HEPATOPROTECTIVE AND AMELIORATIVE EFFECTS OF SELECTED ANTIOXIDANTS ON ALUMINIUM INDUCED TOXICITY ON WISTAR RATS

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ABSTRACT

Hepatoprotective and ameliorative effects of selected antioxidants (Zinc, Selenium, Ginseng, Vitamin A, Vitamin C, and Vitamin E) on aluminium induced toxicity on Wistar Rats was investigated. Forty-eight male Wistar rats were divided into eight groups of six male wistar rats each according to their body weight. Group 1 is the control group. Group 2 received only 200 mg/kg body weight of Aluminium, Group 3 received 14.8mg/kg body weight of zinc + 200 mg/kg body weight of Al (Al+Zn), Group 4 received 100 mg/kg body weight of Selenium + 200mg/kg body weight of Al (Al+Se), Group 5 received 10 mg/kg body weight of Ginseng + 200 mg/kg body weight of Al (Al+Ge), Group 6 received 100 mg/kg body weight of vitamin A + 200 mg/kg body weight of Al (Al+Vit.A, Group 7 received 100mg/kg body weight of vitamin C + 200mg/kg body weight of Al (Al+Vit.C), Group 8 received 100mg/kg body weight of vitamin E + 200mg/kg body weight of Al (Al+Vit.E). All the groups were fed with normal rat chow and water. Administration of aluminium and antioxidants were for six weeks. Liver enzymes - alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), as well as enzymes and markers of oxidative stress; reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) were measured. Liver tissues were also collected for histopathological examination. The results showed that the oral administration of 200 mg/kg body weight of Al caused significant ($p<0.05$) increase in the serum levels of the ALT, AST, ALP. Moreover, Al induced oxidative stress as indicated by a significant increase ($p<0.005$) in the level of MDA with a concomitant decrease in the GSH as well as in the activity of SOD and CAT. Histological examination for liver sections revealed distorted liver architecture, vacuolization of cytoplasm. Administration of antioxidants to Al induced male Wistar rats ameliorated its toxic effect.

Keywords: Aluminium, antioxidants, ameliorate, liver, vitamins, hepatoprotective.

INTRODUCTION

One of the highly abundant elements and most common metal in the environment is aluminium (Camargo *et al*. 2009). Its wide distribution ensures the potential for causing human exposure and harm (Zhang and Zhou, 2005). Al compounds are widely used in medicines as antacid, vaccines, antidiarrhoeals, phosphate binders and allergen injections (Kaehny *et al.* 1997), water purification agents (Newairy *et al*. 2009), food additives and tooth paste (Abbasali *et al*. 2005). It is still a metal of choice in making various kinds of household cookware and storage utensils. Aluminium has many uses, mainly in the form of alloys in packaging, building, construction, transportation and electrical applications. Over 95% of beer and carbonated drinks are packaged in two piece aluminium cans. Human exposure to aluminium comes from food prepared in aluminium utensils and drinking water as well as from pharmaceuticals. The normal average daily intake is 1 to 10 mg for adults (Cuciureanu *et al*. 2000).

Aluminium occurs in the environment in the form of silicates, oxides and hydroxides, combined with other elements such as sodium and fluorine and as complexes with organic matter. Due to its reactivity, aluminium is not found as a free metal in the environment. At pH values greater than 5.5, naturally occurring aluminium compounds exist predominantly in an undissolved form such as $Al(OH)_3$ (gibbsite) or as aluminosilicates. According to (WHO, 1997), the solubility of aluminium in equilibrium with solid phase $Al(OH)_3$ is highly dependent on pH and on complexing agents such as fluoride, silicate, phosphate and organic matter. Aluminium is poorly absorbed following either oral or inhalation exposure and is essentially not absorbed dermally. In plasma 80 to 90% of aluminium binds to transferrin, an iron-transport protein for which there is receptors in many body tissues. Aluminium is removed from blood by the kidneys and excreted in urine (De Voto and Yokel 1994). There were indications that aluminium could induce toxic manifestation such as osteomalacia (Cournot-Witmer *et al.* 1986), gastrointestinal toxicity and alzheimer's disease (Perl and Brody 1980), and changes in the hepatic functions (Demircan *et al.* 1998).

The sources of Al are especially corn, yellow cheese, salt, herbs, spices, tea, cosmetics, aluminium ware and containers. People are exposed to higher than normal levels of Aluminium through environmental pollution with the different aluminium containing compounds. Particulate matters distributed by cement -producing factories contain high amount of Aluminium and populations residing in the vicinity are exposed to the pollution (Shehla *et al*. 2001; Proudfoot. 2009).

Aluminium causes an oxidative stress within brain tissue (Stella *et al*. 2005). Since the elimination half-life of aluminium from the human brain is 7 years, this can result in cumulative damage via the element's interference with neurofilament axonal transport and neurofilament assembly. A possible etiologic link between aluminium exposure and Alzheimer disease emerged from a 1965 study showing that aluminium causes neurofibrillary tangles in the brains of rabbits. Subsequent research has largely failed to support this hypothesis, however. For example, the clinical manifestations and underlying neuropathology of aluminium-induced encephalopathy in dialysis patients bear no resemblance to those of Alzheimer disease (Lidsky 2014). Studies have established that aluminium mediated toxicity in animals or man is mainly due to its ability to

1. Generate reactive oxygen species or free radicals when metabolized
2. Inhibit antioxidant enzymes and other components of the antioxidant system in variety of organs including the liver, testis, kidney, lungs and brain (Yousef *et al.* 2007).

The aim of this study therefore, was to investigate the effects of aluminium on the liver and some oxidative stress parameters and to evaluate the antioxidant and ameliorating property of selected antioxidants.

MATERIALS AND METHODS

Experimental Animals and design

A total of Forty-eight male wistar rats were obtained from the animal house of the Department of Animal and Environmental Biotechnology, University of Port Harcourt, Rivers State, Nigeria. They were housed in separate plastic cages and acclimatized for twenty-one days and feed on conventional rat feed and water. The rats were completely randomized into eight groups of six rats each (control, aluminium, selenium, zinc, ginseng, vitamin A, vitamin C and vitamin E). Rats in the control group were given only their food

and distilled water, the aluminium group was given 200mg/kg of aluminium alongside their food. The other groups which include the selenium, zinc, ginseng, vitamin A, vitamin C and vitamin E were given 200mg/kg of aluminium as well as 100mg/kg, 14.8mg/kg, 10mglkg, 100mg/kg, 100mng/kg and 100mg/kg of the various dosages of antioxidants respectively.

Collection of Blood Samples

The investigation lasted for 6 weeks and two rats were selected randomly from each group and sacrificed. 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 dispensed into the Heparin bottle for biochemical assays

Determination of liver enzymes

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) were analyzed by kinetic methods kits from Randox (United Kingdom) using a double-beam spectrophotometer. Freshly dissected liver from each animal was rapidly fixed in buffered neutral formalin (10%). The tissues were subjected to standard routine histological procedures as described by Brown (2000). The slides were viewed using the light microscope and histopathological changes and observations were recorded at X40 magnification identifying both the normal and the degenerated hepatocytes. The results of the study were reported as mean ± standard error of mean (SEM) of triplicate determinations. Data were analyzed using one way analysis of variance (ANOVA) and differences were considered significant at $p<0.05$.

Determination of Antioxidant Enzymes

The antioxidant enzymes malondialdehyde (MDA), Catalase (CAT), Superoxide dismutase (SOD) and Gluthathione (GSH) were determined. The method for the analysis of these enzymes is described below;

Determination of Catalase Enzyme Activity

Serum catalase activity was determined by measuring the decrease in absorbance at 240nm in a UV recording spectrophotometer by monitoring the decomposition of H_2O_2 for 1, 2, and 3 minutes as described by Usoh *et al*., (2005). The reaction mixture (3 ml) contained 0.1 ml of suitably diluted serum in phosphate buffer (50 mM, pH 7.0) and 2.9ml of 30mM H2O2 in phosphate buffer pH 7.0. An extinction coefficient for H_2O_2 at 240nm of 40.0 M^{-1} cm^{-1} was used for the calculation. The specific activity of catalase has been expressed as moles of H_2O_2 reduced per minute per mg protein.

Determination of Superoxide Dismutase (SOD) Enzyme Activity

Superoxide dismutase activity was assayed by its ability to inhibit the auto oxidation of adrenaline, and determined by the increase in absorbance at 480nm. Whole blood superoxide dismutase was assayed utilizing the technique of Fridovich as described by Usoh *et al*., (2005). 1ml of whole blood was diluted with 9ml of distilled water to make a one in ten dilution of the whole blood. An aliquot of 2.0ml of the diluted blood was added to 2. 5ml of 0.05M carbonate buffer (pH 10.2) and left to equilibrate in the spectrophotometer. The

reaction was started by the addition of 0.3ml freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5ml buffer, 0.3ml of substrate (adrenaline) and 0.2ml of water. The increase in absorbance at 480 nm was monitored for 1, 2, and 3minutes. A single unit of enzyme is defined as the quantity of SOD required to produce 50% inhibition of autoxidation (Usoh *et al*., 2005).

Determination of Malondialdehyde

Malondialdehyde, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of lipid peroxidation reaction. Malondialdehyde has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a red species absorbing at 535nm. 1.0ml of the sample was added to 2.0ml of TCA-TBAHCL (15 % w/v Trichloroacetic acid, 0.375 % w/v Thiobarbituric acid and 0.25 N Hydrochloric acid) reagent and mixed thoroughly. The solution was heated for 15mins in boiling water bath and allowed to cool. The flocculent precipitate formed was removed by centrifugation at 1000g for 10minutes. The absorbance of the sample was measured at 535nm against a blank containing all the reagents minus the lipid. Malondialdehyde concentration of the sample was calculated using an extinction coefficient of $1.56x105M^{-1}cm^{-1}$ (Usoh *et al*., 2005)

Determination of reduced glutathione (GSH)

Glutathione peroxidise (GSH- reduced) was measured in a coupled enzyme system by measuring the decrease of DTNB (5,5-Dithio-bis 2-Nitrobenzoic acid). 0.1ml of sample was added into a test tube containing 1ml of 0.2M Tris EDTA buffer and 0.9ml of 20mM EDTA. 0.1 ml of 10nM DTNB was added and incubated for 30 mins at room temperature. The mixture was centrifuged and absorbance of the supernatant read against distilled water blank at 412nm. The specific activity of GST is expressed as μmol of GSH- DTNB conjugate formed/min/mg protein using an extinction coefficient of $13,600M^{-1}cm^{-1}$ (Usoh *et al*., (2005).

RESULTS

The Activity of Liver Function Enzymes in the Serum of Male Wistar Rats

Data presented in Figures 1- 3 denoted that the administration of Aluminium induced a significant ($p<0.05$) increase in the activity of ALT, AST, ALP as compared to the control group indicating hepatic damage caused by Aluminium. The antioxidants (Zinc, Selenium, Ginseng, Vitamin A, Vitamin C, Vitamin E), when administered concomitantly with Aluminium, demonstrated a potent effect in protecting rats against Al-induced liver damage as evidenced by the reduction in the activity of these liver function biomarker enzymes compared to the Aluminium group.

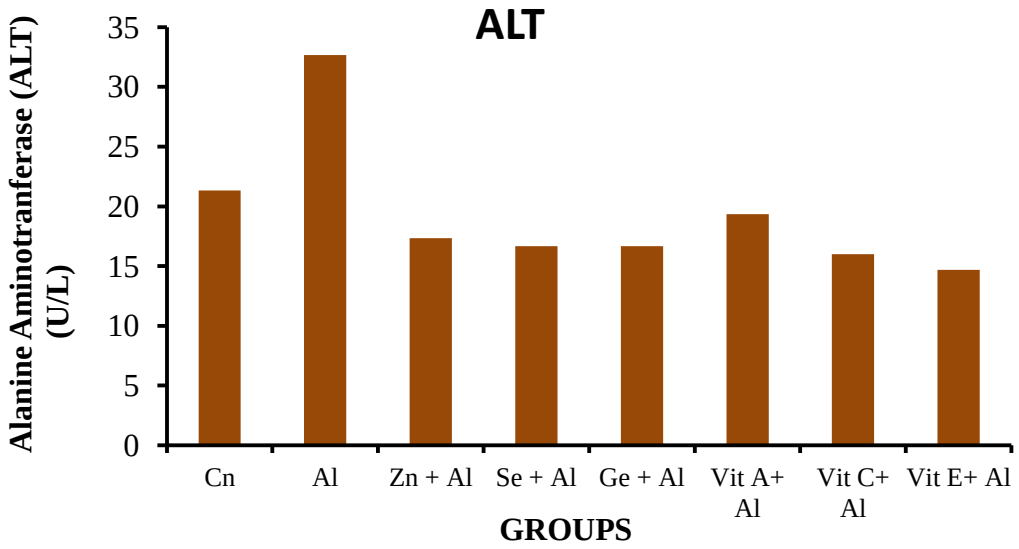


Figure 1 Effect of aluminium on Alanine aminotransferase levels of wistar Rats

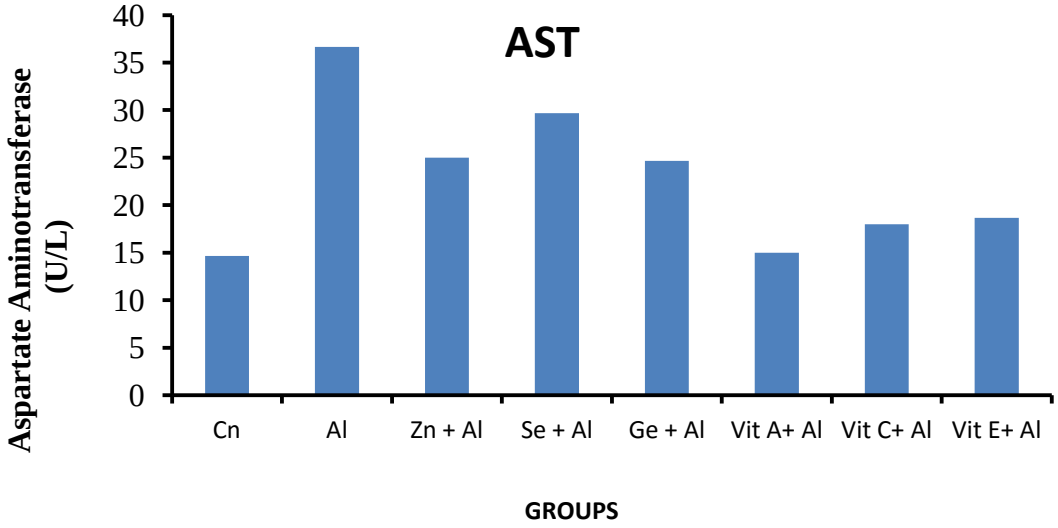


Figure 2 Effect of aluminium on Aspartate aminotransferase levels of wistar Rats

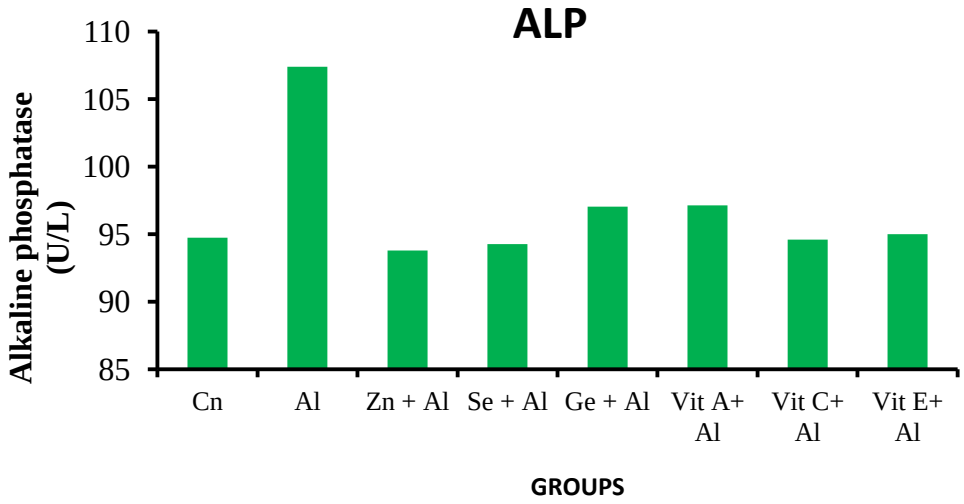


Figure 3 Effect of aluminium on Alkaline phosphatase levels of wistar Rats

The Activity of SOD and CAT in the Liver

The change in the activity of the enzymatic antioxidants, SOD and CAT in liver of control and male Wistar rats are shown in Figure 4.4 and Figure 4.5 The oral administration of Aluminium was associated with a significant ($p<0.05$) decrease in the activities of Catalase (CAT) and SOD compared to the control. Administrating Zinc, Selenium, Ginseng, Vitamin A, Vitamin C, Vitamin E Respectively concomitantly with Aluminium enhanced the enzymatic antioxidative status as demonstrated by the significant ($p<0.05$) increase in the activity of these enzymes in the liver in comparison with that of Aluminium group.

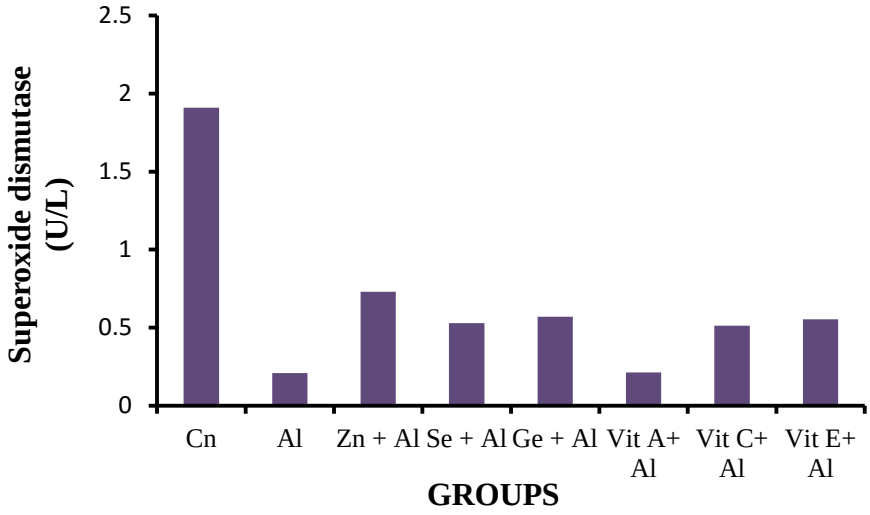


Figure 4 Effect of aluminium on Superoxide dismutase Activity of wistar Rats

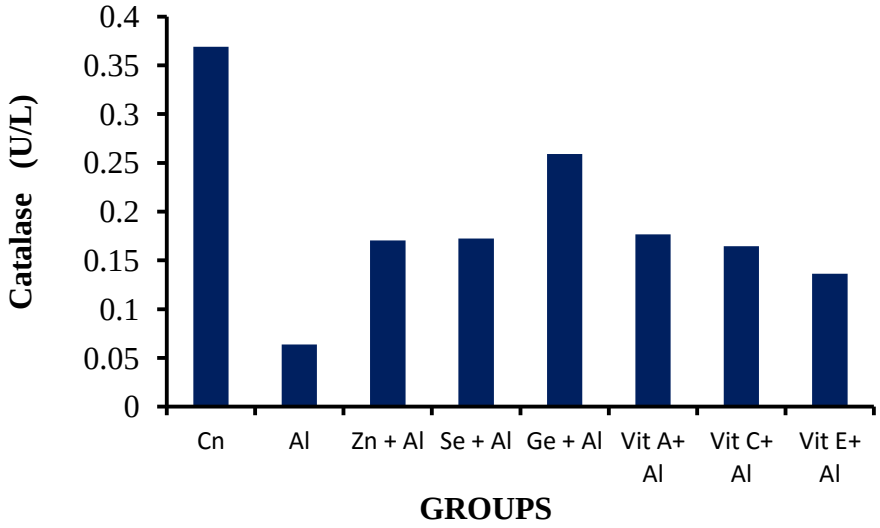


Figure 5 Effect of aluminium on Catalase Activity of wistar Rats

Hepatic MDA Level and GSH Content

Results presented in Figure 4.6 and 4.7 indicated that the level of MDA, an end product of LPO, was significantly ($p<0.05$) increased in Al-treated rats as compared to the control group. Conversely, hepatic GSH content showed significant ($p<0.05$) reduction in rats treated with Aluminium as compared to the control group. Administration of the antioxidants (Zinc, Selenium, Ginseng, Vitamin A, Vitamin C, and Vitamin E) resulted in an insignificant increase in the level of GSH when compared to the control group. Pretreatment with Zinc,

Selenium, Ginseng, Vitamin A, Vitamin C, Vitamin E respectively in Al-induced rats restored the values of MDA and GSH toward the normal value of the control (Figure 4.6 and Figure 4.7).

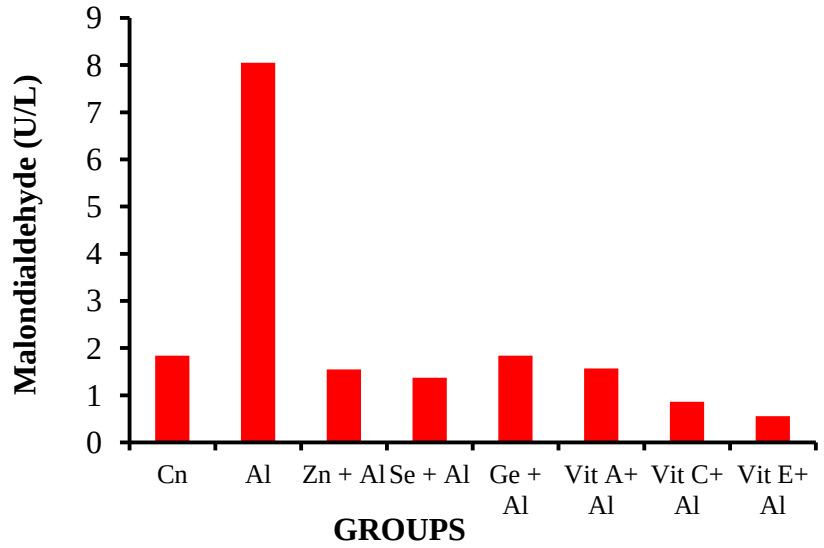


Figure 6 Effect of aluminium on Malondialdehyde Levels of wistar Rats

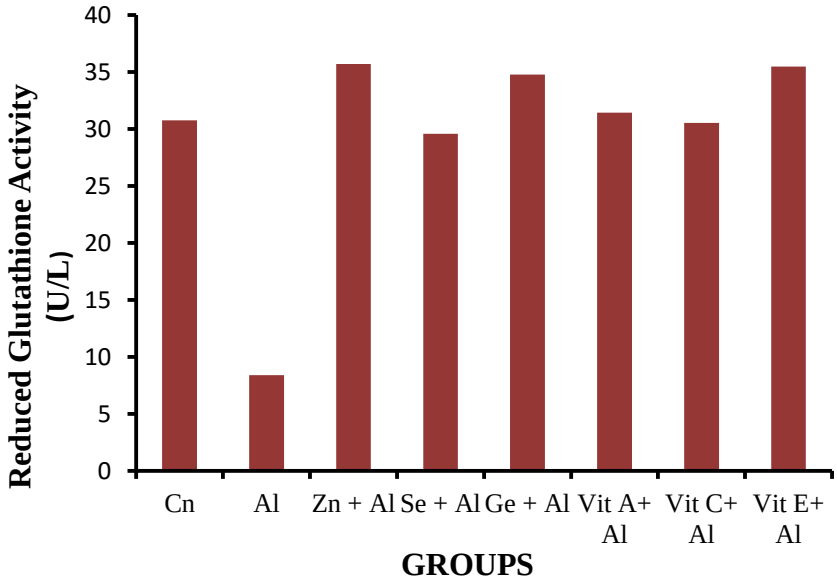
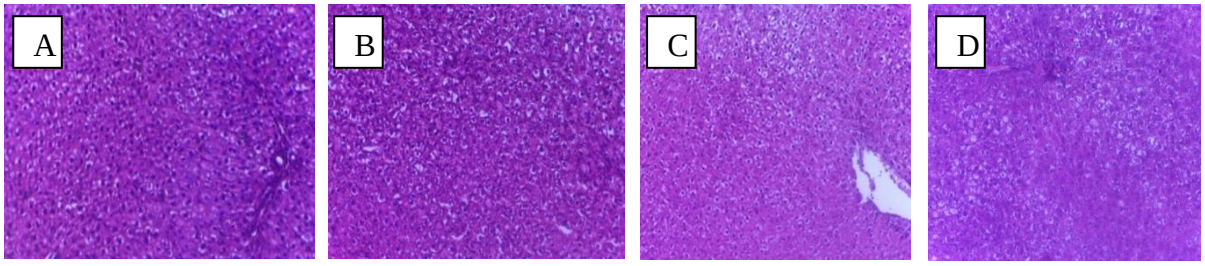


Figure 7 Effect of aluminium on Reduced Glutathione Activity of wistar Rats

Histopathological Examinations of the Liver of Male Wistar Rat



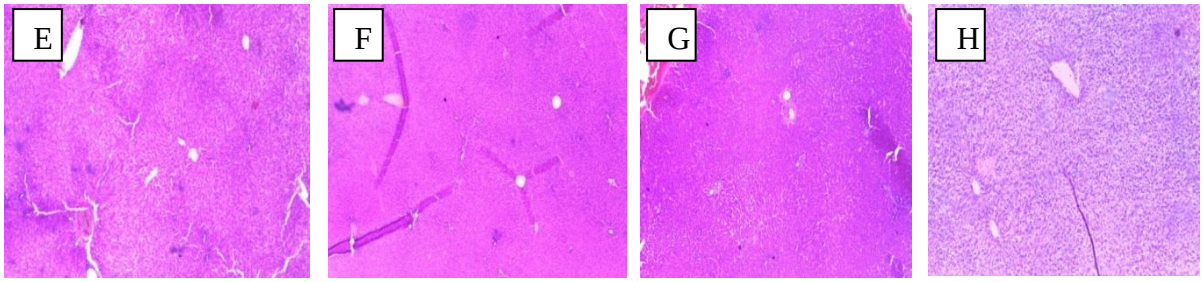


Plate 1: Photomicrograph of hepatocytes of various groups **A**: control group showing normal liver architecture. **B:** rats treated with Aluminium only showing vacuolized hepatocytes. **C:** rats treated with Aluminium and Zinc showing cytoplasmic vacuolations and distortion of lobular architecture. **D:** rats treated with Aluminium and Selenium showing cytoplasmic vacuolations and distorted liver architecture. **E:** rats treated with Aluminium and Ginseng showing normal liver architecture. **F:** rats treated with Aluminium and Vitamin A showing normal liver architecture. **G:** rats treated with Aluminium and Vitamin C showing normal liver architecture. **H:** rats treated with Aluminium and Vitamin E showing normal liver architecture.

DISCUSSION AND CONCLUSION

Aluminum is broadly used in daily life throughout the world. The present study investigated the potential ameliorative effects of some selected antioxidants (Zinc, Selenium, Ginseng, Vitamin A, Vitamin C, Vitamin E) against the possible toxic effects of Aluminium on liver male rats through the evaluation of some biochemical parameters and enzymes in the serum in addition to oxidative stress in this organ.

Exposure to high concentrations of Al can result in its accumulation in the liver and in turn to alterations in the liver function (Nikolov *et al*., 2010). Elevated serum level of AST, ALT and ALP is indicative of liver injury, as these enzymes are present in large quantities in the liver and increases in the serum following hepatocellular damage or injury. In this study, significant ($p< 0.05$) increase in ALT, AST and ALP activities in the serum of rats treated with Aluminium alone compared with the corresponding group of control rats was observed. Serum liver function enzymes including AST, ALT, and ALP showed increased activities after long term administration of Al. Serum ALT level increases when cellular degeneration occurs, which in turn indicates the existence of liver diseases. This was similar with the findings of Ighodaro *et al.* (2102) and Buraimoh, *et al*., (2012), that aluminium has been implicated in liver damage in wistar rats. Result suggests that chronic Aluminium exposure induce hapatoxicity manifested by elevation of liver function enzymes. Amelioration of Al toxicity with some antioxidants respectively (Zinc, Selenium, Ginseng, vitamin A, Vitamin C, Vitamin E) had a significant improvement in the activities in ALT and AST. Therefore, these selected antioxidants can alleviate the Al-mediated hapatoxicty in male Wistar rats.

Reactive oxygen species (ROS) have been implicated in the etiology of several diseases including atherosclerosis, inflammatory conditions, neurodegenerative diseases, cancer, diabetes mellitus, renal, pulmonary, cardiac diseases and the process of aging (Young and Woodside, 2001). It has been reported that the toxic effects associated with Al are related to the generation of ROS which results in oxidative damage to cellular lipids, proteins and DNA (El-Demerdash *et al*., 2004). LPO, as one of the main manifestations of oxidative damage, has been found to be a major contributor in the pathogenesis of many diseases and in the toxicity of many xenobiotics (Anane and Creppy, 2001). The data obtained in the present

study revealed elevation in the level of hepatic MDA as a marker for lipid peroxidation. Several investigations reported that Al has the ability to potentiate iron-mediated lipid peroxidation (Oteiza, 1994; Ohyashiki *et al*., 1998). Disruption in mineral balance through replacing iron ions with Al and the subsequent increase in the amount of the free iron can explain the increased lipid peroxidation. The free iron ions have a strong catalytic power to generate highly reactive hydroxyl radicals from hydrogen peroxide through Fenton's reaction (Ward *et al*., 1978). These radicals are able to initiate lipid peroxidation and cellular damage. These findings agree with Thangarajan *et al.* (2013) who showed that administration of alcoholic seed extract of *Calastrus paniculatus* caused a significant decrease in the level of lipid peroxidation and hence MDA.

The body has antioxidative mechanisms to stabilize oxidative molecules, control lipid oxidation and keep these radicals in balance. When free radicals are generated, the body defends itself from these radicals by endogenous antioxidants (Halliwell, 1994). However, when endogenous antioxidants become insufficient in defence against oxidants, exogenous antioxidants are needed to restore the balance. Endogenous antioxidants, either non-enzymatic (as GSH) or enzymatic (as GPx, SOD and CAT) represents the first line of defence against free radical damage and are crucial for preventing or at least slowing the incidence and progression of diseases (Jacob, 1995). The findings of the present study showed that the administration of Aluminium induced a status of oxidant/antioxidant imbalance as indicated by the increased MDA level and SOD level with a concomitant depletion in GSH content and in the activity of GSH and CAT in the liver tissue. These findings are consistent with previous studies that reported Al intake to be related to alteration in the activity of tissue antioxidant enzymes and promotion of oxidative stress (Yousef, 2004; Nehru & Anand, 2005; Newairy *et al*., 2009). Orihuela *et al*. (2005) reported that high doses of Al are able to induce free radicals and resulted in reduced GSH synthesis by decreasing glutathione-synthase activity. The reduction in the activity of GSH and CAT observed in the present study may be attributed to the reduced synthesis of these enzymes due to higher intracellular concentrations of Al and/or accumulation of free radicals. The data obtained in the present study illustrated that the antioxidants (Zinc, Selenium, Ginseng, Vitamin A, Vitamin C, Vitamin E) restored the oxidant/antioxidant balance as reflected by the decrease in MDA level and the stimulation of the antioxidants GSH, SOD and CAT in the liver.

In conclusion, the present study indicated that oral administration of Aluminium daily for 6 weeks caused hepatic dysfunction, increase in lipid peroxidation and decline in the activity of antioxidant enzymes in the liver. It also induced histological changes in the liver which all are attributed to free radicals production and oxidative stress. Administration of the antioxidants (Zinc, Selenium, Ginseng, Vitamin A, Vitamin C, Vitamin E) prior to Aluminium intoxication minimized its harmful effects and protected the liver against its toxicity. Therefore, supplementation with these antioxidants (Zinc, Selenium, Ginseng, Vitamin A, Vitamin C, and Vitamin E) is useful as a hepatoprotective therapy in cases of intoxication with aluminium.

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