EFFECTS OF FLUORIDE ACCUMULATION ON SOME ENZYMES OF BRAIN AND GASTROCNEMIUS MUSCLE OF MICE

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SUMMARY: This study reports accumulation of fluoride and altered activities of some enzymes involved in free-radical metabolism and membrane function in whole brain and gastrocnemius muscle of female mice treated with NaF (20mg/kg/body weight) for 14 days. The body weight and somatic index were decreased, whereas fluoride levels were significantly increased ($p<0.01$) in both brain and gastrocnemius muscle. The enzymes SOD, GST, and catalase decreased significantly ($p<0.01$) in contrast to XOD activity, which moderately increased. SDH, LDH, AlAT, AAT, and CPK activities and membrane-bound enzymes, *viz* Na^+-K^+, Mg^{++} and Ca^{++}ATPase and AChE were decreased significantly ($p<0.01$) in both brain and gastrocnemius muscle. The effect of fluoride on enzymes of muscle was comparatively larger, which corroborates the greater accumulation of fluoride in muscle than brain. This study therefore shows that both brain and muscle are affected by fluoride with inhibition of some enzymes associated with free-radical metabolism, energy production and transfer, membrane transport, and synaptic transmission, but with an enhanced activity of XOD.

Keywords: Brain fluoride, Enzyme activities, Female mice, Fluoride accumulation, Fluoride toxicity, Gastrocnemius muscle.

INTRODUCTION

Fluorosis caused by excess intake of fluoride is a slow, progressive degenerative disorder, known to affect predominantly the skeletal systems, teeth and also the structure and function of skeletal muscle,[1-3] brain,[4] and spinal cord.[5] Recent studies have shown accumulation of fluoride in the hippocampus of the brain[6] causing degeneration of neurons and decreased aerobic metabolism[7] and altered free-radical metabolism in the liver, kidney, and heart.[8-11] However, the effect of fluoride on neuromuscular tissue is far from clear.

The present study reports the effect of fluoride accumulation on alteration in the organo-somatic index, activities of some enzymes in free-radical metabolism, *viz.* xanthine oxidase (XOD), superoxide dismutase (SOD), catalase and glutathione transferase (GST), membrane bound enzymes like ATPases (Na^+-K^+ATPase, Mg^{++}ATPase and Ca^{++}ATPase), acetylcholinesterase (AChE), succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), alanine aminotransferase (AlAT), aspartate aminotransferase (AAT), and creatine phosphokinase (CPK) in brain and gastrocnemius muscle of female mice treated with sodium fluoride at a dose of 20 mg/kg /body weight for 14 days.

MATERIALS AND METHODS

Twelve healthy, adult female albino mice, *Mus musculus* of Swiss strain, each weighing about 30 ± 2 g, were obtained from the National infrastructural facility for laboratory animals, National Institute of Nutrition, Hyderabad, In-

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dia. The mice were housed in polypropylene cages with stainless steel grill tops and were fed a standard pellet diet (Hindustan Lever Ltd., Bangalore) and given distilled water *ad libitum*. The animals were allowed to acclimatize to the laboratory conditions for four days before experiments began. The mice were randomly divided into two groups of six each, the first group served as controls and were given mammalian physiological saline. The second group animals were injected i.p. with aqueous NaF (20 mg/kg/body weight/day) selected on the basis of the LD_{50} value of fluoride, which is 51.6 mg/kg body weight/day in female mice[12] and maintained for 14 days. The NaF solution (30 mL) was prepared fresh each day in double distilled water. At the end of the 14 day treatment, the animals were sacrificed by cervical dislocation, and the whole brain and gastrocnemius muscle were dissected out, blotted free of blood, transferred to trays maintained at ice-cold conditions and used for analysis. The analysis of the whole brain produced an enzymatic profile of that organ which could be used for further studies on region specific profiles.

Body weight and organo-somatic index: The body weight of each animal was noted before treatment and also on day 15. The weight of gastrocnemius muscle and whole brain of respective groups of animals was recorded. From these values the organo-somatic index (OSI) was calculated by the following formula:

$$\text{Organo-somatic index} = \frac{\text{Weight (g) of the organ}}{\text{Day 15 total body weight (g)}} \times 100$$

Estimation of Fluoride: Fluoride levels in brain and gastrocnemius muscle of control and treated mice were determined by the method of Birkeland[13] with required modifications and are expressed as µg of fluoride/g dry tissue.

The whole brain and gastrocnemius muscle were dissected out, pooled, homogenized, and dried for 24 hours at 105°C. About 200 mg of the dry sample was dissolved in an acid mixture (equal parts of 11.6 M perchloric acid and 14.3 M nitric acid) and neutralized with citrate buffer to a pH 5.5 with an alkaline mixture of 7.8 M sodium hydroxide and 1.0 M trisodium citrate. The process was carried out in a closed compartment. The sample thus obtained was used after appropriate dilutions for recording the fluoride content on a fluorimeter (Orion R 94-09). The values were calculated from a standard curve.

Assay of enzymes

Xanthine oxidase (XOD) activity was assayed by the method of Govindappa and Swami.[14] The homogenate in sucrose solution was centrifuged at 3000 rpm for 15 min, and the activity was expressed as µmoles of formazan formed/mg protein/hr.

Catalase activity in muscle and brain was measured by the method of Chance and Herbert.[15] The homogenates of muscle and brain were prepared in 0.05M phosphate buffer in a glass homogenizer tube equipped with a teflon pestle. The tissue homogenate was centrifuged for 15 min at 10,000 g and the

reaction was started by addition of 30% H_2O_2 solution. The activity of catalase was calculated from the change in absorbance at 240 nm. One unit of catalase activity was calculated by using $k = (2.30/t)(\log A_1/A_2)$ where k is the rate of constant, 't' is time in seconds, A_1 is absorbance at time zero and A_2 is absorbance at 2 min. Specific activity was calculated as Units/mg protein.

Superoxide dismutase (SOD) activity was assayed according to the method of Marklund and Marklund.[16] The homogenates of muscle and whole brain were prepared in Tris (ethylenediamine tetraacetic acid) buffer and centrifuged for 40 min at 105,000 g at 4°C, and the supernatant was used for the enzyme assay. One unit of SOD activity is the amount of the enzyme that inhibits the rate of auto oxidation of pyrogallol by 50% and was expressed as Units/mg protein/min.

Glutathione transferase (GST) activity was estimated by using the substrate, 1-chloro-2,4-dinitrobenzene (CDNB) by the method of Habig *et al*[17] The homogenates of brain and muscle were prepared in Tris HCl buffer and centrifuged at 105,000 g at 4°C for 30 min. The specific activity of GST was expressed as nanomoles of GS-CDNB formed/mg protein/min. One unit of enzyme is defined as the amount of enzyme that catalyzes the formation of 1 nanomole of product/min under assay conditions.

Succinate dehydrogenase (SDH) enzyme activity was determined by the method of Nachlas *et al*[18] and Lactate dehydrogenase (LDH) activity was determined with necessary modifications by the method of Nachlas *et al*[18] The homogenates of muscle and brain were prepared in ice-cold 0.25M sucrose solution, and the homogenate was centrifuged for 20 min at 2500 rpm. The supernatant was used as test sample for enzyme assay. The enzyme activities were calculated from a standard graph and expressed as μmoles of formazan/mg protein/hr.

The activities of alanine amino transferase (AlAT) and aspartate amino transferase (AAT) were assayed by the method of Reitman and Frankel.[19] The homogenates of muscle and brain were prepared in 0.25 M ice-cold sucrose solution and centrifuged for 15 min at 3000 rpm, and the supernatant was used for the enzyme assay. The enzyme activity was expressed as Units/mg protein/min.

The creatine phosphokinase (CPK) enzyme activity was estimated by the method of Gerhardt *et al*[20] The muscle and brain homogenates were prepared in 0.25 M ice-cold sucrose solution and centrifuged in a refrigerated centrifuge at 3000 rpm for 15 min. The supernatant resulting was used for enzyme assay and the activity was expressed as Units/mg protein/min.

The Na^+-K^+ and Mg^{++} ATPase activities were assayed according to Kaplay,[21] and the inorganic phosphate was estimated by the method of Taussky and Shorr.[22] The homogenates of muscle and brain were prepared in 0.25 M ice-cold sucrose solution and centrifuged in a refrigerated centrifuge at 20,000 rpm for 30 min and the supernatant was used for the assay. The difference in the

activity of the enzyme in the absence and presence of ouabain was taken as Na^{+}-K^{+}ATPase and Mg^{++}ATPase activity, respectively. The enzyme activity was expressed as nanomoles of Pi liberated/mg protein/min.

Ca^{++}ATPase activity was assayed by the method of Samaha and Yunis.[23] The homogenates of brain and muscle were prepared in a histidine buffer (pH 7) containing 0.3 M sucrose and 0.01 M histidine, centrifuged at 0°C at 20,000 rpm for 30 min, and the resultant supernatant was used for enzyme assay. The amount of inorganic phosphate liberated was measured by the method of Taussky and Shorr.[22] The enzyme activity was expressed as nanomoles of Pi liberated/mg protein/min.

Acetylcholinesterase activity was estimated by the method of Ellman *et al*[24] The homogenates of muscle and brain were prepared in a buffer containing 1 mM EDTA, 200 mM NaCl and 0.4% Triton X 100 in 0.1 M potassium phosphate buffer (pH 8) and centrifuged at 4°C at 20,000 rpm for 30 min and the supernatant was used for enzyme assay. The activity was determined by using the molar extinction coefficient for the 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) as 1.36×10^4 per mole. AChE activity was expressed as nanomoles of acetylcholine hydrolyzed/mg protein/min. The protein contents in homogenates of all enzymes were determined by the method of Lowry *et al.*[25]

Statistical analysis was carried out using Student's 't' test to compare the differences between control and experimental groups. Data were presented as means ± S.E. of six animals per group.

RESULTS AND DISCUSSION

As shown in Table 1, the body weight of mice treated with sodium fluoride was significantly ($p<0.01$) decreased (14.7%) compared to that of the controls, which is in agreement with earlier findings in rats and mice treated with different concentrations of fluoride.[26] The OSI (organo-somatic index) in fluoride-treated mice also decreased significantly ($p<0.01$) in gastrocnemius muscle (15.32%) as well as in brain (11.28%) compared to controls and was relatively greater in muscle than in brain. This decrease in OSI can be attributed to weight loss, degeneration of structure of organs, and decreased protein levels.

The fluoride content of brain and muscle was significantly ($p<0.01$) increased in fluoride-treated mice. The increase was 8.11% in brain and 13.11% in muscle compared to controls which reveals that muscle was more affected than brain, probably due to the protective role of the blood brain barrier. This study establishes that brain and muscle retain the ingested fluoride, which may in turn interfere with their physiological functions.

It is known that fluoride enhances lipid peroxidation and inhibits the antioxidative enzymes in liver, kidney, and heart of fluoridated mice.[9,11,27] Dierickx and Beer[27] reported that fluoroacetamide inhibits GST activity in rat liver in a dose-dependent manner. Patel and Chinoy[9] concluded that fluoride impaired the functioning of the SOD, GST, and catalase enzymes in ovary of mice. Sun *et al*[11] also reported a decrease in SOD activity in the liver, kidney, and heart of

fluoridated mice. The present results show (Table 2) that the SOD, GST, and catalase activities were decreased in both brain and gastrocnemius muscle in accordance with earlier reports in liver, kidney, and heart,[9,11,27] whereas XOD activity was moderately increased. Thus, the present study further shows that fluoride increases free radical production and at the same time inhibits the antioxidative enzymes SOD, GST, and catalase, which probably make the tissue more susceptible to biochemical injury.

Table 1. Effect of NaF on body weight, organo-somatic index and fluoride levels in brain and gastrocnemius muscle of female mice

Tissues	Control	Experimental	% Change
Body weight (g)	29.66 ± 0.614	25.3 ± 0.321*	-14.70
Organo-somatic index[a]			
Brain	1.356 ± 0.0006	1.203 ± 0.0008*	-11.28
Gastrocnemius muscle	1.005 ± 0.037	0.851 ± 0.04*	-15.32
Fluoride content[b]			
Brain	0.271 ± 0.003	0.293 ± 0.003*	+8.11
Gastrocnemius muscle	0.305 ± 0.003	0.345 ± 0.005*	+13.11

All values are mean ± S.E. of 6 animals.
[a][Organ weight (g) /day 15 body weight (g)] X 100. [b]µg of fluoride/g dry tissue

*The significant values as per student's 't' test are between controls and experimental values at p<0.01.

Table 2. Effect of NaF on XOD, SOD, catalase, and GST activities in brain and gastrocnemius muscle of female mice

Enzyme/Tissues	Control	Experimental	% Change
XOD[a]			
Brain	0.315 ± 0.0008	0.324 ± 0.0007*	+2.85
Gastrocnemius muscle	0.416 ± 0.0007	0.433 ± 0.001*	+4.08
SOD[b]			
Brain	3.29 ± 0.013	2.88 ± 0.015*	-12.46
Gastrocnemius muscle	1.97 ± 0.008	1.71 ± 0.004*	-13.19
Catalase[b]			
Brain	11.58 ± 0.079	9.61 ± 0.193*	-17.01
Gastrocnemius muscle	12.41 ± 0.137	10.7 ± 0.126*	-13.78
GST[c]			
Brain	44.33 ± 0.421	38.33 ± 0.333*	-13.53
Gastrocnemius muscle	51.55 ± 0.421	46.66 ± 0.21*	-9.67

Values are mean ± S.E. of 6 animals. *The significant values as per Student's 't' test are between controls and experimental values at p<0.01.

Enzyme activities: [a]µmoles formazan/mg protein/hr. [b]units/mg protein.
[c]nanomoles GS-CDNB formed/mg protein/min.

A significant decrease ($p<0.01$) was observed in LDH activity (Table 4) in brain as well as gastrocnemius muscle of fluoridated mice compared to controls, and the decrease was greater in muscle than in the brain. These results agree with an earlier report[28] and suggest reduction of glycolytic activities, which may lead to an active pentose phosphate pathway for obtaining energy. The SDH activity was significantly decreased in gastrocnemius muscle ($p<0.05$, 37.19%) and in brain ($p<0.01$, 34.06%) of sodium fluoride-treated mice compared to controls. According to the previous reports, the SDH activity was decreased in pectoralis and gastrocnemius muscles of mice,[29] and in liver and kidney of fluoride-treated golden hamsters.[30] As SDH is a mitochondrial enzyme involved in oxidative metabolism, fluoride may cause changes in the structure and function of mitochondria[9] which alter its metabolism and influence the function of muscle and brain.

Table 3. Effect of NaF on LDH, SDH, ALAT, AAT and CPK activities in brain and gastrocnemius muscle of female mice

Enzyme/Tissues	Control	Experimental	% Change
LDH[a]			
Brain	0.247 ± 0.00006	0.195 ± 0.0009*	-21.05
Gastrocnemius muscle	0.367 ± 0.0005	0.25 ± 0.00007*	-31.88
SDH[a]			
Brain	0.408 ± 0.014	0.269 ± 0.022*	-34.06
Gastrocnemius muscle	0.492 ± 0.035	0.309 ± 0.033*	-37.19
ALAT[b]			
Brain	16.41 ± 0.17	14.35 ± 0.03*	-12.55
Gastrocnemius muscle	25.5 ± 0.154	21.46 ± 0.1 4*	-15.84
AAT[b]			
Brain	26.81 ± 0.289	23.06 ± 0.071*	-13.98
Gastrocnemius muscle	41.45 ± 0.348	37.66 ± 0.21*	-9.14
CPK[b]			
Brain	3.87 ± 0.008	2.72 ± 0.004*	-29.71
Gastrocnemius muscle	5.95 ± 0.013	3.86 ± 0.006*	-35.12

All values are mean ± S.E. of 6 animals.
Enzyme activities: [a]µmoles formazan/mg protein/hr. [b]units/mg protein/min.

*The significant values as per Student's 't' test are between controls and experimental values at $p<0.01$.

Transaminases are reported to be altered by fluorosis. Bogin *et al*[28] found that the mice maintained on water containing 100 ppm NaF showed marked decrease in the levels of alanine amino transferase (AlAT) and aspartate amino transferase (AAT) in liver and AlAT in kidney. In the present study these enzymes decreased significantly ($p<0.01$) in muscle (15.84%, 9.14%) and brain (12.55%, 13.98%) after 14 days exposure to NaF. However rats administered 30 ppm NaF in drinking water developed severe chronic fluorosis and showed in-

creased levels of serum AlAT.[31] Similarly, the activities of both these transaminases were significantly increased in serum of fluorotic human beings.[32]

Table 4. Effect of NaF on Na^+ K^+ ATPase, Mg^{++} ATPase and AchE activities in brain and gastrocnemius muscle of female mice

Enzyme/Tissues	Control	Change	% Change
Na^+ K^+ ATPase[a]			
Brain	114.66 ± 0.8	093.50 ± 1.54*	-18.45
Gastrocnemius muscle	155.33 ± 4.15	106.66 ± 4.58*	-31.33
Mg^{++} ATPase[a]			
Brain	299.66 ± 1.837	274.00 ± 0.632*	-8.56
Gastrocnemius muscle	385.33 ± 0.802	331.50 ± 0.562*	-13.96
Ca^{++} ATPase[a]			
Brain	332.16 ± 0.872	300.33 ±0.421*	-9.58
Gastrocnemius muscle	372.83 ± 1.351	334.33 ± 0.614*	-10.32
AChE[b]			
Brain	0.443 ± 0.007	0.335 ± 0.017*	-24.37
Gastrocnemius muscle	0.152 ± 0.001	0.128 ± 0.002*	-15.78

Values are mean ± S.E. of 6 animals.
Enzyme activities: [a]nanomoles of Pi liberated/mg protein/min.
[b]nanomoles of AchE hydrolyzed/mg protein/min.

*The significant values as per Student's 't' test are between controls and experimental values at $p<0.01$.

The significant decrease of CPK in brain as well as gastrocnemius muscle (Table 3) indicates the effect of fluoride as early as 14 days after administration of sodium fluoride. This may lead to a rise in the serum CPK that could be due to degeneration of muscle fibers and neural tissues. The increased CPK levels in serum after fluoride exposure were also reported earlier.[2,33]

Membrane Na^+-K^+ATPase plays an important role in active transport of Na^+ and K^+ ions across the plasma membrane. The enzyme is present in high concentration in brain and muscle. Murphy and Hoover[34] have reported the inhibition of the enzyme with fluoride exposure. Renal Na^+-K^+ ATPase and Ca^{2+}ATPase activities were inhibited by a single oral administration of NaF in rats[35] and in its reproductive tissues.[36,37] The significant inhibition of Na^+-K^+ATPase by NaF might be another causative factor for the change in neuronal metabolism leading to neuromuscular dysfunction in mice during NaF exposure along with inhibition of AChE. The fluoride-induced effects could also be due to internal injury to the cell membrane,[29] which would affect the activity of membrane bound enzymes like Na^+-K^+ATPase by disturbing membrane fluidity and membrane integrity and altering its permeability which may result in disturbance in the concentration of Mg^{2+}. Pang *et al*[38] have shown decreased Mg^{2+}ATPase activity in skeletal muscle of rat after fluoride exposure. The en-

zyme activity in brain as well as muscle significantly decreased (Table 4) due to the probable Mg and F interactions. Fluoride has been reported to decrease the membrane Ca^{2+}ATPase activity,[37,39] in agreement with the findings of the present study, which may be due to either a direct inhibition or a secondary reaction of fluoride administration.

Chinoy *et al*[40] reported enhanced AChE activity in skeletal muscle after NaF of fluoride treated rats. As shown in Table 4, AChE activity was significantly ($p<0.01$) decreased in gastrocnemius muscle and brain of NaF-treated mice in our study as compared to controls. This discrepancy might be due to differences in mode of administration and species used. Anion hydrolysis products of methyl phosphoric difluoride is also reported to cause an inhibition of cholinesterase in both guinea pigs and rats.[41] Thus the decrease in AChE may interfere with the synaptic transmission in brain and muscle.

In conclusion, this study confirms that fluoride accumulates in the brain and muscle of mice causing stress and inhibiting auto-oxidation mechanisms, thereby resulting in oxidative damage of neural and muscular tissues. Fluoride also inhibits enzymes involved in energy production, transfer, membrane-bound ion transport, and neurotransmission. Thus fluoride accumulation leads to cascading effects resulting in altered functions of brain and muscle. This study also shows that muscle is more susceptible to fluoride uptake than brain, which may be due to the blood brain barrier as well as differential sensitivity of various organs to fluoride toxicity.

REFERENCES

1 Jolly SS, Prasad S, Sharma R. Review of 25 years research on fluoride. Journal Association of Physicians of India 1970;18:459-71.
2 Kaul RD, Susheela AK. Evidence of muscle fibre degeneration in rabbits treated with sodium fluoride. Fluoride 1974;7(4):177-81.
3 Shashi A, Singh JP, Thapar SP. Protein degradation in skeletal muscle of rabbit during experimental fluorosis. Fluoride 1992;25(3):155-8.
4 Shashi A, Singh JP, Thapar SP. Effect of long-term administration of fluoride on levels of protein, free aminoacids and RNA in rabbit brain. Fluoride 1994;27(3):155-9.
5 Franke J. The Spinal cord. In: Symposium on skeletal phase of chronic fluorosis. Fluoride 1976;9(1):30-2.
6 Mullenix PJ, Denbesten PK, Schunior A, Kernan WJ. Neurotoxicity of sodium fluoride in rats. Neurotoxicology and Teratology 1995;17(3):169-77.
7 Spittle B. Comments on the finding of toxin-induced blood vessel inclusions and alterations in neuronal and cerebrovascular integrity following the chronic administration of aluminum fluoride and sodium fluoride. Fluoride 1998;31(2):89-90.
8 Sharma A, Chinoy NJ. Role of free radicals in fluoride-induced toxicity in liver and kidney of mice and its reversal. Fluoride 1998;31(3):S26.
9 Patel D, Chinoy NJ. Influence of fluoride on biological free radical reactions in ovary of mice and its reversal. Fluoride 1998;31(3):S27.

10 Chinoy NJ, Patel D. Influence of fluoride on biological free radicals in ovary of mice and its reversal. Environmental Sciences 1998;6(3):171-84.
11 Sun G, Qiu L, Ding G, Qian C, Zheng Q. Effects of β-carotene and SOD on lipid peroxidation induced by fluoride: An experimental study. Fluoride 1998; 31(3):S29.
12 Pillai KS, Mathai AT, Deshmukh PB, Acute toxicity of fluoride to mice. Fluoride 1987; 20:68-70.
13 Birkel JM. Direct Potentiometric determination of fluoride in soft tooth deposits. Caries Research 1970;4:243-55.
14 Govindappa S, Swami KS. Electrophoretic characteristics of sub-cellular components and their relation to enzyme activities in the amphibian muscle fibre. Indian J Exp Biol 1965;3(4):209-12.
15 Chance B, Herbert. The enzyme substrate compounds of bacterial catalase and peroxides. Biochem J 1950;46:402-14.
16 Marklund S, Marklund G. Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974;47: 469-74.
17 Habig WH, Pabst MJ, Jakoby WD. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974;249:7130-9.
18 Nachlas MM, Margulius SP, Seligman AM. A colorimetric method for the estimation of succinic dehydrogenase activity. J Biol Chem 1960;235:499-503.
19 Reitman A, Frankel S. A Colorimetric method for the determination of serum glutamic oxalo acetate and glutamic pyruvic transaminase. Amer J Clin Pathol 1957;28:56-63.
20 Gerhardt W, Waldenstrom J, Gruber W. EDTA effect of creatine kinase (CK) and on the SCE reagent. Scand J Clin Lab Invest 1979;39:737-42.
21 Kaplay SS. Erythrocyte membrane Na^+-K^+ATPase in protein caloric malnutrition. Amer J Clin Nutr 1978;31:579-84.
22 Taussky HH, Shorr E. A micro colorimetric method for the determination of inorganic phosphorus. J Biol Chem 1953;202:675-85.
23 Samaha EJ, Yunis, FJ. Quantitative and histochemical demonstration of calcium activated mitochondrial ATPase in skeletal muscle. Exp Neurol 1973; 41:431-9.
24 Ellman GL, Courtney UD. Anders Junior V, Featherstone RM. A new and rapid colorimetric determination of AChE activity. Biochem Pharmacol 1961; 7:88-95.
25 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. J Biol Chem 1951;193:265-75.
26 Reddy DR. Skeletal fluorosis In: Venken, PJ, Gryyn GW, editors, Handbook of clinical neurology. Vol. 36. New York: Oxford North Holland Publishing Co; 1979. p. 465-504.
27 Dierickx PJ, De Beer JO. Interaction of fluoroacetamide with rat liver glutathione S-transferases: Evidence for detoxification roles by defluorination. Fluoride 1983;16(3): 145-51.
28 Bogin E, Abrams M, Avidar Y, Israeli B. Effect of fluoride on enzymes from serum, liver, kidney, skeletal and heart muscles of mice. Fluoride 1976; 9(1):42-6.

29 Chinoy NJ, Walimbe AS, Vyas HA, Mangla P. Transient and reversible fluoride toxicity in some soft tissues of female mice. Fluoride 1994;27(4):205-14.
30 William D, Sullivan SJ. The *in vitro* and *in vivo* effects of fluoride on succinic dehydrogenase activity. Fluoride 1969;2(3):168-76.
31 Guan Zhi-zhong, Yang Pei-si, Yu Nai-den, Zhuang Zong-jie. An experimental study of blood biochemical diagnostic indices for chronic fluorosis. Fluoride 1989;22(3):112-8.
32 Plaa GL, Hewitt WR. Quantitative evaluation of indices of hepatotoxicity. In: Plaa GL, Hewitt WR, editors, Toxicology of the liver. New York: Raven Press; 1982; p. 103-21.
33 Wang J, Zheng ZA, Zhang LS, Cao DM, Chen, KZ, Dan Lu. An experimental study for early diagnostic features in fluorosis. Fluoride 1993;26(1):61-5.
34 Murphy AJ, Hoover JC. Inhibition of the Na^{+}-K^{+}ATPase by fluoride. Parallels with its inhibition of the sarcoplasmic reticulum Ca^{+2}ATPase: J Biol Chem 1992;267(24): 16995-7000.
35 Suketa Y, Mikami E. Changes in urinary ion excretion and related renal enzymes activities in fluoride treated rats. Toxicology and Applied Pharmacology 1977;40:551- 9.
36 Suketa Y, Suzuki K, Taki T, Itoh Y, Yamaguchi M, Sakurai T, Tanishita Y. Effect of fluoride on the activities of the Na^{+}/glucose cotransporter and Na^{+}-K^{+}ATPase in brush border and basolateral membranes of rat kidney (*in vitro* and *in vivo*). Biol Pharm Bull 1995;18(2):273-8.
37 Chinoy NJ, Narayana MV, Dalal V, Rawat M, Patel D. Amelioration of fluoride toxicity in some accessory reproductive glands and spermatozoa of rat. Fluoride 1995; 28(2);75-86.
38 Pang YX, Guo YQ, Zhu P, Fu KW, Sun YF, Tang RQ. The effects of fluoride, alone and in combination with selenium, on the morphology and histochemistry of skeletal muscle. Fluoride 1996;29(2):59-62.
39 Missiaen L, Wuytack F, De Smedt H, Vrolix M, Casteels R. AlF_4-reversibly inhibits 'p' type cation - transport ATPases, possibly by interacting with the phosphate-binding site of the ATPase, Biochem J 1988;253(3):827- 33.
40 Chinoy NJ, Sharma M, Mathews M. Beneficial effects of ascorbic acid and calcium on reversal of fluoride toxicity in male rats. Fluoride 1993;26:45-56.
41 Dahl AR, Hobbs CH, Marshall TC. The inhibition of rat and guinea pig cholinesterases by anionic hydrolysis products of methylphosphonic difluoride (Difluoro). Fluoride 1987;20(1):47(Abstract).

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