EVALUATION OF OXIDATIVE STRESS PARAMETERS IN MALE REPRODUCTIVE ORGANS OF SWISS ALBINO MICE EXPOSED TO SODIUM FLUORIDE

Trishna Patel[1], Lata Shahani[2], Nisha Rathore[3]
The IIS University

Abstract

The first fluoride compound which was used in the fluoridation of drinking water is Sodium Fluoride . It is found in the natural environment and is present in soil, food, water, air, vegetation and body. Fluoride dusts and gases are emitted into the atmosphere by many types of industrial operations which include the grinding, drying and electrochemical reduction of metals with fluoride fluxes or melts (WHO, 1984). Fluoride is found in both types of water: surface water and ground water. It produces toxic effects in many soft tissues and organs. The aim of the present study is to evaluate oxidative stress induced by sodium fluoride in testis and epididymis. Male Swiss Albino mice are used as an experimental model. They are divided into two groups. Group I (control) was orally given vehicle only (distilled water). Group II (Treated) was administered with a dose of Sodium Fluoride (10mg/kg b.w.) for 15 days. Animals were autopsied by cervical dislocation on 16th day. Testis and Epididymis were taken out, weighed and examined for biochemical parameters. Biochemical parameters like Protein, Lipid peroxidation, Glutathione reductase, Sodium dismutase and Catalase were performed. The sodium fluoride treatment for 15 days causes significant decrease in values of Protein and non significant decrease in values of GSH, SOD and CAT, non significant increase in values of LPO levels in testis and epididymis as compared to control group.

Key words: Fluoride, Reproductive toxicity, Testis, Epididymis

Introduction:

The monovalent anion derived from the element fluorine is fluoride and it is present in many salts such as sodium fluoride and aluminium fluoride. Dissolution of fluoride salts in water led to release of these ions (Aigueperse *et al*., 2005). For prevention of dental caries fluoride salts or other compounds that release fluoride ion may be added to drinking water (Bassin *et al*., 2006). The distribution of Fluoride is wide in the earth's crust and is released into air, soil and water by industrial, agricultural and other activities in number of geographical locations. Water is the main source of fluoride intake by human population (WHO, 1984; Mason, 1974), air borne fluoride in the vicinity of industries (USEPA, 1980; Smith and Hodge, 1979), foods and beverages besides the rock and soil. The fluoride ion is exposed to public by drinking fluoridated water, dental products like toothpastes, foods and beverages, by inhalation of fluoride compounds from the air, individual dietary and oral hygiene habits and practices (Aigueperse *et al*., 2005; Buzalaf *et al* 2004). Sodium Fluoride has been reported to be toxic to living cells because it can generate reactive free radicals and cause alteration in biochemical indices leading to oxidative stress in a variety of animal species (Chawala *et al* 2008; Burgstahler 2009). The aim of present work is to evaluate the oxidative stress parameters in male reproductive organs of Swiss albino mice exposed to sodium fluoride.

Materials and Methods:

Metal: Sodium fluoride was obtained from Merck Company, Jaipur, India

Experimental Animal:

Swiss Albino Mice 4-6 weeks old were procured from the animal house approved by the Ethical

Committee CPCSEA Registration number No: 1689/PO/9/13/CPCSEA. The animals were maintained on standard diet and water *ad libitum* at the Animal House Laboratory and housed in a temperature- controlled 25± 3.2ºC on light/dark cycle of 12/12 hours and artificially illuminated room, free from any source of chemical contamination. The weight of mice was between 25gm-30gm.

Experimental Design:

Adult male mice (*Musmusculus*) of Swiss strain were divided into two groups with five animals in each group. Group I was administered with distilled water only for 15 days. Second group, was treated with Sodium Fluoride orally (10mg/kg b.w.) for 15 days. On the 16th day male mice were sacrificed, then testis and epididymis were collected for evaluation of body weight, organ weight and biochemical estimation. The dose used was based on the LD50 of fluoride in male mice which is 54.4 mg F/kg bw/day (Pillai *et al* 1987;,1988).

Statistical Analysis:

The data obtained by present set of experiments was subjected to statistical analysis. The statistical calculations are based on biological statistics. The values are expressed as mean ±standard error (SE).The data were analysed statistically using ANOVA. The possibility for obtaining "p" value for a given Degree of Freedom (df) was determined by comparing the "p" values. The "P" values were signified according to the following convention: P<0.05(*) Significant, P<0.01 (**) Highly Significant<0.001 (***) Very Highly Significant.

Evaluation of body weight and organ weight:

The animals were killed by cervical dislocation. The testis and epididymis were taken out and weighed. Organ Weights were reported as (organ weight/body weight x 100) absolute and relative weights. In the case of testis and epididymis, weights were presented as mean of both left and right testis and epididymis of mice.

Results:

1. Effects on body weight and relative sex organs weight.

The effects of daily exposure to NaF(10 mg/kgb.w., orally) for 15 days on body weight and relative organs weight of treated mice are presented in **Table (1)**. It revealed that oral administration of NaF resulted in non-significant difference in body weight and highly significant decrease in testis and epididymis weights compared to control (group 1).

Table 1: Values are presented as mean ± SE. (n=5 mice/ group).Comparison was assessed for significance using one way ANOVA for normal distributed data. P<0.05(*) Significant, P<0.01 (**) Highly Significant, P<0.001 (***) Very Highly Significant.

2. Oxidative Stress Parameters:

The effects of daily exposure to NaF(10 mg/kg b.w., orally) for 15 days on Oxidative stress parameters in testis and epididymis of treated mice are presented in **Table (2) and Table (3)** respectively.

In **Table 2** The sodium fluoride treatment for 15 days showed significant decrease in values of protein and non significant decrease in values of GSH, SOD and CAT in testis, Non-significant increase in LPO level in testis is observed as compared to control values.

In **Table 3** The sodium fluoride treatment for 15 days showed significant decrease in values of protein and non significant decrease in values of GSH, SOD and CAT, non-significant increase in LPO level in epididymis is observed as compared to control values

Table (1): Effect of sodium fluoride (10 mg/kgb.w.) on body weight and relative sex organs weight of exposed mice.

Groups	Body weight	Testis	Epididymis
Control	26.8	.112± .009	.012± .0005
Treated group	27.4	.094±.001**	.009± .0004**

Table (2): Effect of sodium fluoride (10 mg/kg b.w.) in Testis of exposed mice.

Group	Treatment	Protein	LPO	GSH	SOD	CAT
1	Control	20.08 ± 2.19	5.33± 0.87	21.15± .81	10.35 ±2.32	1.27 ± 0.20
2	Sodium Fluoride (10mg/kg b.w.)	9.62 ± 1.09**	7.17 ± 1.13	17.21± .65	7.56± .44	0.80 ± 0.36

Table (3): Effect of sodium fluoride (10 mg/kg b.w.) in Epididymis of exposed mice.

Group	Treatment	Protein	LPO	GSH	SOD	CAT
1	Control	2.50 ± 0.34	5.13 ± 2.26	51.15± .81	88.56 ± 9.09	8.26 ± 3.27
2	Sodium Fluoride (10mg/kg b.w.)	0.87±0.31**	11.78 ± 3.39	47.21± .65	63.02± 5.35	4.97 ± 1.17

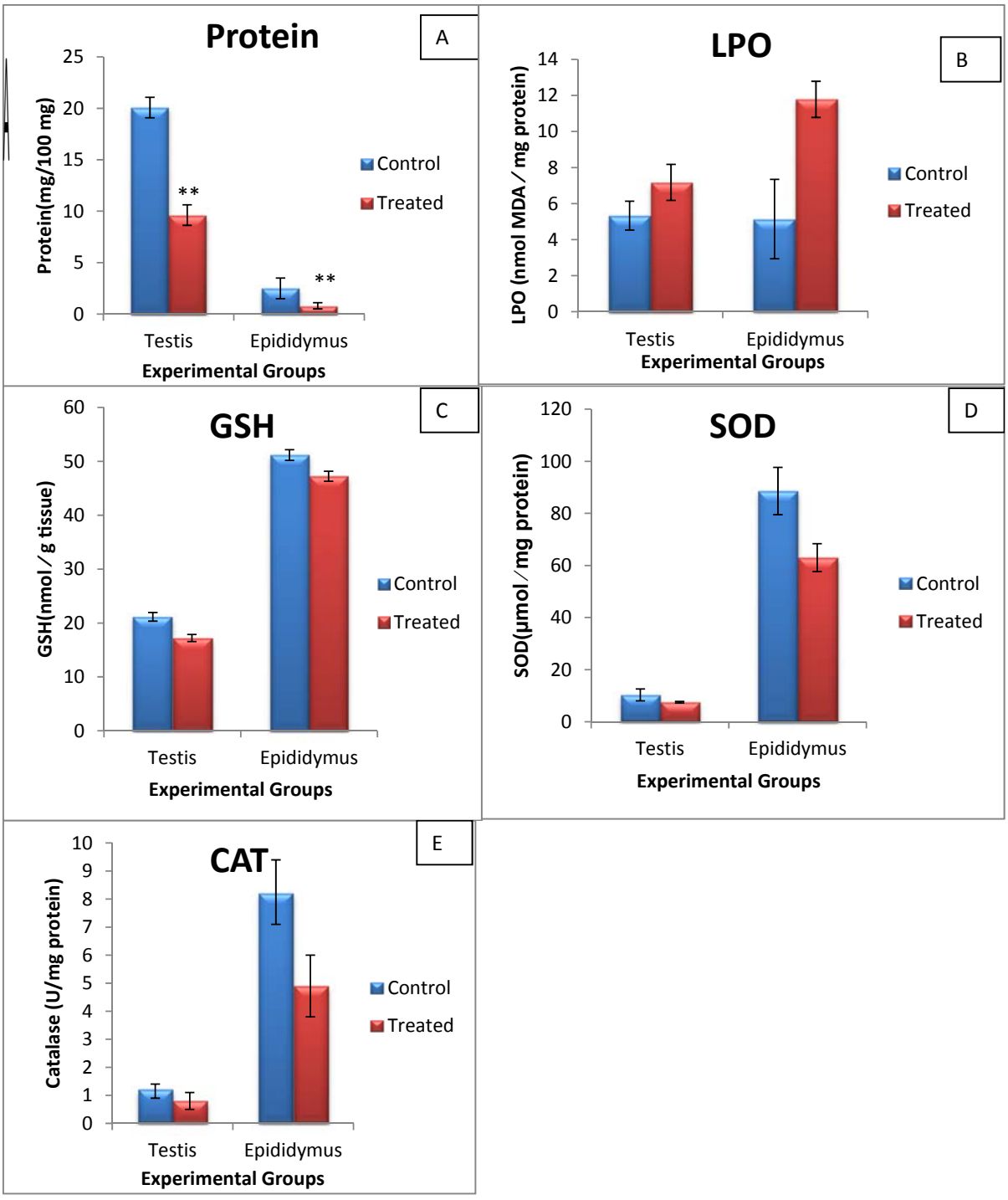

Figure 1: Bar graph showing oxidative stresses parameters level of both the control and treated (Sodium Fluoride for 15 days) group. (a) Protein level (b) LPO level (c) GSH level (d) SOD level (e) CAT level.

Discussion:

The aim of the present study was to evaluate the oxidative stress parameters in male reproductive organs of Swiss albino mice exposed to sodium fluoride at a dose of 10mg/kg body wt**.** The results revealed that a dose of NaF (10 mg/kg body weight) for a period of 15 days produced significant changes in the weight of testis and epididymis. Similar result was obtained by (Rafiq 2000) who also reported significant decline in the weight of testis and epididymis after the treatment of NaF for 30 days at a dose of 10 mg/kg bw.

In the present study protein levels showed significant decrease in the testis and epididymis after 15 days treatment with NaF (10 mg/kg body weight). Similar results were obtained by Chinoy *et al* 1989; Chinoy 1991, 1995, who observed significant decrease in protein levels in cauda epididymis after 30 days of treatment with NaF. This decrease might be due to impairment of protein metabolism (Chinoy *et al* 1989). Several previous studies have also reported that in various soft tissues and serum of rats, mice, rabbits and guinea pigs treated with sodium fluoride at different doses and durations, there is decline in protein levels (Chinoy 1991 a,b; Chinoy and Sequeira, 1989a; Chinoy and Sharma, 1998; Chinoy and Mehta, 1999a,b; Chinoy *et al*., 1991a; 1993a,b; 1994b,c; 1995; 1997a,b; Patel *et al*., 1994).

In the present study no significant change was observed in levels of GSH, SOD, CAT and LPO in testis and epididymis of control and treated mice. Previous studies have reported that NaF induces oxidative stress in reproductive organs by decreasing the levels of various antioxidant enzymes and producing ROS after 30 days of treatment. (Hamza *et al* 2015 Chinoy *et al* 1989; Shivarajashankara *et al* 2002; Chinoy *et al* 2004; Chinoy NJ 2005). The results shown in our study are of 15 days treatment, there is no significant change in the values observed in any of the antioxidant enzymes.

Conclusion: The dose of Sodium Fluoride (10mg/kg b.w.) did not produce significant changes in the oxidative stress parameters after 15 days of treatment in the male reproductive organs of swiss albino mice indicating that the antioxidant defense mechanism is not hampered after short duration of NaF exposure.

References :

1. Aigueperse J., Mollard P., Devilliers D., Chemla M., Faron R., Romano R., Cuer J. (2005) : Fluorine Compounds, Inorganic, *Ullmann's Encyclopedia of Industrial Chemistry*, Weinheim: Wiley-VCH, p 307.
2. Bassin E., Wypij D., Davis R., Mittleman M. (2006) : Age-specific fluoride exposure in drinking water and osteosarcoma (United States).*Cancer Causes Control*, **7** ,421–428.
3. Burgstahler A. (2009) : Recent research on fluoride and oxidative stress [editorial]. *Fluoride*. **42(2)**, 73-74.
4. Buzalaf M., de Almeida B., Cardoso V., Olympio K., FurlaniTde A. (2004) : Total and acid-soluble fluoride content of infant cereals, beverages and biscuits from Brazil. *Food Addit Contam*.**21**, 210-215.
5. Chawla S., Yadav R., Shah D., RaoM. (2008) : Protective action of melatonin against fluoride induced hepatotoxicity in adult female mice. *Fluoride*, **41(1),** 44-51.
6. Chinoy N and Mehta D.(1999b) : Effects of protein supplementation and deficiency on fluoride induced toxicity in reproductive organs of male mice. *Fluoride*, **32(4)**, 204-214.
7. Chinoy N. (1991a) : Effects of fluoride on physiology of some animals and human beings. *Ind. J. Environ. Toxicol*, **1(1)**, 17-32.
8. Chinoy N. (1991b) : Effects of fluoride on some organs of rats and their reversal. *Proc. Zool. Soc., Calcutta*. **44(1),** 11-15.s
9. Chinoy N. (1995) : Role of fluoride in animal systems: A review. In: *Toxicity and Monitoring of Xenobiotics.* pp 13-30.
10. Chinoy N. and Mehta D. (1999a) : Beneficial effects of the amino acids glycine and glutamine on testis of mice treated with sodium fluoride. *Fluoride*, **33(3),** 162-170.
11. Chinoy N. and Sequeria E. (1989a) : Fluoride induced biochemical changes in reproductive organs of male mice. *Fluoride*, **22(2),** 78-85.
12. Chinoy N. and Shah S. (2004) : Biochemical effects of sodium fluoride and arsenic trioxide toxicity and their reversal in the brain of mice. *Fluoride* **37(2)**, 80-87.
13. Chinoy N. and Sharma A. (1998) : Amelioration of fluoride toxicity by vitamin E and D in reproductive functions of male mice. *Fluoride*, **31(4),** 203-216.

14. Chinoy N., Mathews M., Barot V. (1993a) : Toxic effects of sodium fluoride ingestion in mice. *Ind. J. Environ. Toxicol*., **3** ,30-34.
15. Chinoy N., Mehta D, Jhala DD. (2005) : Beneficial effects of a protein rich diet on fluoride induced free radical toxicity in the liver of male mice. *Fluoride*, **38(4)**, 276-83.
16. Chinoy N., Narayana M., Dalal V., Rawat M. and Patel, D. (1995). Amelioration of fluoride toxicity in some accessory reproductive glands and spermatozoa of rat. *Fluoride*., **28(2)**, 75-86.
17. Chinoy N., Patel B., Patel D., Sharma A. (1997b) : Fluoride toxicity in the testis and cauda epididymis of guinea pig and reversal by ascorbate. *Med. Science Res.* **25(2)**, 97-100.
18. Chinoy N., Patel D. (1998) : Influence of fluoride on biological free radicals in ovary of mice and its reversal. *Environ Sci* **6(3)**, 171-84.
19. Chinoy N., Patel S., Bhatt N., Mathews M., (1994c) : Effects of fluoride on some soft tissue functions of fresh water fish *Channa punctatus. Proc. Acad. Environ. Biol*., **3(2)**, 191-196.
20. Chinoy N., Sequeira E. (1989) : Fluoride induced biochemical changes in reproductive organs of male mice. *Fluoride* **2 (2)**, 78-85.
21. Chinoy N., Sequeira E., Narayana M. (1991a) : Effects of vitamin C and calcium on the reversibility of fluoride induced alterations in spermatozoa of rabbit. *Fluoride*, **24(1)**, 29-39.
22. Chinoy N., Sharma M., Mathews M. (1993b) : Beneficial effects of ascrobic acid and calcium on reversal of fluoride toxicity in male rats. *Fluoride*, **26(1**), 45-56.
23. Chinoy N., Shukla S., Walimbe A, Bhattacharya S.(1997a) : Fluoride toxicity on rat testis and cauda epididymal tissue components and its reversal. *Fluoride*., **30(1)**, 41-50.
24. Chinoy N., Walimbe A, Vyas H., Mangla P. (1994b) : Transient and reversible fluoride toxicity in some soft tissues of female mice. *Fluoride*, **27(4)**, 205-214.
25. Hamza R., El-Shenawy N., Ismail H. (2015) : Protective effects of blackberry and quercetin on sodium fluoride-induced oxidative stress and histological changes in the hepatic, renal, testis and brain tissue of male rat. *J Basic Clin Physiol Pharmacol.* **26(3)**:237-51.
26. Mason B. (1974) : Geochemical distribution of elements. In: Encyclopedia *Britannica,Macropaedia* 6, 15th ed., Chicago, Helen Hemingway Benton
27. Patel D., Milind V., Narayana M., Chinoy N.J. (1994) : Effects of sodium fluoride on physiology of female mice and its reversal. *Proc. of Academy of Environ. Biol.* **3(2)**, 197-205.
28. Pillai K., Mathai A., Deshmukh P. (1987), Acute Toxicity of fluoride to mice. *Fluoride*, **20(2)**, 68-70.
29. Pillai K., Mathai A., Deshmukh P. (1988), Effect of subacute dosage of fluoride on male mice. *Toxicology letters.* **44** : 21-29.
30. Rafiq M. (2000) : Studies on fluoride and aluminium toxicity in male mammals and its reversal, Ph.D. Thesis, University School of Sciences Gujarat University, Ahmedabad
31. Shivarajashankara Y., Shivashankara A., Bhat P., Rao S. (2002) : Brain lipid peroxidation and antioxidant systems of young rats in chronic fluoride intoxication. *Fluoride* **35(3)**, 197-203.
32. Smith F. and Hodge H. (1979) : Air-borne fluorides and man. Part I. Crit. *Rev.Environ. Control* , **8**,293-371.
33. US EPA. (1980). Reviews of the environmental effects of pollutants: IX Fluoride, Cincinnati, *US Environmental Protection Agency*, pp. 441.(EPA-600/l-78-050).
34. WHO. (1984). IPCS International Programme on Chemical Safety. Environmental Health Criteria. **36**. *Fluorine and Fluorides*. Published by United Nations Environment Programme, the International Labour Organization, and the World Health Organization, Geneva, 1-136.