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Changes in Antioxidants in the Brain of Fluoride-Treated Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In recent times, fluorosis is gradually becoming a severe problem throughout the globe due to toxic effects of fluoride (F) on plants and animals. Natural geological sources and increased industrialization have contributed greatly to the increasing incidence of fluoride-induced human and animal toxicities. Adverse effects are mainly through the attenuation of antioxidant defense mechanism and chelation of enzymatic cofactors. This present study was carried out to investigate the changes that occur on antioxidants in the brain of male wistar rats after sub-chronic fluoride exposure at varying doses (10 ppm, 20 ppm and 40 ppm). Twenty-four (24) Male Wistar rats with average weight of 120 g were distributed into 4 groups according to dose administration (Control; 10 ppm, 20 ppm and 40 ppm) of 6 animals each. The control groups were given only distilled water while the Test groups were given sodium fluoride at doses mentioned above for 30 days. Overnight fasted animals from each group were sacrificed on the 30th day and the brain removed for studying the antioxidant activities. Catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx) and glutathione-s-transferase (GST) were measured from the homogenized brain supernatants. Results showed that CAT and GPx decreased in activity in respect to the dose being

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applied. Decrease in glutathione peroxidase activity was highest at 20 ppm and 40 ppm while Catalase activity showed a decrease at 10 ppm. Reduced glutathione GSH activity increased in the 10 ppm and 20 ppm but decreased at 40 ppm. Other antioxidant activities measured displayed similar trend with much decrease at higher doses. From our results we can say that fluoride toxicity causes changes in antioxidants level. The implications of these findings are herein discussed.

Keywords: Fluoride; toxicity; catalase; glutathione-s- transferase; glutathione Peroxidase; male wistar rat.

1. INTRODUCTION

In the halide group of the periodic table, fluorine plays a great role due to its small size and high electronegativity. Although the mechanism of fluorine action in biological systems remains unclear but it carries unique chemical and biochemical properties because of its size and reactivity [1]. Fluorine in its ionic form (fluoride) is one of the essential trace elements in the human body [2]. Moderate levels of fluoride ingestion can decrease the incidence of dental caries and promote the development of bones, but at higher doses, a number of adverse effects on human health has been reported [3]. The variability and presence of fluorine depends upon the location. Fluoride is present in the soil within the range of 10-1000 parts per million (ppm). However, in water it ranges from 0.5 to 2000 ppm depending upon the source of water. Fluoride is present in trace amounts in all mineralized tissues of the body such as enamel, dentin, and bone. Fluoride is involved in a number of enzymatic reactions and increase the stability of mineralized tissues and materials by decreasing the solubility of hydroxy-apatite mineral phase present in biomaterials [4,5].

Fluoride is present in trace amounts in all mineralized tissues of the body such as enamel, dentin, and bone. Fluoride is involved in a number of enzymatic reactions [6]. Water is an important media for fluoride delivery where it exist either naturally or added during water fluoridation. Recommended optimal level of fluoride in drinking water is 0.7 mg/l; however, fluoride concentration in water varies based on geographical areas. Fluoride containing dentifrices such as tooth-paste, professionally used varnishes/gels, and mouth rinses, fluoride tooth pastes are available either as low fluoride (500 ppm), standard fluoride (1100-1500 ppm) and high fluoride (>1500 ppm). The mouth rinses have an advantage over toothpastes because of their low viscosity that results in better delivery to least accessible areas of the teeth such as pits and fissures and interproximal areas. Fluoride toxicity can cause neuro-degeneration by crossing the blood brain barrier. Adverse neurological effects were observed in the brain of humans with exposure to fluoride. Different studies have shown fluoride accumulation in the brain of animals exposed to chronic fluoride intake and this accumulation increased as drinking water fluoride content increased [7,8]. Human neurological complications such as paralysis of limbs, vertigo, spasticity and impaired mental intelligent quotient were observed with long term intake of high levels of fluoride.

Fluoride is consumed commonly through the oral cavity and absorbed through the gastrointestinal tract. Other less common routes of fluoride absorption are inhalation and dermal absorption. The principal sources of fluoride are fluoridated water and fluoride containing dental products. The absorption of fluoride starts through the stomach and upper part of the small intestine. In the stomach, the absorption of fluoride depends on the pH of the stomach while in the small intestine fluoride absorption is pH independent and absorption is through facilitated diffusion. Fluoride absorption depends on numerous factors such as stomach pH, the chemical formula of consumed fluoride, presence of food in the stomach, interaction with other food ingredients present in gastrointestinal tract, aluminum, calcium, and magnesium compounds [9]. The unabsorbed fluoride is defecated through feces while the absorbed fluoride is distributed rapidly through the circulation into the intracellular and extracellular fluids and is retained only in the mineralized tissues of the body. Fluoride uptake by mineralized tissues is more efficient in growing children and progressively declines with age. Retention of fluoride in the mineralized tissues of the body is reversible; fluoride is released back slowly when the fluoride level in plasma falls [10]. Fluoride in the plasma is capable of crossing the placenta and is found in placental and fetal tissues. Absorbed fluoride is deposited from serum into mineralized tissues while the remaining is excreted primarily into the urine and to a lesser extent into feces, sweat, saliva, and breast milk.

The excretion of fluoride through the urinary system depends upon several factors like plasma levels of fluoride, glomerular filtration rate (GFR), pH of the urine, and its flow.

Acute fluoride poisoning although occasionally reported could be fatal. Acute fluoride toxicity usually occurs due to accidental consumption of fluoride solution or fluoride salts wrongly perceived as sugar solution or powdered eggs [11]. The symptoms of acute fluoride toxicity depend upon the type and chemical nature of the ingested compound, the age, and the elapsed time between exposure and the beginning of management. For instance, sodium fluoride (NaF) is more toxic as it is more soluble and releases more amounts of fluoride compared to calcium fluoride (CaF) that is a less soluble compound [12]. The acute toxic dose range is 5-8 mg/kg body weight. In the case of acute fluoride toxicity, one or a combination of the following symptoms such as gastric disturbances (nausea, vomiting occasionally with blood, abdominal pain, diarrhea, weakness, and hypocalcaemia) are observed. These symptoms result in generalized or localized muscle tetany especially of hand and feet. In addition, hypotension, bronchospasm, fixed and dilated pupils, and hyperkalemia are also linked with fluoride toxicity, which may result in ventricular arrhythmias and cardiac arrest. Loss of body fluid contributes to an electrolyte imbalance, a state of hypovolemic shock, and decreased blood pressure [13]. Acute fluoride poisoning may induce in some individuals a polyuria resembling diabetes insipidus, which may persist for days to months. In a few instances, the acute polyuric renal failure terminates fatally. A progressive, mixed metabolic and respiratory acidosis may develop because of the failure of renal and respiratory systems, coma and convulsions terminating in death. Due to the economic importance of fluoride and its health implications, this work was therefore undertaken to measure the antioxidants in the brain of rats poisoned with fluoride, and find out its effect on some commonly known antioxidants on male wistar at various concentrations. This is with a view to knowing the extent of damage its toxicity can cause to delicate organs like the brain.

2. MATERIALS AND METHODS

2.1 Materials

The laboratory equipment used include; Centrifuge, Spectrophotometer, Teflon head homogenizer, Sensitive Weighing Balance, Measuring Cylinder, Hand Gloves, Test Tube, Incubator, Beaker, Test Tube Rack, Micro Pipettes, Pipette Tips, Dissecting Set, Needle and Syringe, Refrigerator, Methylated Spirit, Paper Tape, Cotton Wool, pH Meter, Heparinized Tubes, Tissue Paper, Filter Paper, Eppendorf Tubes, Wood Shavings, Plastic Plates, EDTA, Thiobarbituric acid, and all other materials were of analytical grade.

2.2 Experimental Animals

Twenty-four (24) male wistar strain albino rats were divided into four groups with each group comprising of six rats. Group 1 rats were given distilled water for 28 days and taken as control, group 2 rats received 10ppm body weight of Fluoride only, group 3 rats received 20ppm body weight of fluoride only, and group 4 rats received 40ppm body weight of fluoride. After 28 days of fluoride and after an overnight fast, animals were sacrificed by cardiac puncture under light ether anaesthesia into ethylene diamine tetra-acetic acid (EDTA) sample bottles for haematological analysis and heparinised sample bottles for biochemical analysis. Brain, Liver and kidney were removed from the animals for biochemical analyses. Blood samples in heparinized bottles were centrifuged to separate plasma and red blood cells. All samples were stored at -20°C until analysed.

2.3 Preparation of Brain Homogenates

Prior to biochemical analyses, the brain samples were cut into small pieces and homogenized in Phosphate buffer saline (PBS) with a homogenizer to give a 10 % (w/v) brain homogenate. The homogenates were then centrifuged at 12,000 rpm for 15 min. The supernatant obtained was used for the assay of glutathione peroxidase, glutathione-s-transferase and reduced glutathione acid content. (Fortress Diagnostics, United Kingdom) kits is used in determination of quantitative determination of total Glutathione peroxidase (GPx), Glutathione-s-transferase (GST), and reduced glutathione (GSH).

2.3.1 Determination of glutathione peroxidase (GPx)

Glutathione peroxidase (GPx) is an enzyme found in the cytoplasmic and mitochondrial fractions of cells. GPx catalyzes the reduction of hydrogen peroxide and hydroperoxides formed from fatty acids, thus effectively removing toxic

peroxides from living cells. It plays important role in protecting cells from potential damage from free radicals formed by peroxide decomposition. GPx catalyzes the oxidation of Glutathione (GSH) by cumene hydroperoxide. The oxidized glutathione is converted to the reduced form in the presence of glutathione reductase and NADPH. In this reaction the NADPH is oxidized to NADP+ simultaneously. The decrease in absorbance at 340 nm is then measured.

2.3.2 Determination of glutathione-s-transferases (GST)

Glutathione-S-transferases (GST) are a group of enzymes that are important in the detoxication of many different xenobiotics in mammals. The enzymes protect cells against toxicants by conjugating the thiol group of the glutathione to electrophilic xenobiotics, and thereby defend cells against the mutagenic, carcinogenic and toxic effects of the compounds. GST activity was found to be present in plants, insects, yeast, bacteria, and in most mammalian tissues, especially in the liver, which plays in a key role in detoxification. The GST Assay kit utilize CDBN as the substrate. Upon conjugation of the thiol group of glutathione to the CDNB substrate there is an increase in the absorbance at 340 nm.

The assay reaction is performed at room temperature (25^{o}_{C}). Allow all reagents equilibrate to room temperature. An enzyme cocktail was prepared by mixing the following reagent in proportion stated, 330µl of buffer solution, 3µl of glutathione solution, 3µl of substrate CDBN solution, 317µl of the above solution was added to a cuvette, 17µl of sample was added.

2.3.3 Determination of Reduced Glutathione (GSH) assay

Glutathione (glutamylcysteinylglycine or GSH) is a naturally occurring tripeptide whose nucleophilic and reducing properties play a central role in metabolic pathways, as well as in the antioxidant system of most aerobic cells. GSH is required as a coenzyme by a variety of enzymes including glutathione peroxidase, glutathione S- transferase and thioltransferase. GSH also plays a major role in drug metabolism, calcium metabolism, the –glutamylcycle, cell membrane and blood platelet functions. GSH is crucial to a variety of life processes, including the detoxification xenobiotic, maintenance of the –SH level of proteins, thiol- disulfide exchange, removal of hydro peroxides and free radicals, and amino acid transport across membranes. Physiological values for the concentration of intracellular GSH generally range from 1 to 10nM. These spectrophotometric procedures were based on the method of Ellman., 1959, who reported that 5,5-dithiobis – (2-nitrobenzoic acid) is reduced by SH groups to form 1 mole of 2-nitro-5-merccaptobenzoic acid per mole of SH. The nitromercaptobenzoic acid anion has an intense yellow color which when measured at a wavelength of 412 nm can be used to measure SH groups.

2.4 Statistical Analysis

Results are expressed as mean ±S.E.M. The levels of homogeneity among the groups were assessed using One-way Analysis of Variance ANOVA followed by Turkey's test. All analyses were done using Graph Pad Prism Software Version 5.00 and p values <0.05 were considered statistically significant.

3. RESULTS

3.1 Effect of Fluoride Administration on GST Activity

Activity of glutathione- s-transferase in rats exposed to fluoride in their drinking water. Fluoride caused significantly decrease ($P < 0.05$) from 22.74% in the control group to 15.46% of 10ppm sodium fluoride group indicate a decrease. Also, there was an increase of 117.40% in 20ppm sodium fluoride group likewise there is also an increase of 150.78% in 40ppm sodium fluoride group.

3.2 Effect of Fluoride Administration on GSH Activity

The Fig. 2 shows that the exposure of rats to fluoride in their drinking water resulted in significance ($P < 0.05$) elevation of reduced glutathione from 4.82% for the control group to 5.32% of 10ppm sodium fluoride group indicate 10.3% increase. Also, there was an increase in 5.24% in 20ppm sodium fluoride group which indicate 8.7% increase. However, there is also a slight or no decrease of 4.83% in 40ppm sodium fluoride group.

3.3 Effect of Fluoride Administration on CAT Activity

The figure above depicts that the activities of catalase in rats exposed to fluoride in their

drinking water significantly decreases (P< 0.05) from 162.16% for the control group and 153.16% of 10ppm sodium fluoride group indicate a decrease. However, there was an increase by 181% in 20ppm sodium fluoride group likewise there was also an increase by 189.66% in 40ppm sodium fluoride group.

3.4 Effect of Fluoride Administration on GPx Activity

Brain GPx levels of rats treated with fluoride in their drinking water resulted to a significantly decrease (P< 0.05) of glutathione peroxidation activity from 253.75% for the control group to 135.90% of 10ppm sodium fluoride group indicate a decrease. Also, there was a decrease of 125.62% in 20ppm sodium fluoride group likewise there is also a decrease of 125.62% in 40ppm sodium fluoride group.

4. DISCUSSION

Antioxidants play a vital role in scavenging of free radicals produced by Reactive Oxygen Species (ROS), and it is well established that fluoride toxicity is associated with Reactive oxygen species (ROS) induction [14]. In the present study, the results have proved that NaF can enhance brain ROS production levels in a dose-dependent manner. Excessive ROS production can lead to lipid peroxidation [15]. The increased ROS levels may indicate the reduction of cellular antioxidant defenses. The current study showed that NaF decreased activities of antioxidant enzymes CAT, GST, GSH and GPx, which were in line with some earlier studies on the effect of NaF on the antioxidant enzymes [16]. Our results in this study showed that antioxidant enzymes majorly decreased according to the dose being exposed to the test animals of different groups under the same atmospheric conditions for the three studied doses. Decrease in glutathione peroxidase activity was highest for 40 ppm treatment in brain by 18.02%. Catalase activity showed a decrease in 10 ppm by 5.0% and a slight increase by 11.1% and 11.7% in 20 ppm and 40 ppm respectively. Alterations in GST levels were similar to catalase activity with maximum decrease for 10 ppm in brain 47.06%. However, for 20ppm and 40ppm, in brain the GST activities significantly increases. Reduced glutathione GSH activity showed an increase in the 10 ppm 10.3% and 20ppm 8.7%. However, GSH activity on 40ppm showed a slight decrease by 0.11%.

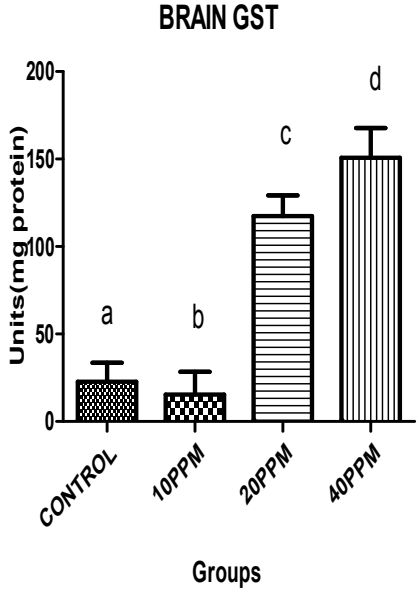

Fig. 1. Effect of fluoride administration on brain GST activity of rats
Values are mean ± SEM and values with different alphabet are significantly different (P<0.05)

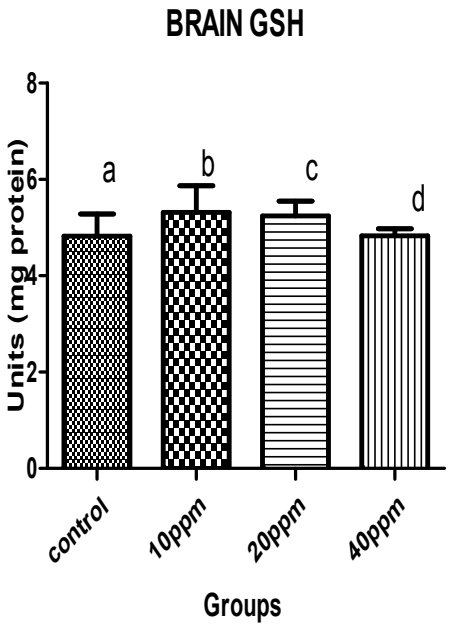

Fig. 2. Effect of fluoride administration on brain GSH activity of rats
Values are mean ± SEM and values with different alphabet are significantly different (P<0.05)

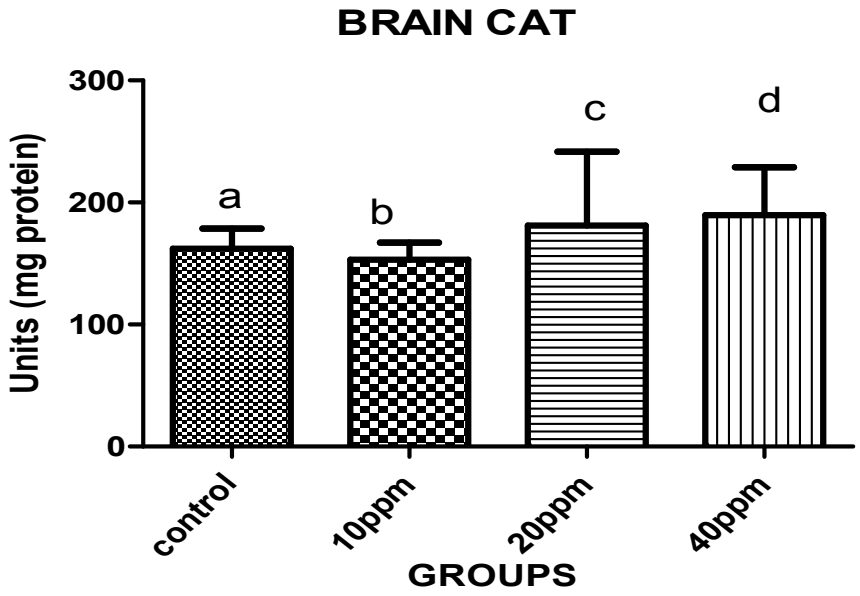

Fig. 3. Effect of fluoride administration on brain CAT activity of rats
Values are mean ± SEM and values with different alphabet are significantly different (P<0.05)

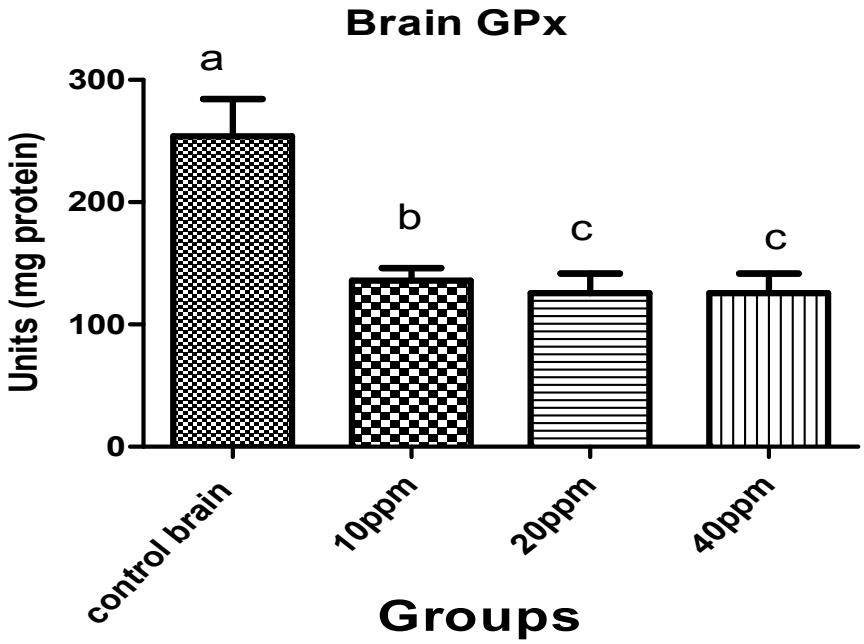

Fig. 4. Effect of fluoride administration on brain GPx activity of rats
Values are mean ± SEM and values with different alphabet are significantly different (P<0.05)

Catalase is the most important antioxidant enzymes, playing a major role in ROS elimination [17]. Non-enzymatic scavengers such as GSH are also involved in scavenging ROS, and the GSH dysfunction could aggravate the organ injury [18]. GPx can promote the reaction between GSH and H_2O_2 in order to achieve the purpose of eliminating peroxide [19]. GST, CAT, GSH, GPx were detected in the present study, therefore, the decreased GPx activities caused by NaF in this study are closely correlated to the reduction of GSH contents. The results showed that these antioxidant enzyme mRNA expression levels were increased in the NaF-treated groups, which were consistent with the reduction of their activities. The above results clearly indicated that NaF not only can promote the ROS production, but also inhibit the antioxidant enzyme mRNA expression in the brain. Then the imbalance between ROS and anti-oxidative function leads to the oxidative stress, which contributes to the occurrence of loose of memory, IQ as well as lack of stability. From our results we concluded that fluoride exposure results in alterations in the antioxidant defense enzymes in a dose related manner and speculate that in sub-acute dose exposures, it may be related to Alzheimer's disease especially when it interacts with aluminum. We also suggest more age related studied with combined exposures to fluoride and aluminum should be carried out with a special focus on Alzheimer's disease.

5. CONCLUSION

Our results evidence that fluoride exposure results in alterations in the antioxidant defense enzymes in a dose related manner and speculate that in sub-acute dose exposures, it may be related to Alzheimer's disease especially when it interacts with aluminum. We also suggest more age related studied with combined exposures to fluoride and aluminum should be carried out with a special focus on Alzheimer's disease.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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