Biochemical Changes in the Liver and the Pancreas of Well-fed and Protein Undernourished Rats Following Fluoride Administration

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ABSTRACT

The biochemical changes in the liver and the pancreas after exposure to 100 ppm fluoride (NaF) in drinking water for one week on Protein Undernourished (PU) and Well-Fed (WF) rats were investigated. The results show that PU induced significant reductions (p<0.05) in body, liver and pancreatic weights. The ingestion of fluoride by both well-fed and PU rats also caused significant reduction (p<0.05) in the body and liver weights except the weight of the pancreas. Moreover, PU increased the level of MDA in both liver and pancreas and has no significant effect on CAT in the liver whereas, its activity was reduced significantly (p<0.05) in the pancreas. The activity of SOD and GSH level were significantly reduced in the liver of PU rats compared to the control but the activity of SOD was increased in the pancreas while no effect was observed in the level of GSH. In the liver, ingestion of fluoride increased the level of MDA in PU rats but the effect was not significant in well fed rats when compared to their respective controls. In the pancreas however, ingestion of fluoride has no effect on the MDA level of both PU and well fed rats when compared to their controls. In addition, ingestion of fluoride significantly reduced the activity of CAT in the liver of both PU and well-fed rats while in the pancreas, CAT activity was significantly reduced in PU only when compared to their respective controls. Fluoride did not affect the activity of SOD in both the liver and the pancreas of well-fed rats but there was reduction in the activity of SOD in the pancreas of PU rats and a significant increase (p<0.05) was observed in the liver. In addition, the ingestion of fluoride had no effect on GSH level in both PU and well fed rats in the liver but significant reductions were observed in the pancreas of both PU and well-fed rats when compared to their respective controls. We conclude that fluoride exerts biochemical effect on lipid peroxidation and antioxidant enzymes of both PU and well-fed rats. This effect varied widely between the liver and the pancreas but it seems that the liver is more sensitive to the toxic assault of fluoride than the pancreas especially in PU rats.

Key words: Fluoride, liver, pancreas, lipid peroxidation, antioxidant, protein undernutrition

INTRODUCTION

Fluoride is ubiquitous in the environment as it is the 13th most abundant element on earth’s crust (Shanthakumari and Subramanian, 2007; Al-Salamah and Nassar, 2009). It is an essential trace element that is beneficial to health if the concentration in drinking water is less than
1.5 mg L\(^{-1}\) but the health may be influenced adversely if excessive fluoride is supplied (Shanthakumari et al., 2004, 2007; Jaganmohan et al., 2010). At higher concentration, serious health hazards arise and the disease caused manifests itself in three forms, namely dental, skeletal and non skeletal fluorosis (Jawed et al., 2006; Shanthakumari and Subramanian, 2007). Fluoride toxicity is the more abundant threat to the common people who are living in the content areas in the globe. Fluoride toxicity will affect all the part of human system leading to the altered life span (Jaganmohan et al., 2010). Studies have shown that accumulation of fluoride altered free-radical metabolism in the liver, kidney and heart (Patel and Chinoy, 1998; Sharma and Chinoy, 1998).

Fluorosis was reported to be prevented through certain interventions if the disease is diagnosed at an early stage and out of these interventions, nutritional measures have much importance. Consumption of diet adequate in protein, calcium, vitamin C, E and other antioxidants can minimize the adverse effects of fluoride (Kaushik et al., 2001).

Protein Undernutrition, (PU), a protein deficiency syndrome is a problem which concerns about half the world’s children (Adenuga et al., 2009). Generally, the antioxidant defense system of PU animals and humans are known to be depressed (Adenuga, 2000) which was due to the imbalance between the production of toxic radicals and their safe disposal (Ashour et al., 1999). Increased generation of Reactive Oxygen Species (ROS) and lipid peroxidation have been found to be involved in the pathogenesis of many disease of known and unknown etiology and in the toxic action of many compounds (Shivarajashankara et al., 2001b).

Studies have been carried out concerning fluorosis in the rats’ tissues such as liver, kidney, pancreas etc. (Shivarajashankara et al., 2002; Guo et al., 2003; Jaganmohan et al., 2010; Al-Omireeni et al., 2011) but rarely in the undernourished state. We attempt in this study to compare the effect of fluoride on well-fed and protein undernourished rats (since it has been reported that consumption of diet adequate in protein minimized the adverse effects of fluoride) on pancreatic and hepatic antioxidative enzymes and the level of MDA in rats exposed to NaF in drinking water.

MATERIALS AND METHODS

Animals, chemicals and diets: Male albino rats (Wistar strain) obtained from the University of Agriculture, Abeokuta, Nigeria were used for the experiment. Diets were prepared as previously described (Adebayo and Adenuga, 2007). The low protein diets contained 5% casein while the normal diet contained 16% casein. All experimental protocol conducted on rats were strictly according to the international guidelines on animal ethics. All reagents including casein were of analytical grade and were obtained from BDH Chemical Ltd., Poole, England and Sigma Co. USA.

Experimental protocol: Animals were allowed to acclimatize for a period of 2 weeks and maintained at 12 h light-dark cycles at room temperature. At the end of the two weeks, animals were randomly assigned to four groups: A, B, C and D with six rats per group and kept in different wire cages. Animals in groups A and C were placed on normal (16% casein) diet and the two other groups (B and D) were placed on low protein (5% casein) diet. Food and water were supplied ad libitum for eleven weeks. At the end of eleventh week, animals in groups B and D were exposed to 100 ppm fluoride (sodium fluoride, NaF) in drinking water for one week (Shivarajashankara et al., 2002). At the end of one week treatment, animals were sacrificed after anesthesia with diethyl ether. The livers and pancreases were removed, homogenized and kept frozen in the freezer at -4°C for analyses.
Preparation of tissues homogenates: Two sets each of liver and pancreas homogenates were prepared. One gram of liver and 0.1 g of pancreas were homogenized in 5 and 1 mL, respectively of 0.01 M phosphate buffer, pH 7.4, kept frozen in the freezer and used for Catalase, Glutathione and Lipid Peroxidation analyses while another set of samples were homogenized in 5 and 1 mL, respectively of isolation buffer (250 mM Sucrose, 5 mM Tris, 1 mM Mercaptoethanol and 0.5 mM Phenylsulphonyl-flouride (PMSF) pH 7.4) and kept frozen in the freezer for Protein determination and Superoxide dismutase analyses.

Lipid peroxidation assay: Hepatic and Pancreatic Lipid Peroxidation assays were carried out by measuring the thiobarbituric acid-reactive (TBAR) products using the procedure of Varshney and Kale (1990). The method is based on formation of pink colored product which has a maximum absorbance at 531.87 nm when Malondialdehyde is treated with 2-thiobarbituric acid.

Glutathione (GSH) assay: Assay for GSH was by the method of Beutler et al. (1963). This method is based on the development of a stable yellow color when 2-nitrobenzoic acids is added to sulfhydryl compounds.

Catalase (CAT) assay: The CAT activity of each liver and pancreas sample was determined by the method of Sinha (1972) but with a slight modification. 0.1 mL of each sample was mixed with 4.9 mL of distilled water. One milliliter of the mixture was added to H2O2-phosphate buffer mixture. The principle is based on the formation of chromic acetate when hydrogen peroxide (H2O2) reacts with dichromate-glacial acetic mixture at 100°C. The decomposition of H2O2 when acted upon by catalase and reduction in the green coloration is measured spectrophotometrically at 570 nm.

Superoxide dismutase (SOD) assay: The SOD activity was determined by the method of Del-Maestro et al. (1983). This assay is based on the ability of SOD to scavenge superoxide anion radical (O2) which, by shortening reaction chains, decreases the overall rate of pyrogallol autoxidation.

Statistical analysis: Statistical analyses were carried out by using one-way Analysis of Variance (ANOVA) at 95% confidence level and differences between means were determined by the use of Duncan multiple range tests (SPSS software, 15).

RESULTS

The results of this investigation as presented on Table 1 show that PU induced significant reductions (p<0.05) in body, liver and pancreatic weights. The ingestion of fluoride by both well-fed and PU rats also caused significant reduction (p<0.05) in the body and liver weights except the weight of the pancreas. As presented on Table 2 and 3, PU increased the level of MDA in both liver and pancreas and has no significant effect on CAT in the liver whereas, its activity was reduced significantly (p<0.05) in the pancreas. The activity of SOD and GSH level were significantly reduced in the liver of PU rats compared to the control but the activity of SOD was increased in the pancreas while no effect was observed in the level of GSH. In the liver, ingestion of fluoride increased the level of MDA in PU rats but the effect was not significant in well fed rats when compared to their respective controls. In the pancreas however, ingestion of fluoride has no effect.
Table 1: Effect of fluoride administration on body, liver and pancreas weights of well-fed and protein undernourished rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
<th>Pancreas weight (g)</th>
<th>Liver weight body weight (g)</th>
<th>Pancreas weight body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>155.3±0.93a</td>
<td>6.17±0.09a</td>
<td>0.63±0.21a</td>
<td>0.04±0.007a</td>
<td>0.004±0.0014a</td>
</tr>
<tr>
<td>PU</td>
<td>110.0±0.21b</td>
<td>4.72±0.02b</td>
<td>0.45±0.02b</td>
<td>0.04±0.008b</td>
<td>0.004±0.0009b</td>
</tr>
<tr>
<td>Control with fluoride</td>
<td>137.7±0.32c</td>
<td>5.79±1.15c</td>
<td>0.52±0.14c</td>
<td>0.04±0.007c</td>
<td>0.003±0.0009c</td>
</tr>
<tr>
<td>PU with fluoride</td>
<td>77.0±0.53d</td>
<td>3.87±0.20d</td>
<td>0.40±0.17d</td>
<td>0.05±0.04d</td>
<td>0.005±0.0004d</td>
</tr>
</tbody>
</table>

Values are presented as Means ± SD. Values with different letters within column differ significantly at p<0.05. Control rats were fed with diet containing 15% casein while pu rats were given diet containing 5% casein. At the end of 11th week, 100 ppm fluoride (NaF) was administered to two of the groups for 1 week. During this period, food and water were supplied *ad libitum* to all animals in each group (n = 6) according to their respective diets.

Table 2: Effect of fluoride administration on MDA levels and antioxidant status in the liver of well-fed and protein undernourished rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Lipid peroxidation (MDA) × 10^3</th>
<th>Catalase activity (units g^-1 tissue 10^6)</th>
<th>Superoxide dismutase (mg SOD m^-1 g^-1 tissue 10^6)</th>
<th>GSH (mg g^-1 tissue protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.42±0.06a</td>
<td>2.08±1.09a</td>
<td>12.83±2.23b</td>
<td>1.48±0.10a</td>
</tr>
<tr>
<td>PU</td>
<td>1.61±0.04a</td>
<td>1.90±0.31a</td>
<td>4.80±1.36a</td>
<td>0.81±0.11a</td>
</tr>
<tr>
<td>Control with fluoride</td>
<td>0.35±0.03a</td>
<td>0.57±0.10a</td>
<td>10.09±2.57b</td>
<td>1.42±0.03a</td>
</tr>
<tr>
<td>PU with fluoride</td>
<td>1.74±0.08a</td>
<td>1.69±0.14a</td>
<td>16.02±4.15b</td>
<td>0.86±0.15a</td>
</tr>
</tbody>
</table>

Values are presented as Means ± SD. Values with different letters within column differ significantly at p<0.05. Control rats were fed with diet containing 15% casein while pu rats were given diet containing 5% casein. At the end of 11th week, 100 ppm fluoride (NaF) was administered to two of the groups for 1 week. During this period, food and water were supplied *ad libitum* to all animals in each group (n = 6) according to their respective diet.

Table 3: Effect of fluoride administration on mda levels and antioxidant status in the pancreas of well-fed and protein undernourished rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Lipid peroxidation (MDA) × 10^3</th>
<th>Catalase activity (units g^-1 tissue 10^6)</th>
<th>Superoxide dismutase (mg SOD m^-1 g^-1 tissue 10^6)</th>
<th>GSH (mg g^-1 tissue protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.02±0.80a</td>
<td>15.08±3.69b</td>
<td>16.02±8.30a</td>
<td>1.56±0.10a</td>
</tr>
<tr>
<td>PU</td>
<td>3.48±2.09b</td>
<td>8.12±2.65b</td>
<td>47.67±0.39a</td>
<td>1.23±0.04a</td>
</tr>
<tr>
<td>Control with fluoride</td>
<td>1.23±0.86b</td>
<td>11.58±4.13b</td>
<td>17.39±10.19b</td>
<td>1.33±0.10a</td>
</tr>
<tr>
<td>PU with fluoride</td>
<td>2.17±1.01a</td>
<td>3.72±1.02a</td>
<td>17.56±8.15a</td>
<td>1.04±0.16a</td>
</tr>
</tbody>
</table>

Values are presented as Means ± SD. Values with different letters within column differ significantly at p<0.05. Control rats were fed with diet containing 15% casein while pu rats were given diet containing 5% casein. At the end of 11th week, 100 ppm fluoride (NaF) was administered to two of the groups for 1 week. During this period, food and water were supplied *ad libitum* to all animals in each group (n = 6) according to their respective diet.

on the MDA level of both PU and well-fed rats when compared to their controls. In addition, ingestion of fluoride significantly reduced the activity of CAT in the liver both PU and well-fed rats while in pancreas, CAT activity was significantly reduced in PU only when compared to their respective controls. Fluoride did not affect the activity of SOD in both the liver and the pancreas of well-fed rats but there was reduction in the activity of SOD in the pancreas of PU rats and a significant increase (p<0.05) was observed in the liver. In addition, the ingestion of fluoride has no effect on GSH level in both PU and well fed rats in the liver but significant reductions were observed in the pancreas of both PU and well-fed rats when compared to their respective controls.

**DISCUSSION**

Fluoride ion is a protoplasmic poison and only a small amount can be tolerated by living cell as it is known to cause several biochemical alterations (Shanthakumari et al., 2004). The decrease
body weight observed in PU rats in comparison to well-fed rats indicates loss of weight due to excessive breakdown of tissue proteins (Chatterjea and Shinde, 2002). Starvation and protein undernutrition result in decreased rate of growth or loss of body weight (Dallman and Spirito, 1972) and this is generally believed to be due to increased generation of ROS (Adebayo et al., 2009, 2011). Ingestion of fluoride further aggravated the reduction in the body weight of both well-fed and PU rats when compared to their respective controls. Also, there were significant reductions in the weights of both the liver and the pancreas of PU rats compared to their well-fed controls. This observation is consistent with various reports on the effect of protein undernutrition (Adebayo et al., 2009; Adegbesan and Adenuga, 2007; Ferreira et al., 2004; Hoppe et al., 2007; Theys et al., 2009; Taleb et al., 1999). However, ingestion of fluoride has no effect on both the weights of the liver and the pancreas of PU and well-fed rats. The observation was further corroborated by the lack of effect of fluoride on liver to body weight and pancreas to body weight ratios of both groups of animals.

The present study, as shown on Table 2 and 3, reported significant increases in MDA level (a marker of lipid peroxidation) in both liver and pancreas of PU rats compared with the well-fed controls. This result is consistent with numerous reports on increased lipid peroxidation in PU rats which suggests a role for free radicals in the pathogenesis of protein-undernutrition (Adebayo and Adenuga, 2007; Adenuga, 2000; Behl and Moosmann, 2002; Rukmini et al., 2004). Numerous studies have examined the relationship between fluoride and free radicals reaction (Guo et al., 2003; Shivarajashankara et al., 2001a, b). The association of fluoride ion with lipid peroxidation processes has been an object of much controversy. Some investigations indicate that excessive fluoride can induce lipid peroxidation which involves polyunsaturated fatty acids (Guan et al., 2000). Kumari and Rao (1991) and Silenko et al. (1992) reported activation, while Jain (1989) found inhibition of lipid peroxidation in the presence of fluoride. Soni et al. (1984) also observed that fluoride inhibits lipid peroxidation in the liver, lungs and testes of rats exposed to fluoride (Chlubek et al., 2003). The present result showed that the levels of lipid peroxidation in the liver and pancreas of well-fed rats given fluoride were not statistically different from the controls. The result of this study on the lipid peroxidation in the pancreas is supported by the report of Chlubek et al. (2003). There was however significant increase in hepatic lipid peroxidation in PU rats following fluoride administration compared with the untreated PU controls while no effect was exerted on the pancreas in PU rats treated with fluoride suggesting that the effect of fluoride on hepatic and pancreatic lipid peroxidation varied in PU rats.

It is generally accepted that CAT, SOD and GSH are able to scavenge free radicals. However, the effect of fluoride on the activities of these antioxidant enzymes and GSH level are controversial (Chlubek et al., 2003). In the present study, while there were significant reductions in SOD activity and GSH level in the liver of PU rats, a significant increase was observed in pancreatic SOD activity and an insignificant effect on the GSH of PU rats compared to their well-fed controls. The differences might be due to variations in the activity of SOD in these tissues as well as their vulnerability to ROS mediated oxidative damage induced by protein undernutrition. It was observed from the study that ingestion of fluoride had no significant effect on the liver SOD activity of well-fed rats compared to the control as well as the GSH levels of both well-fed and PU rats. However, fluoride was able to increase the activity of liver SOD in PU rats. The increase observed in the activity of liver SOD of PU rats might be due to increase expression of SOD in the dismutation of superoxide radicals to produce hydrogen peroxide since SOD is considered as the
first line of defense against oxygen toxicity (Mottley and Mason, 1988). SOD spontaneously
dismutases oxygen (O_2) anion to form O_2 and hydrogen peroxide (H_2O_2). Thus, SOD protects liver
against spontaneous O_2 toxicity and lipid peroxidation (Alvarez et al., 1987).

On the other hand, ingestion of fluoride had no significant effect on the pancreatic SOD activity
of well-fed rats compared to the control whereas pancreatic SOD activity of fluoride-treated PU rats
was significantly lower than that of the PU control. The observation on the response of these tissues
SOD activity and GSH level to fluoride might be due to the variation in fluoride delivery to them
as well as the sensitivity of different tissues to fluoride. The decreased activity of pancreatic SOD
could be explained by the direct and indirect action of fluoride on enzymatic activity. The direct
inhibition of SOD by fluoride may be due to competitive inhibition of the enzyme by fluoride ion.
The proposed mechanism for fluoride ion inhibition of SOD involves its binding to the active site
of Cu on SOD, thus displacing water (Lawson and Yu, 2003). The indirect way of inhibition of SOD
by fluoride may be due to its cytotoxicity associated with general decrease in DNA, RNA and protein
biosynthesis (Song et al., 2002). Thus, it is suggested that excessive fluoride might cause the Cu/Zn
SOD mRNA damage in the pancreas of PU rats resulting in the decrease of SOD biosynthesis.

In addition, there were significant decrease in pancreatic GSH levels of both well-fed and PU
rats given fluoride when compared with their respective controls. GSH functions as a direct free
radical scavenger as a co-substrate for Glutathione Peroxidase (GPx) activity and as a co-factor
for many enzymes. In addition, there were significant decrease in pancreatic GSH levels of both well-
fed and PU rats given fluoride when compared with their respective controls.

Catalase is a heme protein, which decomposes H_2O_2 and protects the tissues from highly reactive
hydroxyl radicals (Chance et al., 1952). In the present study, protein undernutrition had no effect
on CAT activity in liver whereas a significant reduction was observed in the pancreas of PU rats
compared to the control. Fluoride however significantly decreased the hepatic (both in PU and well-
fed rats) and pancreatic catalase activity (in PU rats only) compared to their respective controls. The
reduction in the activity of this enzyme may be due to oxidative stress exerted by fluoride
intoxication.

CONCLUSION

It can be concluded from this study that fluoride exerts biochemical effect on lipid peroxidation
and antioxidant enzymes of both PU and well-fed rats. This effect varied widely between the liver
and the pancreas but it seems that the liver is more sensitive to the toxic assault of fluoride than
the pancreas especially in PU rats.

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diet versus chemical neurotoxins on brain weight, brain lipid peroxidation and antioxidant


