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Protective effects of selenium on fluoride induced alterations in certain enzymes in brain of mice

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Abstract: *This study reports the protective effects of selenium on fluoride induced alterations in the activities of pro-oxidative (xanthine oxidase (XOD), lipid peroxidation (LPO) free radical scavenging, (catalase, superoxide dismutase (SOD), glutathione-s-transferase (GST), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione) and metabolic (glucose-6-phosphate dehydrogenase, alanine amino transferase (ALAT), aspartate aminotransferase (AAT), creatine phosphokinase (CPK), acid phosphatase (AP), alkaline phosphatase (ALP)] enzymes along with fluoride and selenium levels in brain of mice. Animals were divided into control, NaF treated group (20 mg kg^{-1} body wt.$^{-1}$ intraperitonial) and Selenium+NaF treated group (sodium selenite, 5 μg of selenium / 0.2 ml distilled water kg^{-1} body wt.$^{-1}$ day) and were maintained for 14 days on respective treatments. The decreased bodyweight (-11.35%) as well as organosomatic index (-15.1%) of brain in NaF group were recovered in treatment of selenium along with NaF. The increased accumulation of fluoride (32.1%) in brain observed in NaF treated group compared to control was diminished in selenium+NaF treated group. Selenium levels (3.03%) increased in selenium+NaF treated group in compared to decrement in NaF treatment. The SOD (-16.6%), Catalase (-21.5%), GST (-13.72%), GPX (-19.16%), GR (-44.97%) activities and Glutathione (-23%) content in NaF treated group were decreased significantly compared to controls, which were significantly ($p<0.01$) recovered in selenium+NaF group. Increased XOD (10.85%) and LPO (8.61%) levels observed in brain of NaF treated mice were reversed with selenium treatment. Glucose-6-phosphate dehydrogenase (-46.98%), ALAT (-10.44%), AAT (-10.21%), CPK (-27.98%) were decreased and alkaline phosphatase (10.6%), acid phosphatase (24.09%) increased in brain of mice after administration of NaF. All metabolic enzymes were significantly ($p<0.01$) reversed after administration of selenium to the NaF treated group. Thus, the adverse effects of NaF on oxidative and metabolic enzymes of brain were reversible with ameliorative action of selenium supplementation. As evident in this study, the antioxidative nature of selenium coupled with its reversal effect on metabolic enzymes in brain of mice treated with fluoride suggests its use as antidote agent against fluorosis.*

Key words: *Antioxidant enzymes, Mice brain, Metabolic enzymes, NaF, Sodium selenite*
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Introduction

Ingestion of fluoride induces adverse effects not only in teeth and bones but also in different soft tissues like brain, skeletal muscle, kidney and liver (Vani and Reddy, 2000;Guan *et al.,* 2000; Liu *et al.,* 1999; Bishnoi and Arora, 2007) and reproductive functions such as spermatogenesis and steroidogenesis (Pushpalatha *et al.,* 2005; Chinoy and Sharma, 1998). Fluoride content of 0.5 mg l^{-1} in ground water of Hyderabad (AP, India) is sufficient to cause yellowing of teeth and dental problems (Shailaja and Johnson, 2007; Sabal and Khan, 2008). Se is a element belongs to group 16 of the periodic table and in nature it is associated with sulphur in a ratio between 1 : 10^3 and 1 : 10^5 (Roy *et al.,* 2005). The major biological form of selenium is represented by the amino acid selenocysteine. Selenium occurs in both inorganic and organic form. Among the inorganic forms (*i.e.*, selenates, selenides and selenite), the selenide form is more frequently found in the food supply. These selenates and selenites are reduced to selenides in the liver with dimethyl and trimethyl selenide as the end products (Ganther and Hsieh, 1974). The biochemical role of selenium in mammals was clearly established by the discovery that it is part of the active site of the peroxide-destroying enzyme glutathione peroxidase (Rotruck *et al.,* 1973). This enzyme, present in the cytosol and mitochondrial matrix of cells, as well as in body fluids, is formed by four identical subunits each containing one atom of selenium as selenocysteine (Rotruck *et al.,* 1973).

Fluoride is a powerful central nervous system toxin and adversely affects the brain functioning even at low doses (Mullenix *et al.,* 1995). Fluoride induces neuron apoptosis (Lu *et al.,* 2000) and decreased cerebral functions, impaired memory and learning ability (Sun *et al.,* 2000; Wang *et al.,* 2004a,b). Significant increase in neurochemical such as homovanillic acid in the hypothalamus of the mice administered 5 ppm of NaF was observed (Tsunoda *et al.,* 2005). The DNA damage in brain cells of rat and histopathological changes in brain of offspring of exposed rats was observed (Ge *et al.,* 2005).

Selenium (Se) is a cofactor required for the activity of a number of selenoenzymes involved in the stress response, and the maintenance of high tissue antioxidant levels (Beck *et al.,* 2004). Selenium is a potent antioxidant and it nutritionally acts through its various selenoproteins to control the level of cellular hydroperoxides and redox tone of the cell that can damage protein, cell organelle and DNA (Ganther and Hsieh, 1974). It is involved in the regulation and control of the body's antioxidant glutathione and glutathione

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peroxidase system, which plays a major role in the control of ROS (Rotruck *et al.,* 1973). The selenium is also essential dietary trace element, which plays an important role in a number of biological process. Studies on amelioration of toxic effects of fluoride with calcium, magnisium, selenium, Vit.-A, C and D were inconclusive (Pang *et al.,* 1996; Chinoy and Sharma, 93; Chinoy and Sharma, 98; Chinoy and Patel, 2000). A significant rise in skeletal and visceral abnormalities observed in NaF treated rats and these effects were significantly ameliorated in the vitamins D treated groups (Guna Sherlin and Verma, 2001). Oral administration of NaF (40 mg kg^{-1} body wt.) daily from days 6-19 of gestation caused significant reductions in the average number of implantations and average number of live fetuses in rats which were significantly mitigated by co-treatment with vitamins C+D+E (Verma and Guna Sherlin, 2002). The removal of fluoride from water by thermally activated carbon prepared from neem (*Azadirachta indica*) and kikar (*Acacia arabica*) leaves has also been studied (Kumar *et al.,* 2008). The higher dietary levels of fluorine in residents of selenium rich areas are associated with smaller reduction in caries incidence (Hadjimarkos, 1965; Tank and Storvick, 1960). Altered serum enzyme activities and lipids in fluorosis and recovery of these levels were observed when administered the selenium (Zofia Mysliwiec *et al.*, 2002). Effects of sodium selenite and sodium fluoride on oxidative and lysosomal enzyme activity reported in the liver of male rats (Kolodziejczyk *et al.,* 2000). Selenium was found to protect skeletal muscle cells damaged by fluoride with necrosis following a disruption of energy metabolism in the mitochondria with improving mitochondrial membrane stability (Pang *et al.,* 1996). The available information reveals no satisfactory method of treating fluoride patients to relieve them of the disease state and no substance known that completely prevent the toxic effects of increased amounts of ingested fluoride.

Selenium (Se) is a paradoxical element and minor metal essential in amounts varying from trace to parts per million concentrations for humans, animals and plants. The available reports on protective effects of selenium on fluoride induced alterations reveal the very little information particularly involving the activities of pro oxidative, free radical scavenging enzymes and metabolic enzymes in brain of mice. Therfore, this study reports the possible reversal effects of selenium on certain pro-oxidative, free radical scavenging and metabolic enzymes activity changes induced by fluoride along with accumulation of fluoride and selenium in brain of mice. Se in the range of certain concentration had the role of excreting high F, adjusting disorder of free radicals and lipid metabolism, and promoting the recovery of fluorosis in rats with fluorosis and Se could antagonize high F, delay and decrease the occurrence of skeletal fluorosis in rats with fluorosis (Bian *et al.,* 1999, 2000, 2001). The main significance of the study is to evaluate the protective effects of selenium through its antioxidant properties as well as alterations of metabolic enzymes in brain of mice.

Materials and Methods

Adult female albino mice, Mus musculus of Swiss strain, each weighing about 32 ± 2 g, were obtained from National Institute of Nutrition, Hyderabad, India. The mice were housed in polypropylene cages with stainless steel grill tops and were fed with standard pellet diet (Hindustan Lever Ltd., Bangalore) and given distilled water adlibitum. The animals were allowed to acclimatize to the laboratory conditions for four days before experiments began. The mice were randomly divided into 3 groups of six each, the first group served as controls and was given mammalian physiological saline. The second group animals were injected *i.p.* with aqueous sodium floride (NaF) (20 mg kg^{-1} body $wt.^{-1}$ day^{-1}) selected on the basis of the LD_{50} value of fluoride which is 51.6 mg kg^{-1} body $wt.^{-1}$ day^{-1} in female mice (Pillai *et al.,* 1987) and maintained for 14 days. The third group animals were treated orally with sodium selenite (Loba chem) at dose of, 5 µg of sodium selenite / 0.2 ml distilled water kg^{-1} body $wt.^{-1}$ day^{-1}.

Body weight and organo-somatic index: The body weight of each animal was noted before treatment and also on day 15. The weight of whole brain of respective group of animals was recorded. From these values the organo-somatic index (OSI) of brain was calculated dividing organ weight with bodyweight and quotant being multiple by 100 (Krishnaiah and Reddy, 2007).

Estimation of fluoride: Fluoride levels in brain were determined by the method of Birkel, 1970 with required modifications (Vani and Reddy, 2000) and are expressed as µg of fluoride g^{-1} dry tissue. The whole brain was dissected out, pooled, homogenized, and dried for 24 hr at 105°C. About 200 mg of the dry sample was dissolved in an acid mixture (equal parts of 11.6 M perchloric acid

Table - 1: Oxidative enzymes activity in mice brain. Different letters (a, b, c) in the same file indicates that the difference between any two groups is significant, $p<0.01$. Values are mean±SD of six animals

Oxidative enzymes	Control	NaF	NaF+Selinium
Superoxide dismutase (Units mg^{-1} protein)	3.396±0.361[a]	2.83±0.126[b]	3.096±0.095[ab]
Catalase (Units mg^{-1} protein)	11.84± 0.101[a]	9.29± 0.073[b]	10.806±0.073[c]
Xanthine oxidase (Units mg^{-1} formazan per mg protein hr^{-1})	0.304±0.01[a]	0.337±0.007[b]	0.323±0.004[c]
Lipid peroxidation (nano moles of MDA g^{-1} tissue)	18.33± 0.046[a]	19.91±0.058[b]	19.76±0.064[c]
Glutathione-S-transferase (nano moles of GS-CDNB mg^{-1} $protein^{-1}min^{-1}$)	42.32± 0.052[a]	36.51±0.055[b]	38.16±0.088[c]
Glutathione peroxidase (µ moles of NADPH oxidized min^{-1} mg^{-1} protein)	16.59± 0.07[a]	13.41±0.082[b]	15.31±0.083[c]
Glutathione reductase (µ moles of NADPH oxidized min^{-1} mg^{-1} protein)	24.74±0.066[a]	13.63±0.073[b]	19.67±0.075[c]
Glutathione (GSH) (µ g mg^{-1} protein)	13.91± 0.063[a]	10.71±0.052[b]	13.16±0.079[c]

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Table - 2: Metabolic enzymes activity in mice brain. Different letters (a,b,c) in the same file indicates that the difference between any two groups is significant, p<0.01. Values are mean±SD of six animals

Metabolic enzymes	Control	NaF	NaF+Selinium
Glucose-6-phosphate dehydrogenase (μ moles of formazan mg^{-1} protein hr^{-1})	4.81±0.083[a]	2.55±0.05[b]	3.86±0.08[c]
Aspartate amino transferase (Units mg^{-1} protein)	25.36±0.07[a]	22.77±0.07[b]	23.15±0.05[c]
Alanine amino transferase (Units mg^{-1} protein)	18.39±0.05[a]	16.47±0.06[b]	17.26±0.06[c]
Alkaline phosphatase (μ moles of pi mg^{-1} protein hr^{-1})	19.6± 0.05[a]	21.68± 0.06[b]	21.106±0.07[c]
Acid phosphatase (μ moles of pi mg^{-1} protein hr^{-1})	8.26± 0.07[a]	10.25±0.05[b]	10.11±0.07[c]
Creatine phosphokinase (Units mg^{-1} protein)	3.93±0.03[a]	2.83±0.04[b]	3.07±0.05[c]

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).

Selenium accumulation: Selenium determination was carried out (Diaz-Alarcbn *et al.,* 1994) using the atomic absorption spectrometer technique (Perkin-Elmer 1100B). The reduction source was a hollow cathode lamp of selenium used at a wave length of 196 nm and a spectral width of 2 nm.Whole brain collected and dried in an oven at 60°C for 48 hr and were homogenized and samples were mineralized with 5 ml of conc. HNO_3 and heated at 80°C for 1hr in sand bath. Another 5 ml of 4:1 mixture of HNO_3 and $HClO_4$ was added and heating continued for an additional 3 hr until the sample was completely mineralized. Se^{+6} was reduced to Se^{+4} by adding 1 ml of conc. HCl and heating at 100°C for 10 min in a thermo-statted bath. After cooling it was diluted to 10 ml with 1.9 (w/v) distilled water.

Assay of oxidative or free radical enzymes: Xanthine oxidase (XOD) activity was assayed by the method of Govindappa and Swami (1965), and the activity was expressed as micro moles of formazan formed mg^{-1} protein hr^{-1}. Catalase activity in muscle and brain was measured by the method of Chance and Herbert (1950). Specific activity was calculated as Units mg^{-1} protein. Superoxide dismutase (SOD) activity was assayed according to the method of Marklund and Marklund (1974), and units expressed as Units mg^{-1} protein min^{-1}. Glutathione transferase (GST) activity was estimated by using the substrate,1-chloro-2,4-dinitrobenzene (CDNB) by the method of Habig *et al*. (1974) and the specific activity of GST was expressed as nanomoles of GS-CDNB formed mg^{-1} protein min^{-1}. Lipid peroxidation activity was estimated by the method of Bhuyan *et al*. (1981) and units were expressed as nano moles of MDA g tissue. Glutathione peroxidase activity was measured by following the NADPH oxidation by glutathione reductase using hydrogen peroxide (H_2O_2) as substrate as per the method of Martinez *et al.* (1979), units expressed in micro moles of NADPH oxidized/min/mg protein. Glutathione reductase activity was measured according to the method of Carlberg and Mannervik (1981), units expressed in micromoles of NADPH oxidized min^{-1} mg^{-1} protein. Glutathione (GSH) content was estimated as described by Keizo-Meita *et al.*(1983) and expressed as micrograms/ mg protein

Assay of metabolic enzymes: Glucose-6-phosphate dehydrogenase was assayed by the method of Nachlas *et al.* (1960) and units expressed in micromoles of formazan mg^{-1} protein hr^{-1}. Phosphatases (Acid phosphatase, Alkaline phosphatase) activity was estimated by the method of Taussaky and Sherr (1953) and units expressed as micro moles of pi mg^{-1} protein hr^{-1}. Alanine amino transferase (ALAT) and aspartate amino trans ferase (AAT) were assayed by the method of Reitman and Frankel (1957) and units expressed in units mg^{-1} protein. Creatine phosphokinase (CPK) enzyme activity was estimated by the method of Gerhardt *et al.* (1979) and units expressed in units mg^{-1} protein

Statistical analysis: The experimental data were analyzed using statistical software, in which we have used the Tukey's multiple comparision test . Turkey's is the one type of statistical methods for doing data analysis. The problem of multiple comparisons.

Results and Discussion

As seen in Fig. 1 the body weight was decreased in NaF treated group, where as NaF+Se treated group has shown significant (p<0.01) recovery in body weight when compared to control. Organ somatic index of brain of mice (Fig. 2) was decreased significantly in NaF treated group, while NaF+Se treated group has shown maximum recovery when compared to control. The fluoride accumulation increased in brain of NaF treated mice compared to brain of control mice, where as in NaF+Se treatment have shown significant (p<0.01) reversal of fluoride level when compared to NaF treated mice (Fig. 3). Selenium content (Fig. 4) in NaF treated mice was decreased in brain when compared to NaF+Se treated and control group. The decrease of selenium in brain of NaF treated mice was might be due to the diminished level of selenium dependent enzyme GPX in fluoride treated mice.

The alterations in oxidative enzyme activities in brain of mice during administration of NaF and NaF+Se were shown in Table 1. The NaF treatment resulted in significant (p<0.01) enhanced activity of prooxidants LPO, XOD while decreased activities of catalase, SOD, GST, GPX and GSH was found. The supplementation of selenium along with NaF exposure reversed pro and antioxidant systems towards control. As seen in Table 2 alkaline phosphatase and acid phosphatase activities significantly (p<0.01) increased in NaF treatment, where as glucose-6-phosphate dehydrogenase, alanine amino transferase, aspartate amino transferase and creatine

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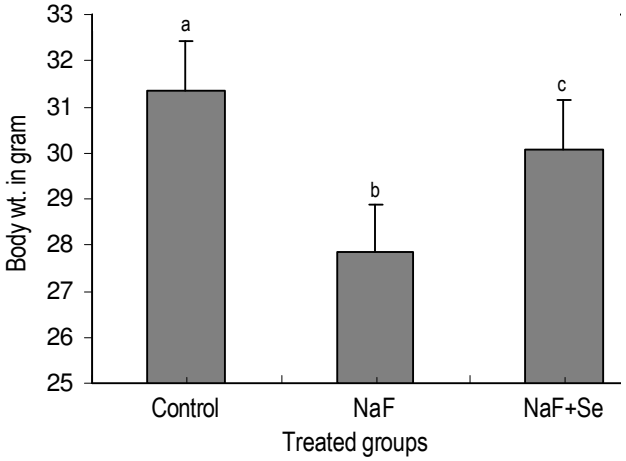

Fig. 1: Effect of selenium on body weight of mice exposed to NaF. Different letters (a, b, c) in the same file indicates that the difference between any two groups is significant, at p<0.01.Values are mean±SD of six animals

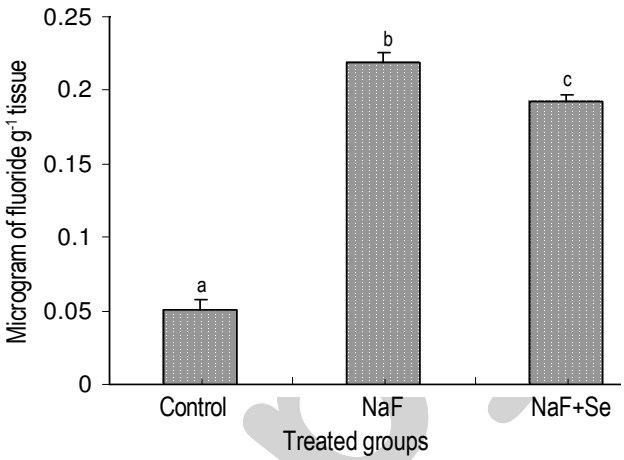

Fig. 3: Fluoride content of mice. Different letters (a, b, c) in the same file indicates that the difference between any two groups is significant at p<0.01. Values are mean±SD of six animals

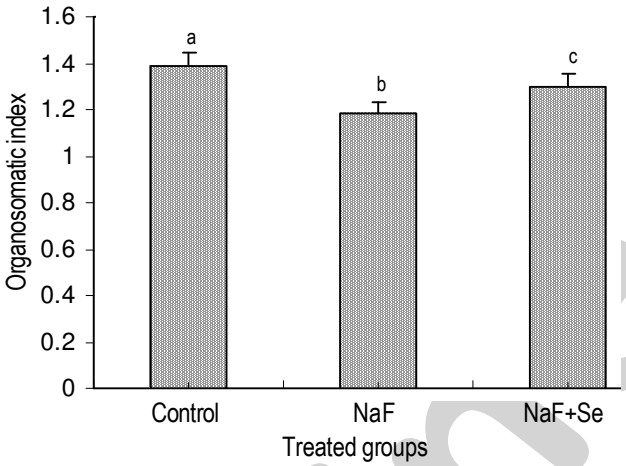

Fig. 2: Organosomatic index of mice. Different letters (a, b, c) in the same file indicates that the difference between any two groups is significant at p<0.01.Values are mean±SD of six animals

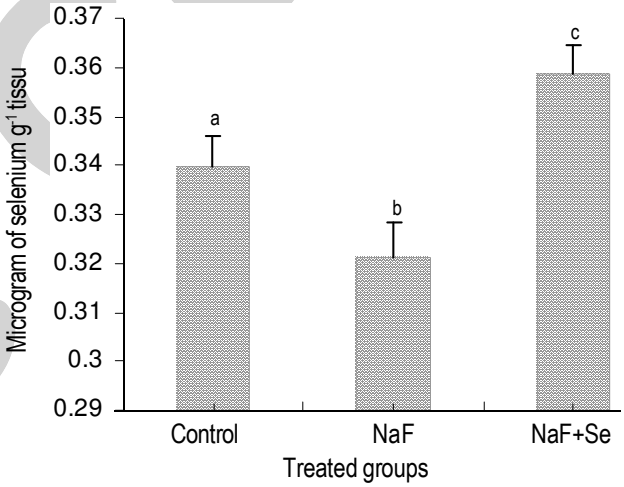

Fig. 4: Selenium content of mice. Different letters (a, b, c) in the same file indicates that the difference between any two groups is significant at p<0.01. Values are mean±SD of six animals

phosphokinase activities were decreased. The simultaneous treatment of NaF+Se resulted in reversal of the metabolic enzymes. Se increases the fluoride excretion and due to the altered activity of GPX enzyme in the cytosol and mitochondrial matrix of cells (Rotruck *et al.,* 1973) reversal of metabolic enzymes might be occurred.

Protective effects of selenium on fluoride induced alterations in free radical scavenging enzymes and metabolic enzymes were found in brain of mice. The significant decrease in body weights of NaF treated mice was reported in fluoride treated animals (Pushpalatha *et al.,* 2005; Vani and Reddy, 2000). The observed accumulation of fluoride in brain is also in conformation with earlier studies (Vani and Reddy, 2000; Shivarajashankara *et al*., 2002). Combined treatment of NaF and selenium showed increased urinary fluoride and decreased liver and kidney fluoride levels (Bian *et al.,* 1999). Selenium is known to afford protective effects on fluoride toxicity (Pang *et al.,* 1996). Sodium selenite administration at a dose of 5 mg kg^{-1} body $wt.^{-1}$ has been shown to have beneficial effect in chronic intoxication with NaF (Grzela, 2000) and sodium selenite administered at 10 mg kg^{-1} body $wt.^{-1}$ 24 hr markedly reduced the serum fluoride. The selenium accumulation was also observed in selenium treatment. Thus, the reversal of body weight, organo somatic index of brain, accumulation of fluoride in brain of mice along with increased selenium content corroborates beneficial effect of selenium treatment.

Fluoride is considered as a prooxidant and disturbs the antioxidant enzyme activities in brain, gastrocnemius muscle of mice (Vani and Reddy, 2000). In the present study the lipid peroxidation was clearly reflected in increased MDA content in the brain in

response to fluoride administration. The LPO activity was reversed in selenium treatment with fluoride. Selenium alleviated lipid peroxidation in fluoride condition (Li *et al.,* 1991). Pretreatment of mice with Se in the diimino proprionitrile model resulted in reduced levels of lipid peroxidation products and inhibited neurobehavioral alterations dose dependently (Al Deeb *et al.,* 1995). XOD activity was increased in NaF treated mice brain, suggesting the generation of super oxide radicals in the tissues. The activity levels of XOD were increased in response to fluoride exposure (Vani and Reddy, 2000). However the activity of XOD was reversed by the administration of selenium to the NaF group might be due to the antioxidant property of selenium. Mice treated with NaF for 14 days revealed decreased SOD, Catalase and GST activities and increased XOD in brain and gastrocnemius muscle (Vani and Reddy, 2000). Decrease in glutathione levels was reported in the liver of male mice during NaF exposure (Chinoy and Patel, 2000). The selenoenzyme glutathione peroxidase, which degrades hydrogen peroxide and is mainly involved in redox defense. Decrease in the activity of GPX occurs in people living in areas of endemic fluorosis (Li and Cao, 1994) and decrease in NaF treated mice (Sharma and Chinoy, 1998). In the present study GPX increased in Selenium+NaF treated group. Increased activity of GPX in selenium treated animals was reported (Combs *et al.,* 1975). Dietary selenium supplementation increases the oxidative stability of tissue by increasing the activity of glutathione peroxidase and also selenium interferes with the redox status by balancing the redox state and controlling the activation of kinases and transcription factors (Savaskan *et al.,* 2003). In fluoride treated animals, glutathione levels were decreased (Sharma and Chinoy, 1998). In the present study glutathione reductase (GR) activity in brain was decreased in NaF treated mice of brain. Similar reports were observed in liver and kidney of fluoride treated mice (Sharma and Chinoy, 1998).

Glucose-6-phosphate dehydrogenase a key enzyme involved in the supply of reduced NADP was decreased in fluoride treated group and significant recovery observed in selenium administered group. Increased activity of alanine amino transferase (ALAT), aspartate amino transferase (AAT) in NaF treated rats and reduced activity reported in selenium treated group (Zofia Mysliwiec *et al.,* 2002). Decreased ALAT and AAT activities were reported in brain and gastrocnemius muscle of mice exposed to NaF (Vani and Reddy, 2000). Creatine phosphokinase decreased in NaF treated mice brain, which confirms the results obtained by Wang *et al.* (1993) and significant recovery was observed in selenium+NaF treated group. Alkaline phosphatase and acid phosphatases were significantly increased in NaF treated brain of mice. Increased testicular activity of acid phosphatase and alkaline phosphatase reported on NaF treatment in male mice (Sudhir and Lall, 1996). The treatments of selenium with NaF have brought about a marked recovery in the activities of acid and alkaline phosphatases.

The present study has shown that the administration of selenium to NaF treated mice was beneficial in promoting the recovery from NaF induced toxicity. The ameliorative effect of the selenium was due to its role in various physiological functions, including as biologically active antioxidant. Effects of Se compounds on brain cells in addition to reducing the fluoride accumulation are elevation of GPX activity, reduction of lipid peroxidation, protection from cell death (Savaskan *et al.,* 2003). Thus, selenium plays a critical role in the maintenance of proper functioning of the nervous system and acts as a potent protective agent for neurons through the expression of selenoproteins, which are mostly involved in regulation of redox status under physiological conditions and in antioxidant defense. Therefore, the future studies should be designed to standardize the proper dosage and treatment regimen for selenium as antidote for fluoride toxicity.

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