Fluoride toxicity on detoxification mechanism mitigated by Vit-C in the selected tissues of Albino rat

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Abstract : The metabolites and antioxidant enzymes involving in detoxification metabolism are severely prone to NaF stress, In India contaminated ground water has a fluoride level up to 25mg/l and continuous ingestion of fluoride contaminated water results in fluoride poisoning. The results of the present study clearly indicate that administration of fluoride causes significant alterations in the antioxidant enzymes in all the tissues of Albino rats. . The animals were orally administered 4.4mg/kg body weight (*i.e.* 1/10th of LD_{50}) of NaF for 28 days with an interval of 24h to induce the toxic effects of fluoride in Albino rats. 200mg/kg/day of vitamin C was given orally.. We observed significant alterations in antioxidant enzymes when compared between NaF exposed rats and NaF + Vitamin C treated rats. Four groups of animals were taken, first group is control, II group was exposed to vit-C for 28 days, III group exposed to NaF for 28 days and IV group exposed to NaF+Vit-C for 28 days. The dosing schedule ranging from 1 to 28 days with an interval of 24 h. was observed. The animals were sacrificed on 29th day by cervical dislocation and the tissues were isolated. and processed immediately for histopathological evaluation and the remaining tissue is stored at -80ºC for further biochemical analysis. The results of the present study clearly indicate that exposure to fluoride levels caused a significant elevation in the LPO and XOD levels. On the other hand SOD, CAT, GPx and GR showed a sharp decline in all the tissues of fluoride intoxicated rats. The alterations in the antioxidant cascade in the fluoride exposed rats clearly indicate the onset of oxidative stress resulting in peroxidation of unsaturated membrane lipids. When a combination of NaF and Vitamin C was used a significant recovery in the antioxidant enzymes. vitamin C can scavenge free radicals directly and can also have positive effects by renewing the antioxidant enzymes which are observed in all the tissues. The results of the present investigation suggest that Vitamin C treatment can ameliorate the toxic effects of NaF related to antioxidant enzymes.

Key words: Sodium Fluoride, Vitamin C, Albino Rats , Detoxification Enzymes.

Fluoride is one of the most ionic and ubiquitously present in environment and is released into nature by water, food, and air. Bioaccumulation of fluoride occurs in crops, fruits and vegetables and WHO reports that now a days daily intake of fluoride is more in the population. An optimum level of fluoride in drinking water should be 0.7 mg/l; however, fluoride concentration in water varies based on geographical areas. Many authors have reported that fluorosis causes severe side effects to skeletal parts and important tissues like brain, liver, kidney and spinal cord [Wang and Li, 2002; Choubisa SL 2016]. There are sufficient reports that 50-80% of absorbed fluoride is eliminated by the kidney and therefore kidney damages are seen in people who ingest fluoride. Liver is highly susceptible to the fluoride intoxication as it is the main organ of detoxification. Various studies demonstrated that fluoride intoxication damages liver and kidney [Wang. & Li, 2002]. Fluorisis is considered as the main public health issue as it has huge effect on liver and kidney. The present study is planned to evaluate the ameliorative role of Vitamin C against fluoride intoxicated rats in different tissues.

Oxygen has double-edged properties, being essential for life; it can also aggravate the damage within the cell by oxidative events. **(**Shinde *et al.,* 2006). A free radical may defined as a molecule or molecular fragments containing one or more unpaired electrons in its outermost atomic or molecular orbital and are capable of independent existence (Halliwell *et al.,* 1999). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are describes free radicals and other non-radical reactive derivatives. The reactivity of radicals is generally stronger than non-radical species though radicals are less stable (Pham-Huy *et al.,* 2008).

Excess production of free radicals or decrease in antioxidant level leads to oxidative stress. It is a harmful process that can be mediates damage to cell structures, including lipids, proteins, RNA and DNA which leads to number of diseases. (Saikat Sen, 2010). Reactive Oxygen Species (ROS) is also referred as active oxygen species (AOS) or reactive oxygen intermediates (ROM). ROS include a number of chemically reactive molecules derive from oxygen (Fridovich 1995; Halliwell, 1999). The first line of cellular defense to ROS is provided by the antioxidant enzymes like SOD, CAT, GPx, GR, GST and XOD. Under normal physiological conditions, a delicate balance exists between the rate of formation of hydrogen peroxide via dismutation of oxygen by SOD activity and the rate of removal of hydrogen peroxide by CAT and GPx.

Materials:

Sodium fluoride (99%) was used as a toxicant supplied by BDH Chemical Division, Bombay.

Animal model : Male *Albino Rats*

Animal selected

Healthy Wister strain *Albino rats* of the same age group 130 ± 10 days and weight 200 ± 10 g were procured as experimental animals for the present study. The rats were procured from Indian Institute of Science (I.I.Sc.), Bangalore. Prior to experimentation the animals were acclimatized and maintained at laboratory conditions in the animal house at 25 ± 2°C with a photoperiod of 12h light and 12h darkness throughout the course of the present study. The rats were fed with standard pellet diet supplied by Sai Durga feeds and foods, Bangalore and water *ad libitum*.

Concentration of NaF selected: The LD_{50} as per the latest reports is 44mg/Kg body weight (Sabine Guth *et al* 2020). In the present investigation $1/10^{th}$ LD_{50} (*i.e.* 4.4 mg/kg body weight) was selected for sub- lethal treatment to the experimental rats to induce the toxic effects of fluoride in *Albino rats.*

Sodium Fluoride stock solution: Stock solution of NaF was prepared in distilled water and it was diluted in such a manner that the experimental rats received $1/10^{th}$ dose of LD_{50} (*i.e.* 4.4 mg/ kg bw/ day.).

Vitamin C stock solution: Stock solution of NaF was prepared in distilled water and it was diluted in such a manner that the experimental rats received 200mg/ kg bw/day (Devendra *et al.* 2009).

Route of administration: Orally through gavage.

Experimental Design: Four groups of albino rats were selected and each group consists of six rats.

Group I : Control rats
Group II : Vitamin C treated albino rats
Group III: NaF (exposed albino rats for 28 days)
Group IV: NaF + Vitamin C (exposed albino rats for 28 days)

Biochemical Assays

Lipid Peroxidation

The assay used in MDA levels as described by the method of Okhaw et al.(1979).

Estimation of Xanthine Oxidase activity: (E.C.1.2.3.2)

Xanthine oxidase activity was estimated by the dye reduction method of Srikanthan and Krishnamurthy, 1955.

Estimation of superoxide dismutase: (E.C.15.1.1)

The activity of SOD was assayed by the reduction of nitro blue tetrazolium. Here the Superoxide was produced by riboflavin mediated photochemical reaction system. Superoxide dismutase activity was determined according to the method of Beachamp and Fridovich (1971).

Estimation of Catalase activity: (E.C.11.1.6)

Catalase activity was measured by a slightly modified version of Aebi (1984).

Glutathione Peroxidase (E.C: 1.11.1.9)

Se-Dependent Glutathione Peroxidase was determined by a modified version Flohe and Gunzler (1984).

Glutathione Reductase (EC-1.6.4.2)

GR activity was determined by a slightly modified method of Carlberg and Mannervik (1985) at 37˚C.

Results and Discussion

The results of the present study clearly indicate that exposure to NaF caused a significant elevation in LPO and XOD. A sharp decline in SOD, CAT, GPx and GR was noticed in experimental rats.

The fluoride levels observes in the liver, kidney, Muscle and Testis of *Albino rats* following their chronic exposure to sodium fluoride are shown in Table 1. From the results it is clear that fluoride intoxicated rats showed a statistically significant increase in the MDA content in all the tissues studied in the present investigation. All the values returned to normalcy in vitamin C supplemented rats. From the results it is clear that fluoride exposed rats showed an increase in the XOD activity in all the tissues studied in the present investigation

The results of the present investigation indicate a decrease in the SOD, CAT, GR and GPx activities that fluoride exposed animals showed a decrease in the SOD activity in all the regions of the brain studied in the present investigation. Maximum decrease in the SOD activity was observed in the animals which were exposed to repeated doses for a longer period of time. The alterations in the antioxidant cascade in the fluoride exposed rats clearly indicate the onset of oxidative stress resulting in peroxidation of unsaturated membrane lipids. It is well known that fluoride impact is a slow and progressive process that cause metabolic, functional and structural damages affecting many tissues particularly musculoskeletal, dental systems, kidney, liver and brain.

The results of the present study suggest that fluoride poisoning induces oxidative stress by depleting intracellular GSH and increasing ROS production. GSH plays a key role in regulating intracellular levels of ROS by scavenging free radicals to maintain the intracellular redox status. Fluoride appears to disturb this key cellular pathway by disrupting mitochondria metabolism. Oxidative stress plays a key role in pathophysiology of many neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, etc. and in the present investigation the author studied the ameliorative effect of vitamin C on the fluoride induced toxicity. Ranpariya *et al*., (2011) studied the neuroprotective activity of *Matricaria recutita* against fluoride-induced stress in rats. Their results showed *Matricaria recutita* results in a dose-dependent neuroprotective activity by significantly decreasing in LPO and increasing SOD, CAT, glutathione and total thiol levels in extract-treated animals as compared with negative control group. Their histopathological studies revealed the potent neuroprotective action of German chamomile against oxidative brain damage. In the present investigation vitamin C supplemented rats showed a similar protective activity.

Apaydin *et al*., (2017) reported that vitamin C can scavenge free radicals directly and can also have positive effects by renewing the antioxidant form of vitamin E indirectly. Vitamin C protects against other toxic contaminants that adversely affect cell function by affecting various signaling pathways. Several authors have reported that vitamin C plays an effective antioxidant role against the toxic effects of cobalt chloride, ozone, methomyl, and fipronil (Badgujar *et al*., 2015; Djeffal *et al*., 2015; Valacchi *et al*., 2015; Ajibade *et al*., 2017).Recent studies reported that vitamin C has the potentiality of anti-hepatotoxicity that is capable of ameliorating liver functions, speculating that therapeutic mechanism relates to normalizing metabolism and blocking inflammatory stress in the liver. In the present investigation vitamin C was found to be helpful against fluoride toxicity.

Table 1: Effect of Sodium Fluoride and its recovery on Detoxification Enzymes in the selected tissues of Albino Rats

	Control	Vit. C (28 D)	NAF (28 D)	NAF + Vit. C (28 D)	F ratio

LPO (L)	7.762[a]± 0.407	8.034[a] ±0.663 (3.39)	9.609± 0.466 (19.22)	7.649[a] ± 0.626 (-1.47)	16.413*
LPO (K)	7.015[b]± 0.435	7.000[b] ±0.548 (-0.21)	8.974± 0.458 (21.84)	7.211[b] ± 0.425 (2.72)	24.850*
LPO (M)	6.961[c]± 0.722	6.857[c] ±0.639 (-1.508)	8.981± 0.306 (22.49)	6.782[c] ± 0.471 (-2.634)	21.637*
LPO. (T)	9.072[d]± 0.481	9.265[d] ±1.183 (2.084)	12.263± 1.801(26.02)	9.066[d] ± 0.653 (-0.062)	11.117*
XOD (L)	0.496[a]±0.034	0.488[a]± 0.033 (-1.674)	0.715± 0.023 (30.55)	0.484[a]± 0.037 (-2.55)	73.378*
XOD (K)	0.700[b]±0.044	0.709[b]± 0.039 (1.161)	0.912± 0.168 (23.18)	0.702[b]± 0.035 (0.18)	7.969*
XOD (M)	0.804[c]±0.043	0.793[c]± 0.022 (-1.347)	1.074± 0.386 (25.16)	0.807[c]± 0.023 (0.46)	2.942*
XOD (T)	0.468[d]±0.481	0.503[d]± 0.048 (7.075)	0.528± 0.027 (11.48)	0.485[d]± 0.015 (3.66)	4.618*
SOD (L)	6.910[a]±0.477	7.033[a]± 0.033 (0.494)	5.796± 0.626 (-19.21)	7.116[a]± 0.385 (2.898)	9.050*
SOD (K)	7.364[b]±0.451	7.428[b]± 0.399 (0.872)	5.934± 0.609 (-24.09)	7.771[b]± 0.330 (5.248)	18.846*
SOD (M)	7.946[c]±0.345	8.090[c]± 0.433 (1.773)	6.187± 0.551 (-28.42)	8.063[c]± 0.385 (1.449)	27.082*
SOD (T)	6.224[d]±0.482	6.471[d]± 0.371 (3.818)	4.870 0.498 (-27.79)	6.666[d]± 0.489 (6.631)	18.476*
CAT (L)	4.877[a]±0.520	5.315[a]± 0.432 (8.238)	4.287±0.453 (-13.76)	5.316[a]±0.516 (8.24)	6.130*
CAT (K)	4.827[b]± 0.201	4.956[b]± 0.529 (2.588)	4.047±0.277 (-19.29)	5.005[b]±0.295 (3.54)	9.939*
CAT (M)	4.977[c]± 0.240	5.313[c]± 0.348 (6.324)	4.068±0.437 (-22.36)	5.078[c]±0.361 (1.99)	14.334*
CAT (T)	4.922[d]± 0.176	4.885[d]± 0.335 (0.758)	4.169±0.324 (18.06)	4.785[d]±0.213 (-2.87)	10.151*
GPx (L)	4.208[a]±0.223	3.976[a]±0.456 (5.842)	3.348±0.431 (25.686)	3.879[a]±0.136(-8.488)	6.864*
GPx (K)	4.162[b]±0.174	4.152[b]±0.212 (2.565)	3.236±0.240 (29.465)	4.078[b]±0.239(-2.736)	18.016*
GPx (M)	4.189[c]±0.314	4.084[c]±0.221(6.324)	4.068±0.437 (22.363)	5.078[c]±0.361 (1.986)	14.334*
GPx (T)	4.287[d]±0.357	4.328[d]±0.203(0.953)	3.417±0.239 (25.478)	4.052[d]±0.139(-5.787)	17.345*
GR (L)	1.768[a]±0.063	1.795[a]±0.116(1.534)	1.380±0.073(28.133)	1.775[a]±0.054(0.394)	37.386*
GR (K)	1.725[b]±0.133	1.676[b]±0.092(2.966)	1.430±0.048(20.688)	1.681[b]±0.048(-2.616)	14.094*
GR (M)	1.852[c]±0.031	1.876[c]±0.061(1.289)	1.458±0.026(26.975)	1.939[c]±0.052 (4.503)	142.647*
GR (T)	1.634[d]±0.081	1.673[d]±0.061(2.325)	1.310±0.096(24.759)	1.573[d]±0.052(-3.892	28.912*

Values expressed in different units are Mean ± SD of six individual animals. Values in parenthesis indicate percent change over control. Mean values with the same superscript do not significantly differ among themselves through S-N-K test.
*p < 0.01
Tissues: L - Liver; K- Kidney; M- Muscle; T-Testis
Metabolites: LPO -Lipid Peroxidase ; XOD - Xanthine Oxidase ; SOD - Superoxide Dismutase;
CAT- Catalase; GPx- Glutathione Peroxidase ; GR- Glutathione Reductase.

Units: LPO- µmoles of MDA/mg protein/min.
XOD- µmoles of formazon formed/mg protein/h.
SOD- superoxide anion reduced/mg protein/min
CAT-µmoles of H_2O_2 degraded/mg protein/min
GPx-µmoles of NADPH oxidized/mg protein/min
GR- µmoles of NADPH oxidized/mg protein/min

CONCLUSION

The present study demonstrate that long term fluoride exposure reduced antioxidant capabilities in the. In addition, significant biochemical and histopathological alterations were induced in the liver, kidneys, muscle and testis in *Albino rats* following chronic fluoride exposure. So, it can be inferred that toxicity of sodium fluoride generates oxidative stress which further drives dysfunctioning of hepato-renal and cardiac functions. Vitamin C protects cells from fluoride dependent oxidative damage by preventing lipid peroxidation, suppressing the formation of reactive oxygen species, and increasing the activity of antioxidant defense system parameters. The use of natural antioxidants is inevitable in order to minimize the harmful effects of NaF. Vitamin C, one of these antioxidants, has been shown to suppress oxidative stress in cells by protecting cells from free radicals. In conclusion, the findings of the present study clearly indicate a significant protective action of vitamin C against fluoride toxicity.Therefore, continual exposure of fluoride in population residing in endemic fluorosis areas in different parts of the world should be of great concern. Public awareness should be created on this aspect and policies should be formulated by the concerned authorities to decline fluoride exposure among residents of endemic fluorosis localities throughout the world.

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References

1. Aebi H., (1984). Catalase. *Methods Enzymol. 105:* 125-126.
2. Apaydin FG., Bas H., Kalender Y.),2017 .(Bendiocarb induced histopathological and biochemical alterations in rat liver and preventive role of vitamins C and E. *Environ Toxicol Pharmacol*;49:148–55.

3. Ajibade TO.,Oyagbemi AA., Omobowale TO.,Asenuga ER.,)2017 .(Adigun KO. Quercetin and vitaminC mitigate cobalt chloride-induced hypertension through reduction in oxidative stress and nuclear factor kappa beta (NF-Kb) expression in experimental rat model. *Biol Trace Elem Res* ;175 (2):347–59.
4. Badgujar PC., Pawar NN., Chandratre GA., Telang AG., Sharma AK.)2015 .(Fipronil induced oxidative stress in kidney and brain of mice: protective effect of vitamin E and

a. vitamin C. *Pestic Biochem Physiol*;118:10–8.

5. Beacham C. and Fridovich, I. (1971). *J. Biol. Chem*. *245:* 4641-464
6. Carlberg, I. and Mannervik, B. (1985). Glutathione Reduction.In Methods in Enzymology.ed., N.O. *Academic press in Orlando. 113*: 484-4
7. Choubisa SL, Choubisa D. (2016) Status of industrial fluoride pollution and its diverse adverse health effects in man and domestic animals in India. *Environ Sci Pollut Res*;23(8):7244– 54.
8. Devendra K.Raia., PrashantK.Raib., SyedI.Rizvia., GeetaWatalb., BechanSharmaa (2009). Carbofuran-induced toxicity in rats: Protective role of vitaminC. *Experimental and Toxicologic Pathology*61531–535
9. Djeffal A., Messarah M., Boumendjel A., Kadeche L., Feki AE.) 2015.(Protective effects of vitamin C and selenium supplementation on methomyl-induced tissue oxidative stress in a adult rats. *Toxicol Ind Health* ;31(1):31–43.
10. Flohe L. and Gunzler, W.A. (1984). Glutathione peroxidase. *Methods Enzymol*. *105:* 115-121.
11. Fridovich I.,(1995).Superoxide adical and superoxide dismutases. *Annu.Rev.Biochem.* 64-97.
12. Halliwell B.,(1999). Antioxidant defense mechanism from the beginning to the end (of the beginning). *Free Radic Res.31:* 261-272.
13. Ohkawa H., Ohishi N. and Yagi K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem. 95:* 351-358.
14. Pham-Huy L.A., He H. and Pham-Huy C. (2008). Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science. 4:* 89-96.
15. Ranpariya V.L,. Parmar S.K., Sheth N.R., and Chandrashekhar V.M. (2011). Neuroprotective activity of Matricaria recutita against fluoride-induced stress in rats Neuroprotective activity of Matricariarecutita against fluoride-induced stress in rats, *Pharmaceutical Biology*, 49:7, 696-701,
16. Sabine Guth., Stephanie Hüser., Angelika Roth., Gisela Degen (2020) Toxicity of fuoride: critical evaluation of evidence for human developmental neurotoxicity in epidemiological studies, animal experiments and in vitro analyses. *Archives of Toxicology 94*:1375–1415
17. Saikat sen, Raja chakraborty, Sridhar C., Reddy, Y.S.R. Biplab, D.e. (2010). Free radicals, Antioxidants, diseases and phytomedicines current status and future prospect. *International journal of pharmaceutical sciences review and research*. *(3):* 91-100.
18. Srikanthan T W. and Krishna Murthy C. (1955). Tetrazolium test for dehydrogenases. *J. Sci. Ind. Res. 14:* 206
19. Shinde V., Dhalwal K., Paradkar A.R., Mahadik K.R., Kadam, S.S., (2006). Evaluation of *in vitro* antioxidant activity of human placental extract, Pharmacologyonline. *3:*172- 179Sies, H. (1991). Oxidative stress: introduction. In: Sies, H. (Ed.), Oxidative Stress: Oxidants and Antioxidants. *Academic Press, San Diego.* 21–48
20. Valacchi G,.,Sticozzi C,.,Belmonte G.,Cervellati F.,Demaude J., Chen N, Krol Y., Oresajo C.)2015.(Vitamin C compound mixtures prevent ozone-induced oxidative damage in human keratinocytes as initial assessment of pollution protection. *PLoS One*;10(8.(
21. Wang, W. & Li, Y. (2002). Environmental epidemiology of fluorine and its effects on health. Soil, water and Environmental Science, 11(4), 383-387