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Ameliorative effect of vitamin e on sodium fluoride induced hepatotoxicity and oxidative stress in male wistar Rats

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Abstract
The present study was to investigate the propensity of Sodium fluoride to induce hepatotoxicity and oxidative stress and also to assess the ameliorative effect of vitamin E. 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 45days. Results obtained showed that mean values of SOD, GSH and catalase revealed significant decrease while there was significant increase in LPO in liver homogenate in Sodium fluoride administration. Enzymatic activities of aminotransferases (AST and ALT) and phosphatase (ALP) in plasma were significantly increased due to Sodium fluoride administration. Further, light microscope investigation revealed that Sodium fluoride exposure induced histopathological alterations in the liver tissues. On the other hand, treatment with Vitamin E alleviated the harmful effect of Sodium fluoride in the combination group. The presence of Vitamin E could diminish the Sodium fluoride induced hepatotoxicity and oxidative stress in male wistar rats as indicated by the result of our study.

Keywords: Hepatotoxicity, oxidative damage, sodium fluoride, vitamin E

1. Introduction
Fluorine is a very reactive non-biodegradable and nonmetallic element. It exists in mostly as fluoride in a combined form with many minerals /element like calcium, aluminum, irons etc. Sodium fluoride is a hazardous-waste by-product from the manufacture of aluminum industries. Fluoride usually found in ground water and has affected many countries of the world. The problem of fluorosis has been reported in various states of India, affecting more than 150,000 villages seriously (Teotia and Teotia, 1991) [23]. It has been established that about 45% of drinking water sources in India are contaminated by fluoride (Teotia and Teotia, 1984) [22]. Fluoride is toxic when consumed in excess but of benefit when consumed within permissible limit **(**Guan *et al*., 2000) [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 (Sharma *et al*., 2010) [18]. 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 (Kalender *et al*., 2004) [8]. 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 hepatotoxicity and oxidative stress of Sodium fluoride and also assessed the ameliorative effect of vitamin E in wistar rats in sub chronic exposure of Sodium fluoride.

Material and methods
Test chemical, animal and experimental design
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). Analytical grade of Sodium fluoride (NaF, Cas no 7681-49-4, purity 97%) was obtained from Hi Media (India) whereas, Vitamin E acetate ($C_{31}H_{52}O_3$, CAS no. 7695-91-2, purity- 95%) used in this study was procured from CENTRAL

DRUG HOUSE, New Delhi. All other chemicals wereused standard analytical grade chemicals and test kits wereprocured from SRL (India), Merk (India), HiMedia (India), BDH, Qualigens, Span diagnostic Ltd. (India) and CDH (India).

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.). Adequate lighting (12 hours light and 12 hour darkness), ventilation, temperature (21±2^{O}C), relative humidity (50±10%) and hygienic conditions were maintained throughout the experiment. 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. After 15 days of acclimatization, the rats were randomly divided in to four groups, each containing 6 male rats. 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.

The experiment continued for 45 days. The biochemical parameters, parameters of oxidative stress and pathomorphological studies were carried out on day 45 of experiment.

Biochemical analysis

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 containing heparin as anticoagulant. Heparinised blood samples were centrifuged at 2000 rpm for 15 min. Plasma was separated and stored at -20ºC for further analysis of biochemical parameters. The biochemical parameters studied were Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP), in the rats of different groups using standard diagnostic kits (Span Diagnostic Ltd., Surat).

Oxidative stress parameters

Rats of all the groups were sacrificed on day 45, the termination of experiment. Feed was withdrawn 24 hours before sacrifice. Liver was collected, washed with ice cold normal saline and weighed and stored at -80ºC until assayed. Estimation of different oxidative stress parameters *viz.* lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase in liver were carried out by double beam UV-VIS spectrophotometer.

Frozen liver samples 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 and Catalase. 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 and Catalase.

The extent of lipid peroxidation was evaluated in terms of MDA (malondialdehyde) production, determined by thiobarbituric acid (TBA) method (Rehman, 1984) [17]. GSH was determined by estimating free –SH groups, using DTNB method of (Sedlak and Lindsay, 1968) [11]. Superoxide dismutase (SOD) was estimated as per the method described by (Madesh and Balasubramanian, 1998) [13]. Whereas catalase was assayed and calculated in tissue homogenate as for the method prescribed by (Bergmeyer, 1983) [2].

Pathomorphological studies

For necropsy six rats of each group were humanely sacrificed by cervical dislocation under anesthesia at the end of 45 days of experimentation. The gross lesions in different organs were carefully recorded and for histopathological studies, tissue samples of liver were preserved in 10% formalin. After proper fixation, paraffin embedded tissue sections of 4-6µ were prepared and stained by routine hematoxyline and eosin technique for histopathological examination (Luna, 1968) [12].

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, 1989) [19].

Results

Biochemical studies

The results of biochemical parameters are summarized and presented in Table 1. Briefly, the mean values of AST, ALT and ALP revealed significant (P<0.01) increase and significant (P<0.05) decrease in the mean values total plasma protein and albumin in the rats of groups-II and group-IV as compared to the rats of group-I and group-III. There was also significant variation in the mean values of AST, ALT and ALP in the rats of group-II as compared to group-IV. Non-significant variation was observed in the mean values of AST, ALT and ALP in rats of groups-IV as compared to the rats of group-I on day 45 of experiment.

Table 1: Changes in values of different biochemical parameters on day 45 in Wistar rats. ((mean ± SEM, N=6)

Biochemical Parameters	Experimental Groups			
	Group I	Group II	Group III	Group IV
AST(IU/L)	68.25^{A}±0.26	125.05^{C}±0.18	66.71^{A}±0.19	105.36^{B}±1.06
ALT(IU/L)	37.64^{A}±0.18	67.49^{C}±0.29	37.15^{A}±0.46	55.50^{B}±0.27
ALP(IU/L)	42.23^{A}±1.0	62.31^{C}±0.57	41.37^{A}±0.85	53.45^{B}±0.61

Mean with different superscript (A, B, C) differing significantly in between the groups, otherwise non-significant.

Oxidative stress parameters

The mean changes of oxidative stress in liver tissue are summarized in Table 2. The mean values of LPO in liver tissue revealed significant (P<0.01) increase and the mean values of GSH, SOD and Catalase showed significant (P<0.01) decrease in the rats of groups-II and group-IV as compared to the rats of group-I & group-III. There was also significant variation in the mean values of LPO, GSH, SOD and Catalase in the rats of group-II as compared to the rats of group-IV and non-significant variation was observed in the mean values of LPO, GSH, SOD and Catalase in the rats of group- IV as compared to the rats of group-I

Table 2: Mean values of different oxidative stress parameters in liver tissue in different experimental groups on day 45 in Wistarrats (mean ± SEM, N=6).

Oxidative Stress Parameters	Experimental Groups			
	Group I	Group II	Group III	Group IV
LPO (nM MDA/g tissue)	68.19^{A}±0.57	85.22^{C}±.0.34	67.14^{A}±0.58	74.10^{B}±0.39
GSH (mMGSH/g tissue)	2.51^{A}±0.03	1.71^{C}±.0.03	2.75^{A}±0.04	2.01^{B}±0.05
SOD (U/ mg of protein)	22.16^{A} ±0.58	14.08^{C} ±.0.57	23.06^{A} ± 0.58	19.11^{B}±0.56
CAT(mMH_2O_2 utilized/min/ mg of protein)	87.11^{A}±0.82	67.10^{C}±0.24	90.13^{A}±0.80	75.11^{B}±0.24

Mean with different superscript (A, B, C) differing significantly in between the groups, otherwise non-significant.

Path morphological observations

In this study, all the 6 rats of each group were sacrificed on day 45 of experimentation. Grossly, the liver of Sodium fluoride toxicity group-II was pale with occasional presence of pinpoint haemorrhages and mottling on the dorsal surface (fig.1). The liver of Vitamin E treated ameliorative rats group -IV was slightly pale as compared to rats of groups-II. The rats of control group–I and rats of group-III did not show distinct morbid lesions in liver on 45 days of post administration.

Histopathologically, the liver of rats of NaF toxicity group –II showed congested sinusoids, central vein and the vessels located in portal areas (fig.3). The hepatocytes showed degenerative changes ranging from cellular swelling to mild to moderate vacuolization (fig.4) with infiltration of large number of mononuclear cells in the portal area (fig.5). Similar microscopic picture were observed in the rats of group-IV, but of mild in nature as compared to the rats of group-II (fig. 6). The rats of control group–I and rats of group-III did not show distinct lesions in liver on 45 days of post administration (fig. 2).

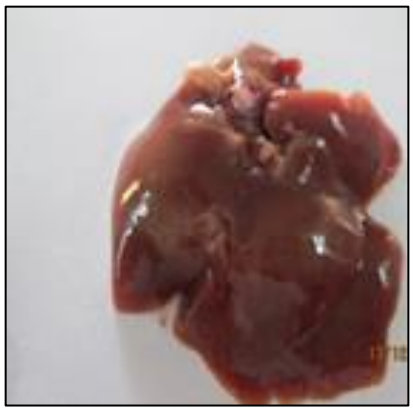

Fig 1: Liver showing paleness and mottling on the dorsal surface in the rat of NaF toxicity group (II).

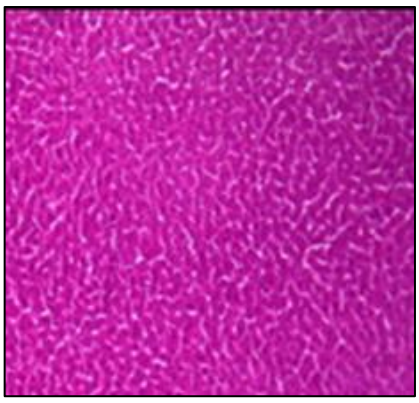

Fig 2: Liver showing normal histoarchitecture of hepatocytes in the rat of control group (I). (H&E 100X)

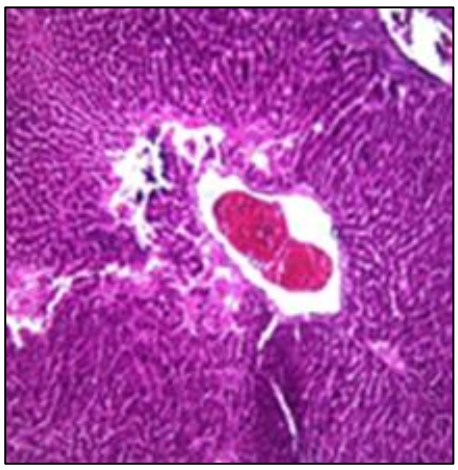

Fig 3: Liver showing congestion of central vein with necrosis of hepatocytes in the rat NaF toxicity group (II).

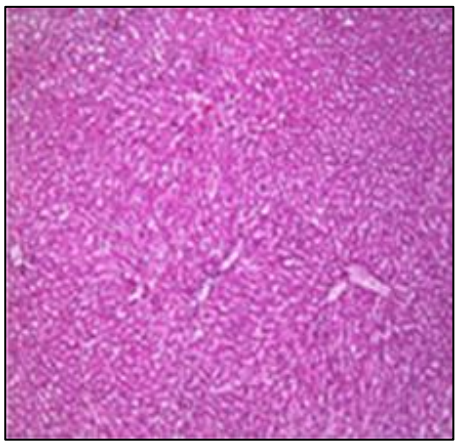

Fig 4: Liver showing vacuolization in hepatocytes in the rat NaF toxicity group (II). (H&E 100X

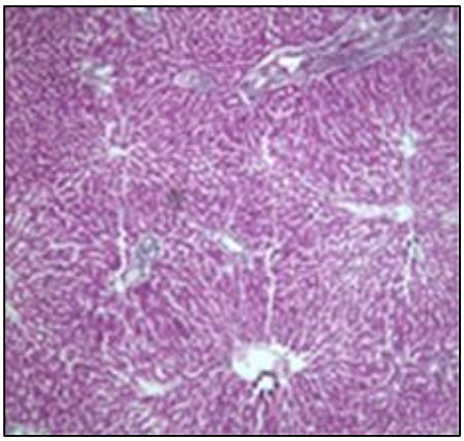

Fig 5: Liver showing infiltration of large number of mononuclear cells in the portal areas in the rat of NaF toxicity group (II).

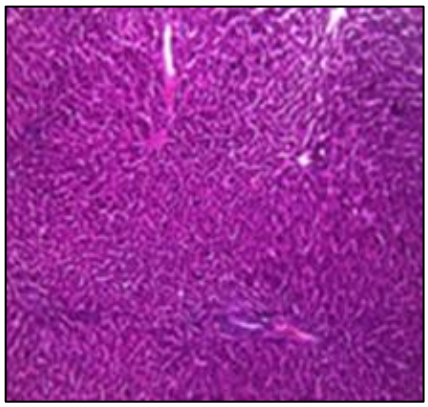

Fig 6: Liver showing mildcongestion of central vein in the rat of Vitamin E treated group (IV).

Discussion

Enzymes like AST and ALT represent the functional status of the liver. ALT activity is related to general hepatocellular dysfunction and AST to mitochondrial damage. Increased aminotransferase (AST and ALT) activity in serum reflects hepatocellular damage leading to leakage of these enzymes into general circulation. The increases in level of ALT, AST and ALP in Fluoride toxicity are in accordance with the findings in the rats (Sharma *et al.,* 2010; Gupta *et al.,* (2013) [18, 5]. In contrast to the present study**,** earlier workers reported that AST and ALT activities did not change as a result of exposure to fluoride in children diagnosed with dental fluorosis (Xiong *et al.,* 2007) [24] and fluoride decreases the ALT activity and increases AST activity in mice (Kanbur *et al.,* 2009) [9]. The increase in the activity of ALP in present study is accordance with (Giri *et al.,* 2015) [3]. Elevated level of serum ALP in the present study can be attributed initially to some patho-physiological changes in liver as a consequence of Fluoride intoxication may be due to damage in membrane permeability of hepatocytes, resulting in leakage of this enzyme into the blood stream.

The present study revealed increased levels of lipid peroxidation, in the liver of sodium fluoride treated rats and our finding are in resonance with the observation of other worker who reported increased free radicals levels in the liver tissues of albino rats (Hassan and Abdel-Aziz, 2010) [6]. Levels of reduced glutathione (GSH) as well as the activities of superoxide dismutase (SOD), and catalase (CAT) are in accordance with earlier studies (Gupta *et al.,* 2013) [5]. Who found decrease in the levels of the glutathione levels with an increase in LPO level activity indicates utilization of GSH for lipid hydroperoxides generated. Thus, under oxidative stress, GSH is consumed by GSH related enzymes to detoxify the peroxides produced due to increased lipid peroxidation. Decreased SOD levels indicate increased utilization of this enzyme for dismutation of excessive superoxide radicals produced due to Fluoride toxicity. Decrease in the activity of catalase in the liver, in Sodium fluoride intoxication may be elucidated by the inadequate supply of nicotinamide adenine dinucleotide phosphate required for catalase activation from its inactivated form.

The findings on the pathomorphological change in the liver of rats were in accordance with the earlier report. Stawiarska *et al*. (2013**)** [20] reported that the 35 days of treatment with NaF (4 mg/kg body weight) to rats resulted in dispersed necrosis and congestion in lobules and infiltration of mononuclear cells in the vicinity of blood vessels. Reham *et al.* (2014) [16] also observed that NaF (10.3 mg/kg body weight) treated mice causes markedly dilated central vein filled by large number of blood cells and surrounded by hepatic cord and showing severe fatty changes of hepatocytes and markedly congested central vein. Akin to our findings (Basha and Rao, 2014) [1] also found that Sodium fluoride treated mice liver exhibited congestion, cellular vacuoles and severe necrosis in hepatocytes, nuclear fragmentation along with nuclear degeneration and hemorrhage in central vein. According to (Ranjan *et al.,* 2009) [15] alterations in transaminases (AST and A LT) could be associated with pathology involving necrosis of liver. The elevated levels of transaminases (AST and ALT) in the present study indicate degenerative and necrotic changes in liver.

As found in this study, supplementation of vitamin E successfully prevented significant changes from NaF to the activity of ALT, AST, ALP, oxidative stress enzymes and pathological changes. The findings in our study are in accordance with (Stawiarska *et al*., 2013) [20] who reported that co-administration of Vitamin E with Sodium fluoride receded congestion and infiltrations of mononuclear cells which were severe in NaF (alone) treated group. (Nair 2004) [14] observed that significant improvement in liver enzymes in vitamin E treated group when compared to those treated with fluoride salt only. Inam *et al.* (2015) [7] reported that fluoride induced hepatotoxicity is prevented by co-administraion by Vitamin E.

According to (Stawiarska *et al*., 2012**)** [21] Vitamin E 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**.** Suppression of kinase C and phospholipase in inflammatory cells substantially diminishes the production of free radicals and their effects.

To conclude with it is inferred that the Sodium fluoride, a potent nonmetallic compound produced pathological changes in the liver. Liver appeared to be target organ due to toxic effects of Sodium fluoride. Administration of vitamin E appeared highly effective as an antioxidant to minimizethe Sodium fluoride induced hepatotoxicity and oxidative stress in male wistar rats.

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