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Impact of Fluoride Toxicity and Ameliorative Effects of Some Antioxidants on Selected Biochemical Indices of Male Rats

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Abstract

The impact of fluoride toxicity and ameliorative effects of some antioxidants on selected biochemical indices of male rats were studied. Thirty-six adult male rats were used in this study and were divided into 6 groups; the study lasted for 6 weeks. Group (I): received normal rodent feed and water and served as control, Group (II):received orally10mg/kg body weight of sodium fluoride (NaF) daily, Group (III): received daily oral dose of sodium fluoride (10mg/kg) + selenium (0.5mg/kg) body weight, Group (IV): received daily oral dose of sodium fluoride (10mg/kg) + zinc (14.8mg/kg) body weight, Group (V): received daily oral dose of sodium fluoride (10mg/kg) + ginseng (10mg/kg) body weight, Group (VI): received oral dose of sodium fluoride (10mg/kg) + 100mg/kg body weight of vitamin E. At the end of the 4^{th} and 6^{th} week, liver function enzymes: alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST); total and conjugated bilirubin, urea and creatinine concentrations were assayed. Result showed that the rats that received sodium fluoride alone had a significant increase ($p<0.05$) in ALP, ALT, AST, total and conjugated bilirubin and also a significant increase ($p<0.05$) in the urea and creatinine concentrations compared to the control group. Concomitant administration of sodium fluoride + selenium, sodium fluoride+ zinc, sodium fluoride + ginseng, sodium fluoride + vitamin E showed a significant reversal ($p<0.05$) of the toxic effect of NaF on the treated animals. The result showed that NaF induced severe toxic changes in the liver and kidney functions while the antioxidants provided partial and complete amelioration against these toxic effects.

1. Introduction

The acute effects of the ingestion of massive doses of fluoride are, first those of an irritant poison, and later become apparent in enzyme system, such as those engaged in metabolism, energetic cellular respiration and in endocrine function. Investigation has demonstrated the effect of fluoride on soft body organ kidney. The kidney is a site for potential fluoride toxicity, since it can be exposed to relatively high concentration of fluoride. Fluoride in kidney is associated with structural and biochemical changes. Although fluoride intake is necessary for the development of teeth and body skeleton but requirements are in traces. However, a few recent studies indicated that more fluoride

intake might cause toxic effects in animals and human being [1]. Fluoride intake (fluorosis) usually occurs in two forms; endemic fluorosis, related to intake of drinking water with high fluoride contents [2] and industrial fluorosis, related to exposure to air rich in fluoride contents [3]. Fluorosis has been found to cause severe side effects, not only to skeletal parts of the body [4] but also to the soft tissues like brain, liver, kidney and spinal cord [5].

Earlier work [6] reported that 50-80% of absorbed fluoride is eliminated by the kidneys indicating the chances of kidney damages due to fluorosis. Liver is the main organ for fluoride detoxification and, therefore, is highly susceptible to the fluoride intoxication [5]. Various studies demonstrated that elevated levels of serum hepatic and renal enzymes have been found following fluoride intoxication indicating degenerative and inflammatory damages to the liver and kidney [5, 7, 8]. Other histopathological findings include hyperplasia, dilatations of hepatic sinusoids and accumulation of amorphous and crystalline bodies in the hepatocytes around the hepatic vein. It has been found that fluoride intoxication induced hepatic hemangiosarcoma, hepatocellular adenoma and carcinoma, metastatic lung tumors, and Zymbal's gland tumors in rats [9]. Many studies have shown that elevated concentrations of fluoride can occur in the kidney as it has a major route in removal of fluoride from the body [8, 10]. Fluoride nephrotoxicity causes pathological changes in the glomeruli and in the proximal, distal, and collecting tubules of experimental animals [11]. Another report [12] on the effects of withdrawal upon cessation of NaF ingestion and of administering ascorbic acid (AA) and/or calcium (Ca^{2+}) showed that NaF treatment caused a significant elevation in serum fluoride levels with a simultaneous rise in Ca^{2+} levels. However, there was a significant recovery from NaF-induced toxicity which occurred following administration of ascorbic acid and/or calcium, while combined treatment (AA+Ca^{2+}) for 70 days manifested a synergistic effect. The transient fluoride induced effects were reversible. In contrast, [13] suggested that chronic Fluoride ingestion may affect Ca^{2+}homeostasis and decreases Ca^{2+} uptake by rat kidney membranes.

Selenium is one of the essential trace elements for both human and animals. Scientists documented the vital role of selenium in numerous biological functions mainly through its antioxidant effects. They also claim that it has an immunomodulatory, anticarcinogenic and antiatherogenic activities [14, 15]. Vitamin E (Vit E) is believed to exert its protective effect at the cellular-molecular level, primarily through destruction of cell damaging free radical oxygen species [16]. Zinc antagonizes oxidative stress, apoptosis, and cell cycle changes induced by excess fluoride [17]. Ginseng (the root of Panax ginseng) is one of the most commonly used herbal medicines in Asian and Western countries. Studies have shown a wide range of beneficial effects of ginseng against human diseases [18, 19].

Considering that fluorosis is a public health issue and the very fact that fluoride exposure has a definite effect on the liver and kidney. The following study was planned to observe the toxic effect induced by sodium fluoride on the liver and kidney and to evaluate the ameliorative role, if any of selenium, zinc, ginseng and vitamin E against these toxic effects on adult male rats.

2. Methods

2.1. Drugs

Sodium fluoride was obtained in the form of white powder from JoeChem Ventures, Rumuchakara, Choba Road, Port-Harcourt, Nigeria. Selenium, Zinc, Ginseng and Vitamin E in the form of supplement drugs, were obtained from Chux Medical, 1 Alogu Road Alakahia Port- Harcourt, Nigeria. These drugs were freshly prepared by dissolving each of them in distilled water and given by oral gavage.

2.2. Animals

Sexually mature male rats weighing 150-200gm were obtained from the Animal House of College of Natural and Applied Sciences, Department of Animal and Environmental Biology, University of Port Harcourt. The animals were divided into six equal groups, caged with standardization of the environmental conditions. After two weeks acclimatization period on guinea growers mash (Port Harcourt Flour Mills, Port Harcourt, Nigeria), static bioassay tests were conducted to find out the value of LD_{50} (chronic toxicity test).

2.3. Experimental Design

Thirty-six adult male rats were used in this study and were divided equally into 6 groups; Group (I): received normal rodent feed and water and served as control, Group (II): received orally10mg/kg body weight of sodium fluoride (NaF) daily, Group (III): received daily oral dose of sodium fluoride (10mg/kg) + selenium (0.5mg/kg) body weight, Group (IV): received daily oral dose of sodium fluoride (10mg/kg) + zinc (14.8mg/kg) body weight, Group (V): received daily oral dose of sodium fluoride (10mg/kg) + ginseng (10mg/kg) body weight, Group (VI): received oral dose of sodium fluoride (10mg/kg) + 100mg/kg body weight of vitamin E. The present study reveals the lethal dose for the 50% of the populations for 4 weeks was 10mg/kg so it was undertaken for study. Biochemical indices were assayed at the end of the 4^{th} and 6^{th} week.

2.4. Collection and Analysis of Sample

The animals to be sacrificed were first anaesthetized with chloroform (inhalational anesthesia) followed by cervical dislocation. Each animal was then placed on a dissecting slab and then cut along the thorax down the abdominal region; blood was collected via cardiac puncture and dispense into the Heparin bottle for biochemical assays (ALT, AST and ALP). ALT, AST and ALP were analyzed by kinetic methods

kits from Randox (United Kingdom) using a double-beam spectrophotometer. All other reagents were of analytical grade.

2.5. Statistical Analysis of Data

The data for toxicological screening were analyzed for statistical differences between treatment groups, by means of one-way ANOVA and post hoc LSD, on SPSS 19. In all p value of less than 0.05 ($p \leq 0.05$) was considered to be significant. Data are presented as mean ± SD (standard deviation).

3. Results

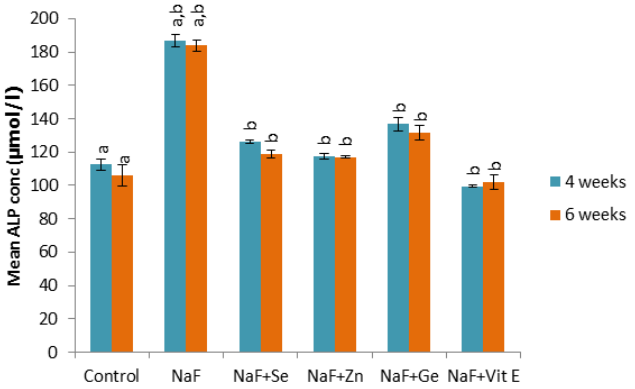

Figure 1. *Alkaline phosphatase concentration of treated groups of NaF induced toxicity.*

NaF = Sodium Fluoride, Se = Selenium, Zn = Zinc, Ge = Ginseng, Vit E = Vitamin E. n = 3, per group/week. Bars with same colour but different letters (a,b) are significantly different at p<0.05, a represents significant difference when group I (control group) are compared with group II (NaF group) at p<0.05, b represents significant difference when group II (NaF group) are compared with the antioxidant treated groups at p<0.05.Values without letters indicate no significant difference when compared with the control and antioxidant groups.

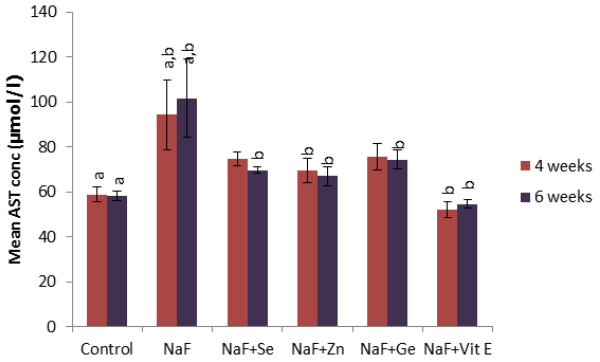

Figure 2. *Aspartate aminotransferase concentration of treated groups of NaF induced toxicity.*

NaF = Sodium Fluoride, Se = Selenium, Zn = Zinc, Ge = Ginseng, Vit E = Vitamin E. n = 3, per group/week. Bars with same colour but different letters (a,b) are significantly different at p<0.05, a represents significant difference when group I (control group) are compared with group II (NaF group) at p<0.05, b represents significant difference when group II (NaF group) are compared with the antioxidant treated groups at p<0.05.Values without letters indicate no significant difference when compared with the control and antioxidant groups.

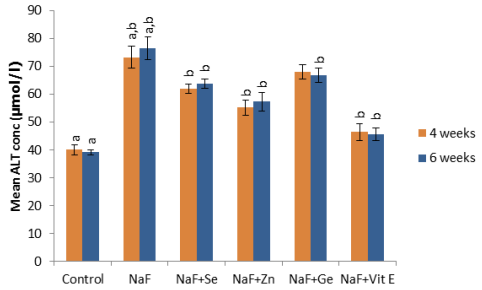

Figure 3. *Alanine aminotransferase concentration of treated groups of NaF induced toxicity.*

NaF = Sodium Fluoride, Se = Selenium, Zn = Zinc, Ge = Ginseng, Vit E = Vitamin E. n = 3, per group/week. Bars with same colour but different letters (a,b) are significantly different at p<0.05, a represents significant difference when group I (control group) are compared with group II (NaF group) at p<0.05, b represents significant difference when group II (NaF group) are compared with the antioxidant treated groups at p<0.05. Values without letters indicate no significant difference when compared with the control and antioxidant groups.

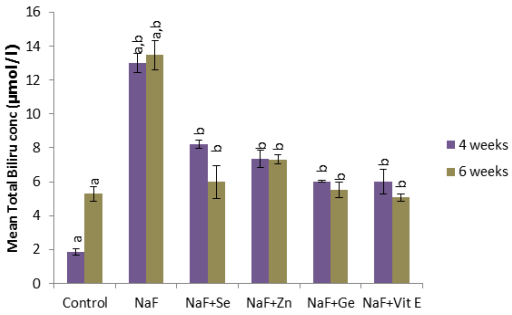

Figure 4. *Total Bilirubin concentration of the treated groups of NaF induced toxicity.*

NaF = Sodium Fluoride, Se = Selenium, Zn = Zinc, Ge = Ginseng, Vit E = Vitamin E. n = 3, per group/week. Bars with same colour but different letters (a,b) are significantly different at p<0.05, a represents significant difference when group I (control group) are compared with group II (NaF group) at p<0.05, b represents significant difference when group II (NaF group) are compared with the antioxidant treated groups at p<0.05.Values without letters indicate no significant difference when compared with the control and antioxidant groups.

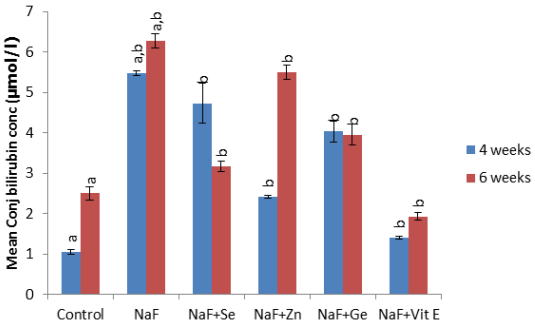

Figure 5. *Conjugated bilirubin concentration of the treated groups of NaF induced toxicity.*

NaF = Sodium Fluoride, Se = Selenium, Zn = Zinc, Ge = Ginseng, Vit E = Vitamin E. n = 3, per group/week. Bars with same colour but different letters (a,b) are significantly different at p<0.05, a represents significant difference when group I (control group) was compared with group II (NaF group) at p<0.05, b represents significant difference when group II (NaF group) was compared with the antioxidant treated groups at p<0.05.Values without letters indicate no significant difference when compared with the control and antioxidant groups.

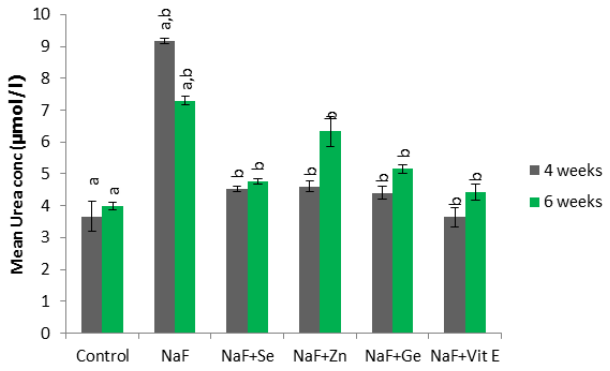

Figure 6. *Urea concentration of treated groups of NaF induced toxicity.*

NaF = Sodium Fluoride, Se = Selenium, Zn = Zinc, Ge = Ginseng, Vit E = Vitamin E. n = 3, per group/week. Bars with same colour but different letters (a,b) are significantly different at p<0.05, a represents significant difference when group I (control group) are compared with group II (NaF group) at p<0.05, b represents significant difference when group II (NaF group) are compared with the antioxidant treated groups at p<0.05.Values without letters indicate no significant difference when compared with the control and antioxidant groups.

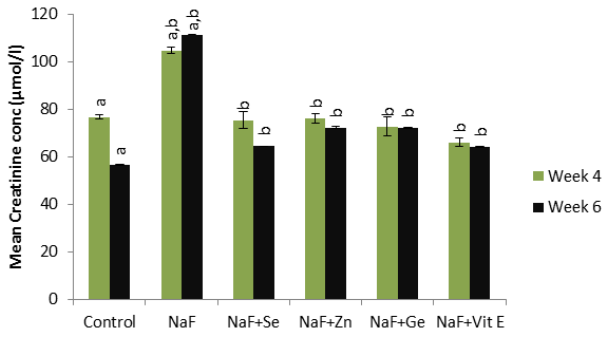

Figure 7. *Creatinine concentration of the treated rats of NaF induced toxicity.*

NaF = Sodium Fluoride, Se = Selenium, Zn = Zinc, Ge = Ginseng, Vit E = Vitamin E. n = 3, per group/week. Bars with same colour but different letters (a,b) are significantly different at p<0.05, a represents significant difference when group I (control group) are compared with group II (NaF group) at p<0.05, b represents significant difference when group II (NaF group) are compared with the antioxidant treated groups at p<0.05.Values without letters indicate no significant difference when compared with the control and antioxidant groups.

4. Discussion

The consumption of food stuffs and drinking water is the principal route of exposure to fluoride. When a large proportion of fluoride is ingested and inhaled by humans or laboratory animals, then it is rapidly absorbed through the gastrointestinal tract. Absorbed fluoride is carried by the blood, causes metabolic disturbances in the body and is excreted via the renal system [20]. The present study provides us information about the serum enzymes which are basic indicators of liver and kidney functioning at different fluoride exposure. It will be helpful to devise some strategies to protect the liver, kidneys and skeleton before they are fully damaged. In this present study, after 4 weeks, there was a significant increase in the urea concentration of the NaF-treated group (9.17 ± 0.07) when compared to the control group (3.68 ± 0.49) but there was a significant decrease in the NaF+Vit E-treated group (3.64 ± 0.31), NaF+Se-treated group (4.54 ± 0.09), NaF+Ge-treated (4.40 ± 0.20) and NaF+Zn-treated group (4.60 ± 0.17) compared to the NaF-treated group (9.17 ± 0.07). After 6 weeks, there was still a significant increase in the urea concentration of the NaF-treated group (7.30 ± 0.15) when compared to the control group (4.00 ± 0.13) but a significant decrease in the NaF+Vit E-treated group (4.43 ± 0.25), NaF+Se-treated group (4.77 ± 0.08), NaF+Ge-treated group (5.17 ± 0.13) and NaF+Zn-treated group (6.33 ± 0.47)Results obtained at the end of 6 weeks showed a significant increase ($p<0.05$) in ALP activity of the NaF-treated group (183.86±3.55) when compared to the control group (106.30±6.32) while a significant decrease ($p<0.05$) was observed in the NaF+Vit E-treated group (101.80±4.25), NaF+Se-treated group (118.66±2.25), NaF+Ge-treated group (131.46±4.39) and NaF+Zn-treated group (117.13±0.80) when compared to the NaF-treated group (186.46±3.69). Most significant amelioration was observed in the NaF+Vit E-treated group compared to other antioxidant treated groups.

Results for ALT activity obtained after 4 weeks revealed a significant increase ($p<0.05$) in the NaF-treated group (73.30±3.83) when compared to the control group (40.03±1.83). However, there was a significant decrease ($p<0.05$) in the NaF+Vit E-treated group (46.36±3.05), NaF+Se-treated group (61.96±1.71), NaF+Zn-treated group (55.16±2.85) but no significant difference ($p>0.05$) in the NaF+Ge-treated group (68.00±2.45) when compared to NaF-treated group (73.30±3.83). Results obtained after 6 weeks showed that there was a significant increase ($p<0.05$) in ALT activity of the NaF-treated group (76.56±4.15) when compared to the control group (39.16±0.89). Similarly, a significant decrease ($p<0.05$) in the NaF+Vit E-treated group (45.60±2.30), NaF+Se-treated group (63.70±1.73), NaF+Ge-treated group (66.73±2.61) and NaF+Zn-treated group (57.26±3.46) compared to the NaF-treated group (73.30±3.83). Most significant amelioration was observed in the NaF+Vit E-treated group when compared to other treated groups.

For AST activity, results after 4 weeks showed a significant increase ($p<0.05$) in the NaF-treated group (94.30±15.62) when compared to the control group (58.86±3.20).A significant decrease ($p<0.05$) was observed in the NaF+Vit E-treated group (52.13±3.49) and NaF+Zn-treated group (69.43±5.54) but no significant difference ($p>0.05$) in NaF+Se-treated group (74.73±2.91) and NaF+Ge-treated group (75.60±5.83) when compared to NaF-treated group (94.30±15.62). Extension of treatment to 6 weeks showed a significant increase ($p<0.05$) in the AST activity of the NaF-treated group (101.66±17.36) when compared to the control group (58.23±2.31). However, there was significant decrease ($p<0.05$) in the NaF+Vit E-treated group (54.53±1.95), NaF+Se-treated group (69.60±1.49), NaF+Ge-treated group (74.43±4.35) and NaF+Zn-treated group (67.10±4.29) when compared to the NaF-treated group

(101.66±17.36). Most significant amelioration was observed in the NaF+Vit E-treated group compared to other treated groups.

Blood examination is a good way of assessing the health status of animals as it plays a vital role in physiological, nutritional and pathological status of an organism [30]. Serum ALP in rats treated with the antioxidants were lower ($p<0.05$) compared to the controls. ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum [22, 31]; it is therefore an ectoenzyme of the plasma membrane [32]. It is often used to assess the integrity of the plasma membrane [33], such that in the tissue and serum would indicate likely damage to the external cell boundaries (plasma membrane). An increase in ALP level may query the possibility of membrane damage, because ALP is a membrane bound enzyme [34, 35]. High levels of serum ALP activity is usually noticed in liver damage, cancer and heart infections [36]. Consequently significant decrease ($p < 0.05$) in serum ALP observed in the rats treated with the antioxidants may be an indication of the drug actions of these antioxidants compared to those in the NaF groups. The tissue activities of the transaminases (AST and ALT) enzyme are markers for the functions and integrity of the heart and liver [37, 38]. They rearrange the building blocks of proteins. It is released from damaged liver cells [39, 40]. Elevation of these enzymes in the serum have been reported to indicate cellular damage, tissue necrosis, as well as a calculated risk for cardiovascular diseases, with higher risk of cardiovascular disease and elevated myocardial infarction being attributed to elevation of ALT and AST respectively [41]. Results suggest that the antioxidants restored damage to the plasma membranes in the rats, further lending credence to earlier observations in serum.

5. Conclusion

From this experimental study it is concluded that fluoride toxicity produce definite alteration in some biochemical parameters of the liver and kidney, which was duration dependent. Administration of some selected antioxidants revealed both partial and complete significant recovery in all the parameters which suggests that effects induced by NaF treatment were transient and reversible.

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