an antioxidant, by reduction of free radicals GSH is recovered that also resynthesis of oxidative enzymes SOD and catalase. These results are in agreement with earlier studies in rats [30]. From overall data it is concluded that vitamin E administration during fluoride intoxication is quite advantageous as it is a potent

antioxidant and check lipid peroxidation not only in kidney but other tissues too [16].

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