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ORIGINAL ARTICLE

PROTECTIVE EFFECT OF VITAMIN E ON FLUORIDE INDUCED HEPATOTOXICITY

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ABSTRACT

Fluoride salts generate free oxygen radicals which cause lipid peroxidation resulting in cell membrane damage and toxicity. Morphologically toxicity to liver is manifested as ballooning degeneration of cells, necrosis and infiltration of mononuclear cells in hepatic lobules.

Aims and Objectives: The present experimental study is designed to investigate the toxic changes in the liver of sodium fluoride treated mice and evaluate the protection provided by vitamin E. Four groups with 12 animals in each are used.

Materials and Methods: Group A was given olive oil 6.67 ml/kg/day orally for 30 days. Group B was given sodium fluoride 10 mg/kg/day dissolved in distilled water + olive oil 6.67 ml/kg/day both orally for 30 days. Group C was given sodium fluoride 10 mg/kg/day dissolved in distilled water and vitamin E 15 mg/kg/day dissolved in olive oil both orally for 30 days. Group D was given vitamin E 15 mg/kg/ day dissolved in olive oil orally for 30 days.

Results: On histological examination, animals of group B showed altered structure of hepatic lobules with deranged radiating hepatocytes, many of which manifested ballooning degeneration and statistically significant increase in their size (p-value < 0.001), vacuolation, presence of necrotic foci and periportal inflammation. These deranged histological findings were correlated with elevated levels of liver enzymes in serum. Co-administration of vitamin E and sodium fluoride to animals of group C showed statistically significant prevention of signs of degeneration in hepatic lobules.

Conclusion: Fluoride induced hepatotoxicity is effectively prevented by co-administration of Vitamin E treatment.

Key Words: Sodium Fluoride, Hepatotoxicity, Vitamin E.

INTRODUCTION

Fluoride occurs naturally in ground water and some amount is added to drinking water to meet the required level.[1] It was discovered in early 1940s that people who lived in areas where drinking water supplies had naturally occurring fluoride level of approximately 1mg per liter of water (1 ppm) had fewer incidence of dental caries.[2] This indicated that fluoride affords protection against dental caries. Consequently, fluoride was added to water to increase its level to approximately 1 mg per liter (1 ppm) of water to avoid tooth decay.[3]

Exposure to sodium fluoride (NaF) occurs invariably through number of sources including water, drugs, pesticides, fertilizer and products related to dental care, infant formula milks, drinks prepared with fluoridated water,[4] fish[5] and tea leaves[6] are also responsible for exposure to excess fluoride. Fluoride contaminated groundwater has been reported in Asian countries. Endemic fluorosis is a public health problem in India.[7] More than 20 provinces in China are affected from fluorosis of variable severity.[8] The fluoride concentration of 1 to 25 mg or more per litre have been found in various regions of Turkey.[9] The concentration of fluoride in groundwater of the district Tharparker, Sindh, was found to be between 0.93 – 11.8 ppm [10]. Jhang, Chakwal, Khoshab and Mianwali also have the higher levels of fluoride in Pakistan.[11]

Endemic fluorosis in Pakistan was first reported in Kalalanwala, Kasur district, in July 2000, when more than 400 local inhabitants were diagnosed with bone disease which included osteosclerosis, arrest of bone growth and calcification at the site of tendon insertion. About 72 cases were under 15 years of age.[12]

A number of studies indicate that fluoride can cause deleterious effects in animal liver.[13-15] Widespread degenerative changes varying from ballooning of cell to necrosis and accumulation of mononuclear cells in hepatic lobules were observed in fluoride toxicity.[14] It has also been reported in previous studies that the fluoride treated animals showed mononuclear cells in

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portal canals, and sporadic areas of necrosis within individual lobules. These changes were accompanied by swelling of Kupffer cells in congested sinusoidal vessels.[16] The determination of transaminase activity in serum is a common means of detecting liver damage. Previous study showed that serum ALT and AST in the experimental rats exposed to fluoride were reported to be elevated when compared to the control group.[17]

Anuradha reported that fluoride can cause damage to mitochondria, decreasing cellular respiration and also increasing the release of cytochrome C and the initiation of the caspase cascade; this leads to opening of the permeability transition pore in HL – 60 cells.[18] Opening of the permeability pore in mitochondria of rat's liver, results in its swelling and cell death through apoptosis or necrosis [19]. Fluoride is also considered to increase the production of reactive oxygen species and free radicals[14,20] which attack double bonds of polyunsaturated lipids in cell membranes. The lipid – radical interactions yield peroxides, which themselves are unstable and reactive. In this way an autocatalytic chain reaction ensues which causes disruption of membranes of cell and organelles, oxidation of proteins with resultant loss of enzymes.[21] Shivarajashankara showed that lipid peroxidation was increased in sodium fluoride treated mice and pups, as shown by high liver malondialdehyde levels, while serum antioxidant status showed a substantial decline.[22]

The toxic effect of fluoride on different systems had been extensively studied in the past; the effect of antioxidants in ameliorating the fluoride induced toxicity is also well studied, however much work on protective effect of vitamin E on fluoride induced histological changes in the liver has not been done. Consequently, the present study was designed to investigate the preventive effect of vitamin E on histological structure of mouse liver treated with sodium fluoride and correlate it with effect on levels of liver enzymes.

MATERIALS AND METHODS

This study was conducted at the Experimental Research Laboratory of University of Health Sciences, Lahore, after approval from the ethical committee of the University. Forty eight adult male albino mice, 6-8 weeks of age and weighing 30 ± 5 g were obtained from National Institute of Health, Islamabad; the animals were housed in cages and kept in controlled environment at room temperature of 23 ± 2°C, humidity of 50 ± 5% and with light and dark cycles of 12 hours each. Animals were fed on standard rodent diet and were given water *ad libitum and* were acclimatized for a period of one week.

Animal Groups

Animals were randomly divided into four experimental groups A, B, C and D, each having 12 animals. All animals in each group were given identification marks which were verified every time before giving the dose.

Group A served as a control and was given olive oil 6.67 ml/kg/day orally for 30 days.

Group B was given sodium fluoride (Riedel-de-Haen company) 10 mg/kg/day dissolved in distilled water + olive oil 6.67 ml/kg/day both orally for 30 days.

Group C was given sodium fluoride 10 mg/kg/day dissolved in distilled water and vitamin E (Sigma) 15 mg/kg/day in 6.67 ml of olive oil both orally for 30 days.

Group D was given only vitamin E 15mg/kg/day dissolved in olive oil orally for 30 days (Table 1).

Blood was drawn by cardiac puncture for analysis of serum ALT and AST. Animals were sacrificed and liver was dissected out. 3 – 5 mm pieces of liver were obtained and immediately put into a bottle containing 10% formalin and fixed for a period of 48 hours.

The liver pieces were processed in the automatic tissue processor and paraffin blocks were prepared. Sections 4 μm thick were obtained using rotary microtome. The slides, after staining with haematoxylin and eosin, were examined with light microscope using different power of magnifications as of X10 and X20.

SPSS 18 was used for statistical analysis. For quantitative variables Mean (± S.D) was calculated. One

Table 1: *Experimental design showing various experimental groups, intervention its dosage and its duration.*

Groups (n = 12)	*Intervention and Route*	*Dosage*	*Duration of Intervention*	*Day of Sacrifice*
A	Olive oil orally	6.67 ml/kg/day	30 days	31ist
B	Sodium fluoride orally + Olive oil orally	10 mg/kg/day dissolved in distilled water 6.67 ml/kg/day	30 days 30 days	31ist
C	Sodium fluoride orally + Vitamin E orally	10 mg/kg/day dissolved in distilled water + 15 mg/kg/day dissolved in olive oil	30 days	31ist
D	Vitamin E orally	15 mg/kg/day dissolved in olive oil	30 days	31ist

Table 2: *Showing comparison of general architecture of hepatic lobule among groups.*

General Pattern of Hepatic Lobule	*Group of Animals*				*Total (n = 48)*	*p-value*
	A Group (n = 12)	*B Group (n = 12)*	*C Group (n = 12)*	*D Group (n = 12)*		
Deranged	0 (0%)	12 (100%)	1 (8.3%)	0 (0%)	13 (27.1%)	0001*
Near to normal	0 (0%)	0 (0%)	4 (33.3%)	0 (0%)	4 (8.33%)	
Normal	12 (100%)	0 (0%)	7 (58.3%)	12 (100%)	31 (64.58%)	
Total	12	12	12	12	48	

*Fisher exact p-value ≤ 0.05 is statistically significant

Table 3: *Showing comparison of mean of levels of ALT and AST among groups.*

Group of Animal	*Group A Mean ± SD*	*Group B Mean ± SD*	*Group C Mean ± SD*	*Group D Mean ± SD*	*p-value*
ALT	40.42 ± 6.882	134.67 ± 3.526	59.08 ± 16.223	41.67 ± 8.294	0.001*
AST	80.92 ± 8.295	223.08 ± 7.594	127 ± 22.794	95.08 ± 12.631	0.001*

*p-value ≤ 0.05 is statistically significant

Way ANOVA was applied to show the statistical difference in means among groups. Post hoc Tukey test was applied to show which group's mean differs statistically significantly.

Fisher Exact test was used to observe association between qualitative variables and groups.

RESULTS

The histological sections of liver of the animals from control group A showed normal hepatolobular architecture; hepatocytes were arranged as radiating rows of polyhedral cells from central vein to periphery of the lobule to the portal areas. The sinusoids were present between cords of hepatocytes, lined by endothelial cells having flattened dark nuclei and Kupffer cells with nuclei projecting into the lumen (Fig. 1). These sinusoids ultimately open into the central vein (Fig. 1). The portal areas were present at the periphery of the typical hepatic lobule characterized by presence of a branch each of portal vein, bile ductule and hepatic artery (Fig. 1).

Hepatocytes were polygonal in shape and possessed well defined plasma lemma. Each cell had a central nucleus with a distinct nuclear membrane and one or two prominent nucleoli; few binucleated cells were also observed (Fig. 1).

The histological sections of liver of group B animals showed deranged hepatolobular pattern *(p-value < 0.001)*. Hepatocytes appeared to be swollen with statistically significant increase in size *(p-value < 0.001)* and were empty looking due to presence of vacuoles in them, the nuclei were darkly stained with clumping of chromatin material and, nucleoli were not clearly evident, indicating pyknotic changes. Areas of necrosis were also obvious and were indicated by increased eosinophilia, pyknosis and disintegrating nuclei. Sinusoidal spaces were reduced and Kupffer cells increased, but the difference was not statistically significant (p-value = 0.06). The periportal areas in the hepatic lobules showed varying degrees of inflammation as evident by mononuclear leukocytic infiltrates (Fig. 3).

In the histological section of group C, the liver architecture was generally well preserved (Fig. 4) and was

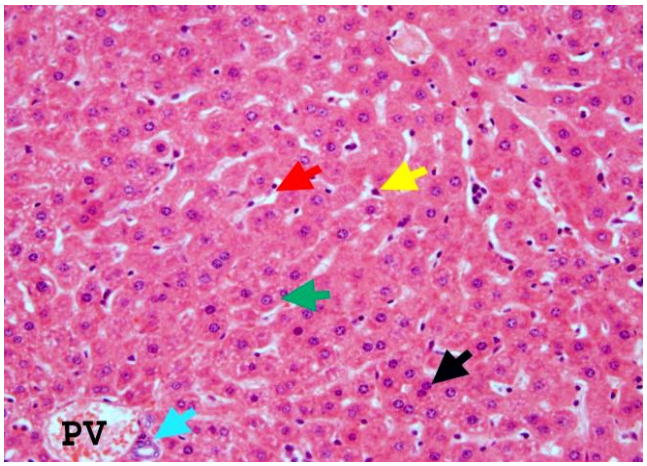

Fig. 1: *Photomicrograph of liver section from group A, showing cords of hepatocytes radiating peripherally (green arrow), sinusoidal spaces are present between the hepatocytes (red arrow) and Kupffer cells (yellow arrow), portal areas consisting of branch of portal vein (PV), bile ductile (turquoise arrow) H & E. X200.*

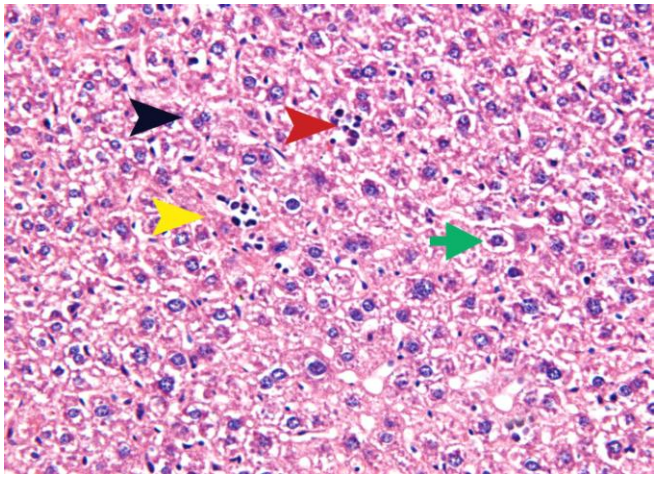

Fig. 2: *Photomicrograph of section of liver of group B showing swollen hepatocytes (green arrow), necrosis (yellow arrow head), inflammatory cells (red arrow head), binucleated hepatocytes (black arrow head). X200, H & E stain.*

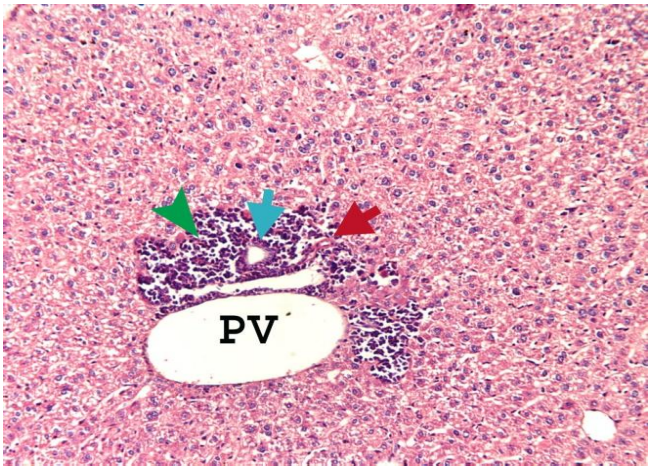

Fig. 3: *Photomicrograph of liver section from group B, showing branch of portal vein (PV), bile ductule (turquoise arrow) and branch of hepatic artery (red arrow), periportal inflammatory cells (green arrow head). H & E. X100.*

comparable to group A, showing normal architecture (Fig. 1) which was unlike that of group B which showed deranged architecture (Fig. 2). Further, hepatocytes in group C appeared normal in shape and form and showed the usual arrangement of cords radiating from central vein to portal areas in the periphery of each hepatic lobule (Fig. 4); between the cords of hepatocytes were the sinusoids lined by endothelial cells having flattened dark nuclei and projecting nuclei of Kupffer cells; the number of lining cells was comparable to those of groups A and B. Sinusoids were seen to be present between anastomosing cords of hepatocytes and then opening into the lumen of central vein.

The hepatocytes of group C contained small vacu-

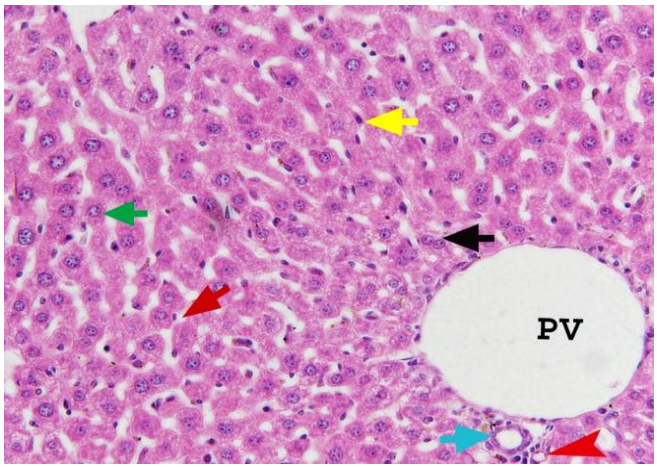

Fig. 4: *Photomicrograph of liver section from group C, showing normal architecture with cords of hepatocytes radiating peripherally (green arrow), Sinusoidal spaces are present between the hepatocytes (red arrow) lined by endothelium and kupffer cells (yellow arrow), portal areas consisting of branch of portal vein (PV), Bile ductile (turquoise arrow) and branch of hepatic artery (red arrow head). H & E stain X 200.*

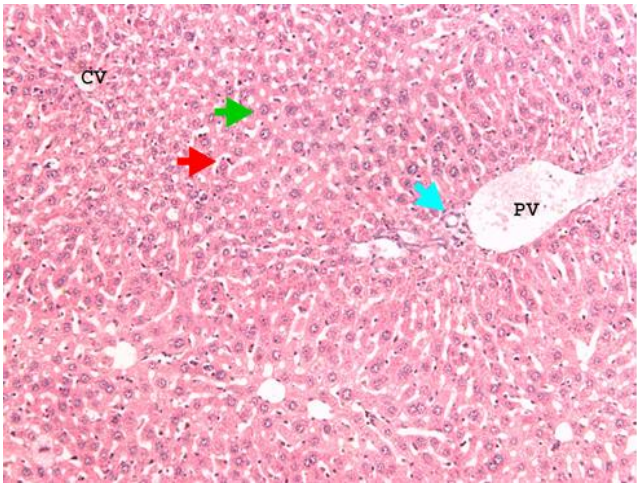

Fig. 5: *Photomicrograph of histological section from liver of group D animal, showing normal architecture with central vein (CV), cords of hepatocytes radiating peripherally (green arrow), Sinusoidal spaces are present between the hepatocytes (red arrow), portal areas consisting of portal vein (PV) and bile duct (turquoise arrow) visible at the periphery. H & E stain X 100.*

oles, which were smaller and lesser in number (Fig. 4) than those of group B (Fig. 2); these, however, were comparable to those in group A (Fig. 1). Mean size of hepatocyte in the group C showed statistically significant prevention in swelling of hepatocyte when compared to group B *(p-value < 0.001)* and was comparable to those of group A. In the histological section of group

D, the liver architecture was hardly disturbed and was nearly comparable to group A and C (Fig. 5). The values of the enzymes (ALT and AST) when compared among groups showed statistically significant difference (*p-value < 0.001)* (Table 3).

DISCUSSION

In the present investigation the mice of group A showed normal architecture of hepatic lobules with hepatocytes arranged as cords of polyhedral cells radiating from central vein to the periphery. Between the cords of hepatocytes the sinusoids were lined with endothelial cells having flattened dark nuclei and Kupffer cells with nuclei projecting into the sinusoids which opened into the central vein.

Group B mice showed statistically significant deranged pattern of hepatic lobule with swollen hepatocytes and loss of radial arrangement of cords *(p-value < 0.001)*. Sinusoidal spaces are reduced; periportal areas were infiltrated with inflammatory cells. The results of the current study are comparable to those reported earlier in which the architecture of liver lobule was reported to be deranged upon treating animals with sodium fluoride for variable periods of time ranging from 35 to 90 days.[23,24] In the present study it was observed that histological preparation from group B animals showed characteristic changes ranging from cellular swelling to necrosis, accumulation of mononuclear cells in hepatic lobules and portal areas. Hepatocytic hyperplasia was indicated by statistically significant increase in number of binucleated cells. Kupffer cells were not significantly increased when all the groups were compared with p-value < 0.06; similar changes in the liver lobules of animals treated with fluoride were reported earlier also.[24,25] Current findings also corroborate with those of the earlier studies in which necrosis of hepatocytes, pyknotic nuclei and deranged hepatic cords were reported in fluoride-treated adult rats.[16,20,24] Finding of current investigation are also comparable with an earlier study in which rabbits were treated with fluoride at a dose of 10 mg/kg for 15 weeks; focal necrosis of hepatocytes with hyperplasia at places was observed, however, no significant increase in the number of Kupffer cells was reported.[26]

Number of studies had shown that the toxic effects of fluoride on the liver morphology could be ameliorated or prevented by the use of different antioxidants supplements.[27-29] Among antioxidants vitamin E has been widely used to afford protection against hepatotoxicity produced by various toxic substances. Previous studies have shown that antioxidant supplements could protect the liver tissues from oxidation stress and prevent damage under various toxic conditions.[28-30] In the current study the animals of group C were concomitantly treated with sodium fluoride and vitamin E; histological preparation of the liver from these animals showed the results which were comparable to those from groups A and D; whereas, there was statistically significant improvement in the structure of hepatic lobule in group C when compared with those from group B, treated with fluoride alone (Table 2). In similar studies, structure of pancreas affected by fluoride was reported to have been restored by vitamin E.[30] The result of the current investigation showed that simultaneous administration of NaF and vitamin E to mice significantly prevented necrosis of hepatocytes. A study reported similar findings with co-administration NaF 4 mg/rat/day and 3 mg/rat/day vitamin E for 5 weeks.[24] Nair showed that the administration of sodium fluoride and arsenic trioxide to rats for 30 days caused severe zonal necrosis. In the same study during the withdrawal period administration of vitamin E showed amelioration in necrosis of hepatocytes [27]. Vitamin E suppresses the activity of enzymes such as protein kinase C and phospholipase A2 inflammatory cells (monocytes); this also is reported to substantially diminish the production of free radicals and their effects.[24]

The current study showed elevated levels of both ALT and AST in group B animals. Many authors reported elevated levels of ALT after fluoride toxicosis.[31,32] Elevated enzyme levels were related to the damaged liver cells.[33]

In our study administration of vitamin E showed near to normal levels of AST and ALT enzymes in NaF and vitamin E treated group as compared to those treated with NaF alone. Nair reported significant improvement in liver enzymes in vitamin E treated group when compared to those treated with fluoride salt only.[32]

This study showed that sodium fluoride induced changes in the liver of the mouse, as shown by disrupted architecture of liver, increase in size of hepatocytes, vacuolation, necrosis and periportal inflammation were restored to nearly normal upon concurrent administration of vitamin E. From the present study it is concluded that sodium fluoride induced damages in liver of the mouse were effectively prevented by Vitamin E.

It is ***concluded*** that our observations confirmed the previous findings on hepatotoxicity induced by fluoride; our results also have shown that vitamin E is effective in preventing changes in histological structure and biochemical indices of liver in mice, since vitamin E is protective against hepatotoxins, cheap and easily available, it can be used in endemically affected areas.

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