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RESEARCH ARTICLE

ALTERATIONS OF DETOXIFICATION ENZYME LEVELS IN DIFFERENT TISSUES OF SODIUM FLUORIDE (NaF) TREATED ALBINO MICE

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Abstract

This study reports the effect of Sodium fluoride (NaF) on different tissues i.e. brain, liver and kidney of mice during NaF exposure. The animals are divided into three batches each containing five individuals among which 1st batch served as control, while remaining two batches were treated with 5 and 10ppm NaF for three months respectively. Then the animals were sacrificed and the brain, liver and kidney were isolated for the biochemical assay. Significant changes were observed in the NaF treated animals when compared to the control. Between the two doses the animals exposed to high dose showed significant changes compared to low dose exposed animals. Among the biochemical parameters studied Lipid peroxidation (LP) level, Glutathione s-transferase (GST) and Xanthine oxidase (XOD) activities were increased in all the tissues of the animals exposed to NaF compared to the control, besides decrease in Catalase (CAT) and Superoxide dismutase (SOD) activity. All metabolic enzymes were significantly ($p<0.05$) altered during treated with two different concentrations of NaF.

Introduction

The main route of exposure to fluoride is via ingestion, although inhalation exposure may occur in certain industrial situations [1]. The toxic effects of Sodium fluoride (NaF) on mice are well documented [2]. NaF toxicity on mice depends very much on increasing F concentration, exposure time, as well as water temperature and uptake of F directly via food [3,4]. 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 [5]. NaF causes adverse biological effects such as changes in carbohydrate, lipid, and protein metabolism, reproduction, impairment, reduced embryonic and development of life stages, and alteration of size and growth[6] and is therefore a potent hazardous pollutant[6]. Fluoride is a powerful central nervous system toxin and adversely affects the brain functioning even at low doses [7]. The DNA damage in brain cells of rat and histopathological changes in brain of offspring of exposed rats was observed [8]. Fluoride is naturally present in water sources and drinking water as they are released from the runoff of fluoride-containing rocks and soils and leach into groundwater [9]. Fluoride induces neuron apoptosis [10]and decreased cerebral functions, impaired memory and learning ability [11].

Material and Methods

Procurement and maintenance of animals:

Female adult Wistar mice weighing 35±5 grams were used as the experimental animals in the present investigation. The mice were purchased from the Indian Institute of Science (IISc), Bangalore, maintained in the animal house in polypropylene cages under laboratory conditions of 28 ± 2^0C temperature with photoperiod of 12 hours light and 12 hours dark and 75% relative humidity. The mice were fed with standard pellet diet (Hindustan Lever Ltd., Mumbai) and water *ad labium*. The mice were maintained according to the ethical guidelines for animal protection and welfare bearing the CPCSEA.

The mice were randomly divided into 3 groups having 5 in each group and were treated as follows:
Group I: Received saline (control).
Group II: 5ppm NaF.
Group III: 10 ppm NaF.
NaF was dissolved in distilled water and given to the animals for three months, through drinking water.

Isolation of tissues
After a stipulated duration of exposure the animals were sacrificed by cervical dislocation and different tissues were immediately isolated, frozen in liquid nitrogen and stored at -40^{0}C until analysis.

Biochemical Analysis:
MDA content [Lipid Peroxidation (LP)]:

This assay is used to determine MDA levels as described by Ohkawa et al. (1979) [12]. The kidney tissue was homogenized (5% - w/v) in 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenates were centrifuged at 10,000 rpm for 10 min at 0^0 C in cold centrifuge. The separated supernatant part was used for the estimation. 200 µl of the tissue extract was added to 50 µl of 8.1% sodium dodecyle sulphate (SDS), vortexed and incubated for 10 min at room temperature. 375 µl of twenty percent acetic acid (pH 3.5) and 375 µl of thiobarbituric acid (0.6%) were added and placed in a boiling water bath for 60 min. the samples were allowed to cool at room temperature. A mixture of 1,25 ml of butanol:phyridine (15:1) was added, vortexed and centrifuged at 1000 rpm for 5 min. The colored layer (500 µl) was measured at 532 nm using 1,1,3,3-tetraethoxypropane as a standard. The values were expressed in µ moles of malondialdehyde formed / gram wet weight of the tissue.

Glutathione – S – tranferase (GST – EC: 2.5.1.18):

Glutathione-S-tranferase activity was measured with its conventional substrate, 1-Chloro 2, 4-Dinitro Benzene (CDNB) at 340 nm as per the method of Habig et al.(1974) [13]. The kidney tissue was homogenized in 50 mM ice cold Tris-HCl buffer (pH 7.4) containing 0.2 M sucrose and centrifuged at 16,000 g for 45 at 40C. The pellet was discarded and the supernatant was used as the enzyme source. The reaction mixture in a total volume of 3 ml contained 2.4 ml of 0.3 M potassium phosphate buffer (pH 6.9), 0.1 ml of 30 mM CDNB, 0.1 ml of 30mM GSH and 0.4 ml of enzyme source. The reaction was initiated by the addition of glutathione and the absorbance was read at 340 nm against the reangent blank and the activity was expressed in µ moles of thioether formed / mg protein / min.

Catalase (CAT – EC: 1.11.1.6):

Catalase activity was measured by a slightly modified version of Aebi (1984) [14] at room temperature. The kidney tissue was homogenized in ice cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v). The homogenates were centrifuged at 10,000 rpm for 10 min at 0^o C in cold centrifuge. The resulting supernatant was used as enzyme source. 10 µl of 100% EtOH was added to 100 µl of tissue extract and then placed in an ice bath for 30 min. After 30 min the tubes were kept at room temperature followed by the addition of 10 µl of Triton X-100 RS. In a cuvette containing 200 µl of phosphate buffer and 50 µl of tissue extract was added 250 µl of 0.066 M H2O2 (in phosphate buffer) and decreases in optical density measured at 240 nm for 60 s in a UV spectrophotometer. The molar extinction coefficient of 43.6 M cm-1 was used to determine CAT activity. One unit of activity is equal to the moles of H_2O_2degraded / mg protein / min.

Superoxide dismutase :(SOD-EC: 1.15.1.6):

Superoxide dismutase activity was determined according to the method of Misra and Fridovich (1972) [15] at room temperature. The Brain tissue was homogenized in ice cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v). The homogenates were centrifuged at 10,000 rpm for 10 min at 4^0 C in ice cold centrifuge. The supernatant was separated and used for enzyme assay. 100 µl of tissue extract was added to 880 µl (0.05 M, PH 10.2, containing 0.1 mM EDTA) carbonate buffer and 20 µl of 30 mM epinephrine (in 0.05% acetic acid) was added to the mixture and measured the optical density values at 480 nm for 4 min on a Hitachi U-2000 Spectrophotometer. Activity expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50% , which is equal to 1 unit activity.

Xanthine Oxidase (XOD):

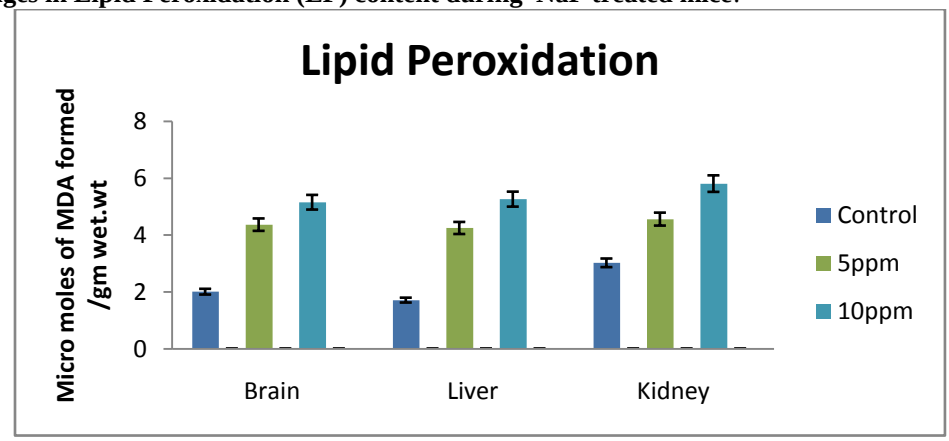
Xanthine oxidase activity was assayed by the dye reduction method of Srikanthan and Krishnamurthy (1955)[16]. The assay mixture contained 100 µ moles of sodium phosphate buffer (pH 7.4), 50 µ moles of xanthine, 0.1 µ moles of NAD, 0.4 µ moles of INT and the enzyme source. The reaction was initiated by the addition of 20 mg of enzyme source and incubated at 37 ^{0}C for 30 min. The reaction was stopped by the addition of 5 ml glacial acetic acid and the formazan formed was extracted into 5 ml of toluene and read at 495 nm against toluene blank. The activity was expressed in µ moles of formazan formed / mg protein / hour.

Statistical analysis:
All observations were carried out with six separate replicates from each group. The mean, standard error (SE) and Analysis of Variance (ANOVA) were done using SPSS statistical software (11.5 ver.) for different biochemical assays. Difference between control and experimental were considered as significant at $P<0.05$.

Results and Discussion

As seen in Fig. 1 the Lipid peroxidation content was increased in NaF treated group when compared to control. The highest elevation was observed in kidney when compared to the Liver and Brain. The proportionate increase in NaF concentrations also. Lipid peroxidation was clearly reflected in increased MDA content in the Kidney, Liver and Brain in response to NaF administration [17]. Lipid peroxidation products and inhibited neurobehavioral alterations dose dependently [18].

Fig .1 : Changes in Lipid Peroxidation (LP) content during NaF treated mice.



The Glutathione s-transferase (GST) activity(Fig.2) was increased in NaF treated group when compared to control. The highest elevation was observed in Brain when compared to the Kidney and Liver. The proportionate increase in NaF concentrations also. GSTs are part of phase II metabolism of xenobiotics, a process which removes toxic compounds. To make compounds more hydrophilic, it conjugates GSH to electrophilic compounds and improves their excretability [19]. GST is the most interesting because of structural similarity to sulfite, a known carcinogen [20]. Further investigation may implicate the inhibition of this detoxifying enzyme as a mechanism.

Fig .2 : Changes in Glutathione s-transferase (GST) activity during NaF treated mice.

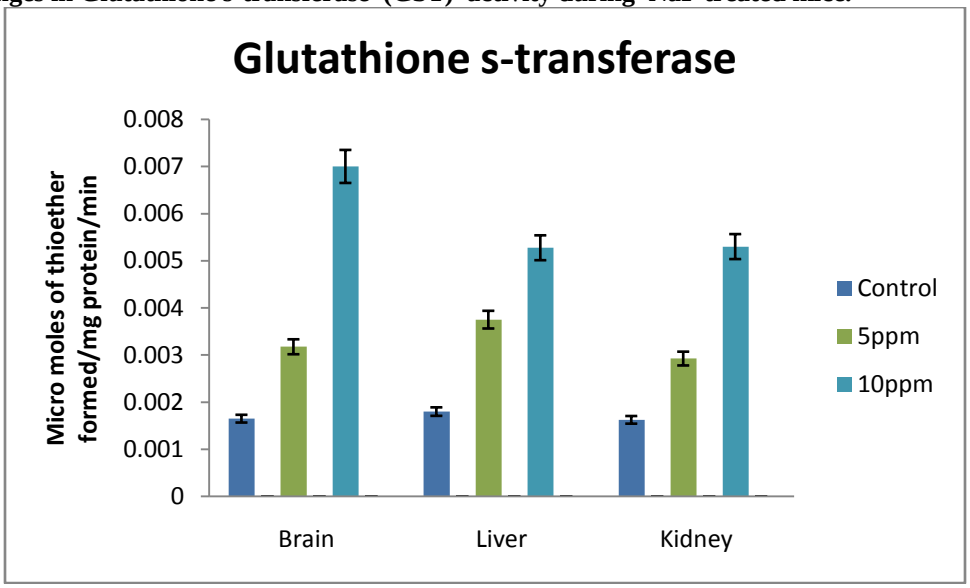
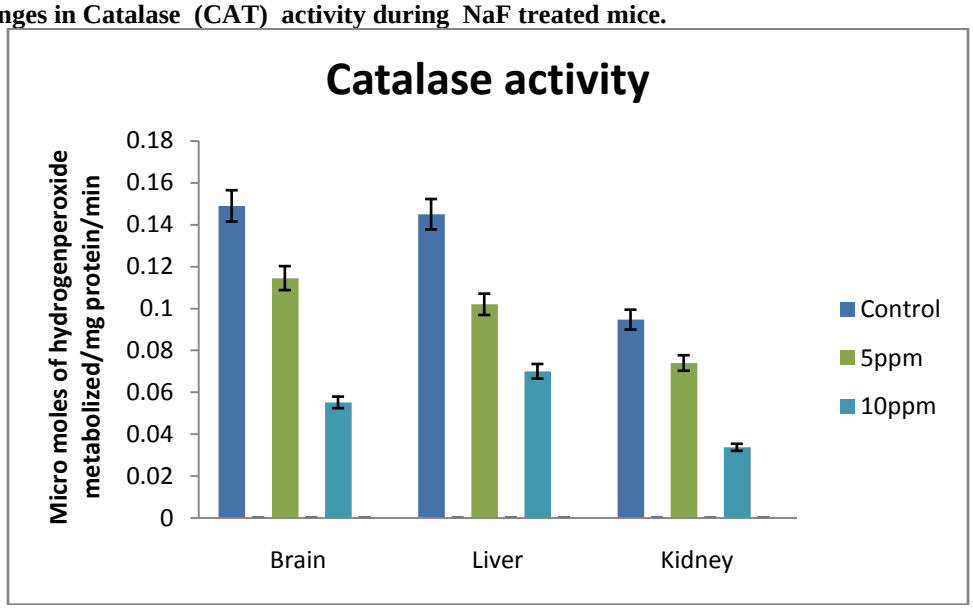
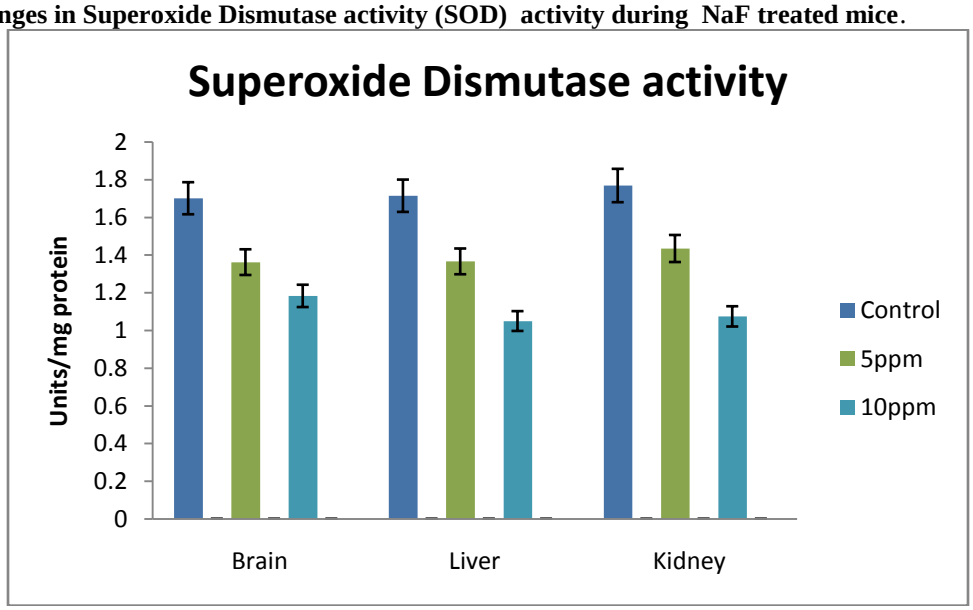


Fig .3 : Changes in Catalase (CAT) activity during NaF treated mice.



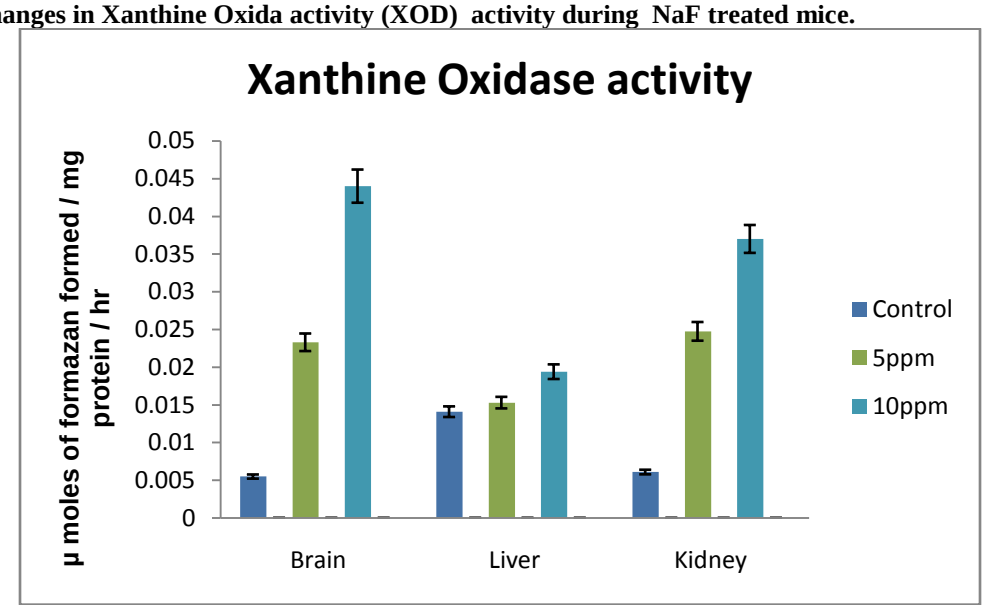
The Catalase (CAT) activity(Fig.3) was decreased in NaF treated group when compared to control. The highest diminution was observed in Kidney when compared to the Brain and Liver. The proportionate decrease in NaF concentrations also. Decrease in Catalase activity was reported in the liver of male mice during NaF exposure [21]. Catalase activity was significantly (P<005) decreased in kidney.

Fig .4 : Changes in Superoxide Dismutase activity (SOD) activity during NaF treated mice.



The Superoxide Dismutase activity (SOD) activity(FIG.4) was decreased in NaF treated group when compared to control. The highest diminution was observed in Liver when compared to the Kidney and Brain. The proportionate decrease in NaF concentrations also. Mice treated with NaF for 14 days revealed decreased SOD, Catalase and GST activities and increased XOD in brain and gastrocnemius muscle [22].-was reported.

Fig .5 : Changes in Xanthine Oxida activity (XOD) activity during NaF treated mice.



The Xanthine Oxidase activity (XOD) activity (Fig.5) was increased in NaF treated group when compared to control. The highest elevation was observed in Brain when compared to the Kidney and Liver. However, similar reports were observed by the administration of NaF [23] earlier.Hence, it may be concluded that these two concentrations 5ppm and 10ppm of NaF have nerotoxic, hepatotoxic and nephrotoxic activity and cause perceptible changes in the antioxidant and detoxification system. Therefore, the future studies shall be designed to mitigate for fluoride toxicity.

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