RECOVERY FROM FLUORIDE AND ALUMINIUM INDUCED FREE RADICAL LIVER TOXICITY IN MICE

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SUMMARY: Adult male Swiss strain albino mice (*Mus musculus*) were administered orally sodium fluoride (NaF, 10 mg/kg bw), aluminium chloride ($AlCl_3$, 200 mg/kg bw), or both together for a period of 30 days to study the induction of free radical toxicity in their liver. The effects of withdrawal of treatment (30 days) as well as the beneficial effects, if any, of vitamin C (15 mg/animal/day) or vitamin E (2 mg/animal/day) administered alone were also investigated. Fluoride, alone and in combination with aluminium, significantly (P<0.001) impaired the production of free radical scavengers, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione (GSH), and total ascorbic acid (TAA), thereby increasing hepatic lipid peroxidation and thus making the tissue more susceptible to injury. Cessation of the NaF and $AlCl_3$ treatments for the next 30 days brought about measurable recovery of most of these parameters in the liver as compared to the treated group of mice. However, pronounced or even complete recovery occurred in all parameters (P<0.001) by administration of ascorbic acid (vitamin C) or vitamin E during the 30-day recovery period. The results show that mice liver function can recover after fluoride and aluminium induced intoxication by the mitigating effects of vitamins C and E.

Key words: Aluminium and liver; Ascorbic acid; Fluoride and liver; Free radicals; Toxicity reversal; Vitamin C; Vitamin E.

INTRODUCTION

Free radicals and lipid peroxidation are known to play an important role in fluorosis.[1] Fluoride induced hepatotoxicity due to the formation of free radicals and decreased activity of the antioxidant system in hepatocytes of animals and humans have been reported.[2-5] Fluoride exposure also induces histopathological changes in liver involving focal necrosis, infiltration of leucocytes, swelling of Kupffer cells, extensive vacuolisation, hemorrhagic areas, ultrastructural alterations in hepatocytes, and increased apoptosis in animals and humans.[6-12]

Treatment of rats and mice with aluminium chloride or citrate results in an accumulation of aluminium in several organelles of their hepatocytes causing lesions in liver. Carbohydrate and protein metabolism of the liver is also affected[9,13] together with elevation of serum transaminases indicating liver damage.[12,14-16]

The present investigations were undertaken to study free radical toxicity in the liver of male mice induced by fluoride and aluminium treatments, both individually and together, and to examine the possible recovery of liver function by the antioxidant vitamins C and E.

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MATERIALS AND METHODS

Animals: Healthy, adult Swiss strain male albino mice (*Mus musculus*) weighing between 25 and 35 g were used for the experiments. The animals were obtained from Cadila Health Care, Ahmedabad, India, under Registration No. 167/1999/CPCSEA from the Ministry of Social Justice and Empowerment, Government of India and Committee for the Purpose of Control and Supervision of Experiments on Animals, Chennai, India. They were maintained on standard chow and water (0.6-1.0 ppm F) was given *ad libitum*. They were housed in an air-conditioned animal house at 26 ± 2 ºC and exposed to 10–12 hours of light per day.

Exposure treatments: The animals were divided into seven major groups and caged separately as shown in the Table 1.

Table 1. Experimental protocol

Group	Treatment and dose (10 to 12 animals in each group)	Duration (days)	Day of autopsy
IA	Control + distilled water (DW)	-	*
IB	Control + olive oil (OO) (0.2 mL/animal/day)	30	31st
IC	Control + ascorbic acid (AA) (15 mg/animal/day)	30	31st
ID	Control + vitamin E (vit. E) (2 mg/0.2mL olive oil/animal/day)	30	31st
II	NaF - treated (10 mg/kg body wt)	30	31st
III	Aluminium chloride ($AlCl_3$) (200mg/kg body wt)	30	31st
IV	NaF+ $AlCl_3$ treated (10mg + 200 mg/kg body wt)	30	31st
V	Same as in Group IV then withdrawal for further 30 days (Withdr.)	30+30	61st
VI	Same as in Group IV + AA for 30 days	30 + 30	61st
VII	Same as in Group IV + vit E for 30 days	30 + 30	61st

* Sacrificed along with treated mice

All treatments were given orally with a hypodermic syringe attached to an angular needle. Animals in Groups IA-ID served as controls (untreated, vehicle treated, and positive controls). Sodium fluoride (NaF) (Loba Chemie, Mumbai, 99% purity) was administered to Group II, IV–VII animals at a dose of 10 mg/kg body weight for 30 days. Aluminium chloride ($AlCl_3$) (S.D. Fine Chem. Ltd., Boisor 401501, India, 99.5% purity) was administered in water to Group III–VII animals at a dose of 200 mg/kg body weight for 30 days. The above doses were

established on the basis of LD_{50} values *viz*, 54.4 mg/kg body weight for NaF and 4 g/kg body weight for $AlCl_3$, respectively.[17-19] The Group IV animals were fed NaF and $AlCl_3$ orally in combination (doses as above) for 30 days.

To study the reversibility of the induced effects, the treatment of Group IV animals was withdrawn for 30 days. These were Group V animals and continued to be maintained on standard food and water *ad libitum*. During the 30-day withdrawal period, animals in Group VI were administered ascorbic acid (AA, vitamin C) (15 mg/animal/day) (Loba Chemie, Mumbai, 99% purity), and animals in Group VII were given vitamin E (Tocopherol acetate) (2 mg/animal/day) (E. Merck, India Ltd. Mumbai, 99% purity). The doses of vitamin C and E were based on earlier work.[9,20]

At the end of each treatment, the animals were weighed on an animal weighing balance (Ohaus, USA) and sacrificed by cervical dislocation. The liver was dissected out carefully, blotted free of blood, and utilized for the study of various parameters.

Biochemical studies of lipid peroxide,[21] superoxide dismutase (E.C.1.1.15.11),[22] catalase (E.C. 1.11.1.6),[23] glutathione peroxidase (E.C.1.11.1.9),[24] glutathione,[25] and ascorbic acid[26] were carried out on the liver of control and all treated mice by the methods cited.

Statistical analysis: For all biochemical analyses, a minimum of 8–10 replicates were performed for each parameter. The data were statistically analysed by Student's t test and Analysis of Variance (ANOVA).

RESULTS

Results of the various treatments are shown in Tables 2 and 3 below.

A significant ($P<0.001$) decline was observed in the levels of glutathione, total and reduced ascorbic acid (TAA, RAA) and activity of glutathione peroxidase, in liver of mice in the treatment Groups II–IV as compared to controls (Groups IA to ID). However, the levels of lipid peroxides (LPO) and dehydroascorbic acid were significantly ($P<0.001$) increased by the fluoride or aluminium treatments given either singly or in combination (Tables 2 and 3). Withdrawal of the treatments for 30 days showed significant ($P<0.01$) recovery in some parameters, except RAA, DHA, TAA, and SOD, which were not recovered compared to Group IV. Administration of vitamins C and E (Groups VI and VII) showed significant ($P<0.001$) recovery in all the parameters, thus manifesting marked ameliorative effects as compared to the treated Group IV mice.

DISCUSSION

Fluoride treatment caused free radical toxicity by a decrease in the activities of free radical scavenging enzymes, *viz,* SOD, GSH-Px, and catalase, but it increased lipid peroxidation in the liver of male mice in corroboration of earlier studies on male and female rodents treated with NaF.[1,3-5,9,10,27-30] A decrease in polyunsaturated lipids and total phospholipids but an increase in saturated lipids in the liver of rats[31] given NaF in drinking water suggests oxidative stress in that organ. However, some workers have reported no significant changes in antioxidant enzymes in fluoride treated rat pancreas[32] or in

rabbits intoxicated with fluoride or in skeletal fluorosis patients.[33] But these studies[32,33] do not conclusively rule out the oxidative stress hypothesis and suggest that more controlled studies are needed in this direction.[34]

Table 2. Lipid peroxide (LPO) level (nanomoles of MDA/mg tissue wt/60 min), superoxide dismutase (SOD) (units/mg protein), catalase (units/min/mg protein), and glutathione peroxidase (GSH-Px) (nanomoles of NADPH oxidized/min/mg protein) activity in liver of mice in Groups I–VII[a]

Group	Treatment	LPO	SOD	CATALASE	GSH-Px
IA	Control + DW	33.12 ± 0.19	0.52 ± 0.003	35.71 ± 0.12	18.70 ± 0.23
IB	Control + OO	32.85 ± 0.24	0.52 ± 0.003	35.37 ± 0.14	18.81 ± 0.21
IC	Control + AA	32.79 ± 0.21	0.52 ± 0.004	35.48 ± 0.19	18.68 ± 0.10
ID	Control + vit. E	32.75 ± 0.22	0.51 ± 0.004	35.67 ± 0.09	18.67 ± 0.13
II	NaF	42.06 ± 0.27§	0.46 ± 0.005§	31.22 ± 0.22§	15.83 ± 0.15§
III	$AlCl_3$	53.47 ± 0.41§	0.42 ± 0.005§	29.27 ± 0.16§	13.91 ± 0.11§
IV	NaF + $AlCl_3$	51.08 ± 0.31§	0.35 ± 0.005§	26.90 ± 0.17§	11.34 ± 0.13§
V	Withdrawal	49.11 ± 0.22‡	0.40 ± 0.004	27.83 ± 0.23‡	12.19 ± 0.13‡
VI	Withdr. + AA	35.48 ± 0.16§	0.50 ± 0.004§	33.10 ± 0.17§	17.10 ± 0.18§
VII	Withdr. + vit. E	35.44 ± 0.08§	0.50 ± 0.006§	33.02 ± 0.06§	17.02 ± 0.16§

[a]Data are expressed as mean ± S.E. * P<0.05; † P<0.02; ‡ P<0.01; § P<0.001; no sign = Not significant.
Comparisons between: Group I with Groups II or III or IV individually; Group IV with Groups V or VI or VII individually.

The treatments with aluminium and with NaF + $AlCl_3$ also resulted in effects similar to those produced by NaF alone. On the whole, at the level administered, aluminium chloride seemed to be comparatively more effective in causing oxidative stress than NaF.

A decrease in levels of total ascorbic acid and reduced ascorbic acid (TAA and RAA), concomitant with an increase in dehydroascorbic acid (DHA) in the liver of treated male mice in the present study is in agreement with earlier work.[9,27] It is thus evident that both aluminium and fluoride cause disturbances in the utilization and probably synthesis of ascorbic acid leading to change in its metabolism which might be influenced by the decrease in glutathione (GSH) levels. A decrease in GSH but an increase in its oxidized form GSSG in blood of NaF-exposed rats has been reported.[35] Similarly, GSH decreased in human hepatocytes *in vitro*.[6] Since GSH is involved in the mechanism of detoxification[36] by

scavenging free radicals in rat liver[28,37] and in the conversion of dehydroascorbic acid into the reduced form (RAA),[38] its decrease might also cause toxicity.

Table 3. Glutathione (g/100 mg fresh tissue wt), total, dehydro and reduced ascorbic acid (TAA, DHA and RAA) (mg/g fresh tissue wt) levels in liver of mice in Groups I-VII[a]

Group	Treatment	Glutathione	RAA	DHA	TAA
IA	Control + DW	86.17 ± 0.19	4.18 ± 0.008	2.54 ± 0.006	6.73 ± 0.008
IB	Control + OO	84.61 ± 0.22	4.18 ± 0.007	2.52 ± 0.005	6.71 ± 0.006
IC	Control + AA	85.03 ± 0.20	4.20 ± 0.006	2.56 ± 0.005	6.77 ± 0.005
ID	Control + vit. E	84.99 ± 0.32	4.20 ± 0.005	2.56 ± 0.004	6.76 ± 0.004
II	NaF	71.34 ± 0.31§	3.50 ± 0.005§	2.88 ± 0.004§	6.39 ± 0.004§
III	$AlCl_3$	60.31 ± 0.22§	3.22 ± 0.010§	2.91 ± 0.004§	6.14 ± 0.010§
IV	NaF + $AlCl_3$	52.72 ± 0.32§	3.00 ± 0.008§	2.98 ± 0.004§	5.99 ± 0.006§
V	Withdrawal	58.69 ± 0.21‡	3.07 ± 0.006	2.93 ± 0.004	6.01 ± 0.009
VI	Withdr. + AA	82.44 ± 0.18§	3.93 ± 0.004§	2.75 ± 0.003§	6.68 ± 0.003§
VII	Withdr. + vit. E	81.68 ± 0.28§	4.18 ± 0.006§	2.57 ± 0.004§	6.75 ± 0.003§

[a]Data are expressed as mean ± S.E. * P<0.05; † P<0.02; ‡ P<0.01; § P<0.001; no sign = Not significant.
Comparisons between: Group I with Groups II or III or IV individually; Group IV with Groups V or VI or VII individually.

The withdrawal of treatments with NaF, $AlCl_3$, and NaF + $AlCl_3$ was conducive for recovery of some parameters in the mice liver as also reported in rats.[11]

Supplementation with vitamins C and E during the withdrawal period caused significant recovery in liver parameters, which is attributed to their antioxidant action[38,39] and their synergistic action,[40] since the free radical scavenging property of vitamin E is enhanced in presence of vitamin C. The beneficial effects of a protein supplemented diet in mice liver[41] and the protective action of SOD and vitamin E against free radical toxicity are also reported.[42]

In conclusion, the present study revealed that ascorbic acid (vitamin C) and vitamin E are capable of completely, or almost completely, mitigating liver toxicity in mice induced by fluoride and aluminium.

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REFERENCES

1 Sun GF, Shen HY, Ding GY. Effects of extraneous GSH on toxicity and metabolism of fluoride [abstract]. In: Proceedings of the XXth Conference of the International Society for Fluoride Research; 1994 Sept 5–9; Beijing, China. p. 156-7.

2 Li J, Cao S. Recent studies on endemic fluorosis in China. Fluoride 1994;27(3):125-8.

3 Guo XY, Sun GF, Sun YC. Oxidative stress from fluoride-induced hepatotoxicity in rats. Fluoride 2003;36(1):25-9.

4 Shivashankara AR, Shivarajashankara YM, Bhat PG, Rao SH. Lipid peroxidation and antioxidant defense systems in liver of rats in chronic fluoride toxicity. Bull Environ Contam Toxicol 2002;68(4):612-6.

5 Karimov K, Inoiatova FKh, Inoiatov FSH. Toxic effects of various water pollutants on structural and functional parameters of hepatocytes. Vopr Med Khim 2002;48(2):174-9. [in Russian, abstract in Fluoride 2003;36(1):61.]

6 Wang A, Xia T, Ran P, Bai Y, Yang K, Chen X. Effects of selenium and fluoride on apoptosis and lipid peroxidation in human hepatocytes. Zhonghua Yu Fang Yi Xue Za Zhi 2002; 36(4):235-8. [in Chinese, abstract in Fluoride 2003;36(1):45-6.]

7 Kapoor V, Prasad T, Bhatia KC. Effect of dietary fluorine on histopathological changes in calves. Fluoride 1993;26(2):105-10.

8 Shashi A, Thapar SP. Histopathology of fluoride-induced hepatotoxicity in rabbits. Fluoride 2001;34(1):34-42.

9 Chinoy NJ. Studies on fluoride, aluminium and arsenic toxicity in mammals and amelioration by some antidotes. In: Tripathi G, editor. Modern Trends in Experimental Biology. New Delhi: CBS Publisher; 2002. p. 164-93.

10 Kołodziejczyk L, Put A, Grzela P. Liver morphology and histochemistry in rats resulting from ingestion of sodium selenite and sodium fluoride. Fluoride 2000;33(1):6-16.

11 Dąbrowska E, Szynaka B. Effect of fluoride on the ultrastructure of rat liver [abstract]. Fluoride 2000;33(1):S8.

12 Sharma A, Chinoy NJ. Fluoride induced ultrastructural and histopathological changes in liver of mice and its reversal by antidotes [abstract]. In: Proceedings of International Conference On Probing in Biological Systems; 2000 Feb 7–11; Mumbai, India. p. 113.

13 Chinoy NJ, Bhattacharya S, Chawla S. Effect of aluminium on some soft tissues of male mice and its reversal [abstract]. In: Proceedings of International Conference On Probing in Biological Systems; 2000 Feb 7–11; Mumbai, India. p. 112.

14 Klein GL, Heyman MB, Lee TC, Miller NL, Marathe G, Gourley WK, et al. Aluminium-associated hepatobiliary dysfunction in rats: relationship to dosage and duration of exposure. Pediatr Res 1988;23:275-8.

15 Roy AK, Talukder G, Sharma A. Similar effects *in vivo* of two aluminium salts on the liver, kidney, bone and brain of *Rattus norvegicus*. Bull Environ Contam Toxicol 1991;47:288-95.

16 Van der voet GB. Aluminium in Biology and Medicine. New York: John Wiley and Sons; 1992. p. 109-22.

17 Pillai KS, Mathai AT, Deshmukh PB. Effect of subacute dosage of fluoride on male mice. Toxicol Lett 1988;44:21-9.

18 Chinoy NJ, Bhattacharya S. Effects of single dose of aluminium chloride on some reproductive organs and fertility in male mice. Indian J Environ Toxicol 1996;6(1):10-3.

19 Chinoy NJ, Bhattacharya S. Effect of chronic administration of aluminium chloride on reproductive functions of testis and some accessory sex organs of male mice. Indian J Environ Toxicol 1997;7(1):12-5.

20 Chinoy NJ, Sharma A. Amelioration of fluoride toxicity by vitamins E and D in reproductive functions of male mice. Fluoride 1998;31(4):203-16.

21 Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.

22 Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Ind J Biochem Biophys 1984;21(4):1302.

23 Luck H. A spectrophotometric method for the estimation of catalase. In: Bergmeyer HU, editor. Methods of Enzymatic Analysis. London: Academic Press; 1963. p. 886-7.
24 Pagila DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:15869.
25 Grünert RR, Phillips PH. A modification of the nitroprusside method of analysis for glutathione. Arch Biochem 1951;30:217-25.
26 Roe JH, Küether CA. The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenyl- hydrazine derivatives of dehydroascorbic acid. J Biol Chem 1943;147:399-407.
27 Sharma A, Chinoy NJ. Role of free radicals in fluoride-induced toxicity in liver and kidney of mice and its reversal [abstract]. Fluoride1998;31(3):S26.
28 Liang G, Zhang XM, Zhao H, Tang JC, Li YY, Li FJ, et al. The effects of GSH taken orally on lipid peroxidation induced by fluoride [abstract]. In: Proceedings of the PAN Asia Pacific Conference on Fluoride and Arsenic Research; 1999 Aug 16–20; Shenyang, China. p. 55.
29 Inkielewicz I, Krechniak J. Fluoride effects on glutathione peroxidase and lipid peroxidation in rats. Fluoride 2004;37(1):7-12.
30 Chinoy NJ. Fluoride stress on antioxidant defence systems [editorial review]. Fluoride 2003;36:138-41.
31 Wang YN, Xiao KQ, Liu JL. Dallner G, Guan ZZ. Effect of long term fluoride exposure on lipid composition in rat liver. Toxicology 2000;146(2-3):161-9.
32 Chlubek D, Grucka-Mamczar E, Birkner E, Polaniak R, Stawiarska-Pieta B, Duliban H. Activity of pancreatic antioxidant enzymes and malondialdehyde concentrations in rats with hyperglycemia caused by fluoride intoxication. J Trace Elem Med Biol 2003;17:57-60.
33 Reddy GB, Khandare AL, Reddy PY, Rao GS, Balakrishna N, Srivalli I. Antioxidant defense system and lipid peroxidation in patients with skeletal fluorosis and in fluoride-intoxicated rabbits. Toxicol Sci 2003;72:363-8.
34 Chlubek D. Fluoride and oxidative stress [editorial review]. Fluoride 2003;36:217-28.
35 Kaushik T, Shyam R, Vats P, Suri S, Kumria MML, Sharma PC, Singh SN. Glutathione metabolism in rats exposed to high-fluoride water and effect of Spirulina treatment. Fluoride 2001;34(2):132-8.
36 Meister A, Anderson ME. Glutathione. Annu Rev Biochem 1983;52:711-60.
37 Satsangi K, Dua KK. Preventive effects of few dietary nutrients against acute aluminium toxicity in mice [abstract]. In: Proceedings of the International Conference on Probing in Biological Systems; 2000 Feb 7–11; Mumbai, India. p. 186-7.
38 Chinoy NJ. Ascorbic acid turnover in animal and human tissues. J. Anim Morphol Physiol 1978;Silver Jubilee Volume:65-85.
39 Basu TK, Dickerson JW. In: Vitamins in Human Health and Disease. Wallingford, UK: CAB International; 1996. p. 214-27.
40 Nair SB, Jhala DD, Chinoy NJ. Beneficial effects of certain antidotes in mitigating fluoride and/or arsenic induced hepatotoxicity in mice. Fluoride 2004;37(2):60-70.
41 Chinoy NJ, Mehta D. Beneficial effects of protein-supplemented diet on fluoride-induced toxicity in liver of male mice [abstract]. Fluoride 2000;33(1);S7-8.
42 Wang ZC, Fu D, Wang YP, Guan DH, Li XD, Yan JL, et al. Effects of free radical on the development of fluorosis and the protective effects by superoxide dismutase and vitamin E [abstract]. In: Proceedings of the XXth Conference of the International Society for Fluoride Research; 1994 Sept 5–9; Beijing, China. p. 145-6.

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