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RENAL BIOMARKERS AND HISTOMORPHOLOGICAL ALTERATIONS IN *RATTUS NORVEGICUS* WISTAR STRAIN EXPOSED TO HEMATITE

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ABSTRACT

Hematite (iron ore) is one of the heavy metals causing environmental hazards and damage to the body. This study examined the effect of iron ore on renal function biomarkers and histo-architecture in Wistar Rats. A total of 20 male albino Wistar rats subdivided into four groups of 5 rats each were used. Group A served as control while B, C and D were experimental groups. B and C received 3mg/kg b.wt and 4.5mg/kg bwt of iron ore while D received 4.5mg/kg b.wt of iron ore and Vitamin E for 28 days respectively, after which the animals were sacrificed to harvest the kidneys for histological analysis. Blood samples were also collected in plain bottles for renal function biomarker analysis. Data obtained were expressed as mean ± SEM while One way Analysis of Variance (ANOVA) was used to compare means, with level of significance accepted at $p<0.05$. Results showed significant increase of Urea, Creatinine, Sodium ion and Bicarbonate ion ($p<0.05$) in a dose dependent manner, but a decrease of potassium ion and chloride ion in like manner. A dose dependent histo-architectural distortion of the kidney was also observed. However, cconcomitant administration of Vitamin E modulated the renotoxic potentials of the iron ore.

Key Words: Hematite, Heavy metals, Renal biomarkers, Renal histomorphology

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INTRODUCTION

Due to industrialization, a lot of waste products are released to the environments which are toxic to human health. These toxic agents include heavy metals like cadmium, copper, lead, chromium and mercury; all of which are important environmental pollutants, particularly in areas with high anthropogenic pressure (Msaky *et al.,* 1990). The mobilization of heavy metals into the earth surface by human activity has become an important process in the geochemical cycling of these metals (Bilos *et al.,* 2001).

Contamination of garden soils by metals may be widespread in urban areas due to previous industrial activity and the use of fossil fuels (Chronopoulos *et al.,* 1997). Heavy metals may enter the human body through inhalation of iron ore dust particles, direct ingestion of soil and consumption of food plants/vegetables grown in iron ore metal contaminated environment (Cambra *et al.,* 1999). Exposure to possible toxic metals from dust inhalation or soil ingestion is estimated simply as the concentration of a contaminant measured in soil multiplied by the quantity of iron ore dust inhaled or soil ingested (Konz *et al.,* 1989). This is a conservative approach to estimate dose because the bioaccessibility of heavy metals such as iron ore, lead, chromium, cadmium and copper absorbed or ingested soil particle is not 100% (Ruby *et al.,* 1999).

Iron ore is important as it serves as a raw material for the manufacture of metallic plates, iron/steel rods, building materials, poles and also vital in galvanization and electroplating of metals (Warnock et al., 2004). Iron compounds serve some medicinal purposes in the provision of remedy for combating complications of anaemia when the haemoglobin level declines (Milman *et al.,* 2007).

However, studies on iron ore toxicity in human have shown some level of reduction in the renal and hepatic function (Berglund *et al.,* 1994).Certain agents that have been implicated in kidney damage includes iron ore, ferric oxide, iron oxide, lead, cadmium and chromium (Hotz *et al.,* 1999) and some of these agents have been used in manufacturing with possible carcinogenic consequences.

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This study therefore, examines the changes in the renal function biomarkers and renal histo-architecture in albino Wistar rats treated with iron ore.

MATERIALS AND METHODS

Chemicals and drugs: The iron ore used for this experiment was collected from Itakpe, Kogi State, Nigeria, in a coarse form and pounded into fine powdered particles with the aid of a laboratory ceramic mortar and pistle after which it was weighed and mixed with distilled water. The α-Tocopherol (Vitamin E) used for this study was purchased from Sigma Chemicals Co. St Louis, USA.

Animals: Twenty adult male *Rattus norvegicus* Wistar strain weighing 190 ± 30g procured from the Animal House, Faculty of Basic Medical Science, Delta State University, Abraka, were used for the study after ethical clearance from Institutional Ethical Committee was obtained. They were kept in cages under standard laboratory conditions and fed with commercial rat feed (supplied by Ewu Flour Mill, Ewu, Edo State, Nigeria) and water ad libitum. Prior to the study the animals were allowed to adapt to laboratory conditions for seven days.

Experimental protocol: Animals were randomly assigned into four groups of five each. Group A served as normal control and received water (p.o. daily for 28 days); Group B received 3 mg/kg b.wt. p.o. daily for 28 days; Group C received 4.5mg/kg p.o. daily for 28 days, while Group 4 received 4.5mg/kg p.o. daily for 28 days + Vitamin E (40 mg/kg once daily for 28 days).

Sample collection: Twenty-four hours after administration of the last dose of iron ore, the rats were sacrificed by cervical dislocation. Blood was collected by heart puncture and transferred into a plane tubes. Serum was obtained by centrifugation and used for the estimation of renal electrolytes, urea and creatinine. The kidneys were also harvested and cleaned thoroughly with 0.9% sodium chloride solution (saline) and cut into micro pieces fixed in 10 % formalin for histopathological examinations.

Biochemical Analysis: Serum urea was measured by enzymatic colorimetric method as described by Cheesbrough (1991). Serum creatinine was measured by colorimetric method according to Cheesbrough (1991).

Histopathology of kidney: The histological preparation of the kidney of rats was done by the procedure described by Humason (1972). The tissues were isolated and gently rinsed with physiological saline solution (0.9%). They were fixed in Bouin's fluid (25 ml 40 % formaldehyde, 75 ml saturated aqueous picric acid, and glacial acetic acid) for 24 hours. Overnight running water was used to remove the fixative. Then the tissues were processed for dehydration using ethyl alcohol. The tissues were passed through alcohol containing 30%, 50%, 70%, 80%, 90%, 95% and absolute alcohols. Then the tissues were cleaned in methyl benzoate and embedded in paraffin wax. Sections of about 5 µ thickness were cut using rotatory microtome. The sections were stained with hematoxylin and counter stained with eosin dissolved in 95% alcohol. After dehydration and cleaning, the sections were mounted. Photomicrographs of the sections were taken using photomicrographing equipment.

Statistical Analysis: Data were represented in mean ± SEM. The evaluation of data for statistical significance between control and experimental groups was done using ANOVA. Statistical software, SPSS 20 was used to analyse the data. A *p-value* of less than 0.05 ($p<0.05$) was accepted as statistically significant.

RESULTS

Effect of Iron Ore on Urea level:

Iron ore caused a dose dependent significant ($p<0.05$) increase in the urea level when compared with the control. Vitamin E decreased the urea level of animals treated with 4.5mg/kg iron ore but this was not significant compared with the 4.5mg/kg group

Effect of Iron Ore on Creatinine level

Increase in doses of iron ore caused progressive and significant ($p<0.05$) increase in Creatinine level in rats treated with 4.5mg/kg iron ore. It was observed that Vitamin E slightly reversed the effects of 4.5mg/kg iron ore though with no significance.

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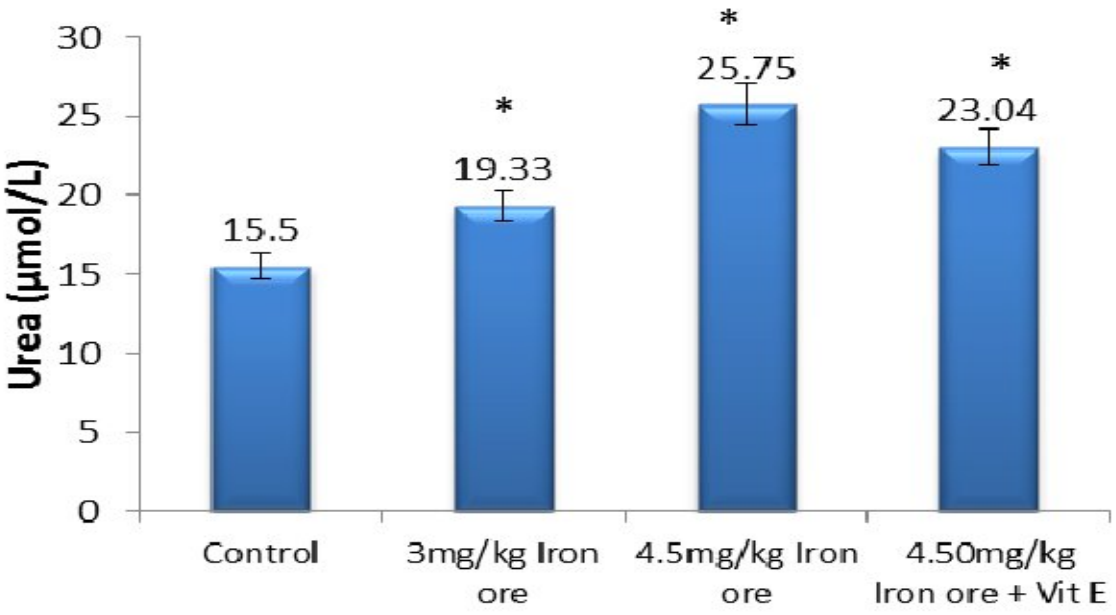

Fig 1 Effect of Iron ore on the Renal Urea *(n=5)*
*(*p < 0.05 compared with control group)*

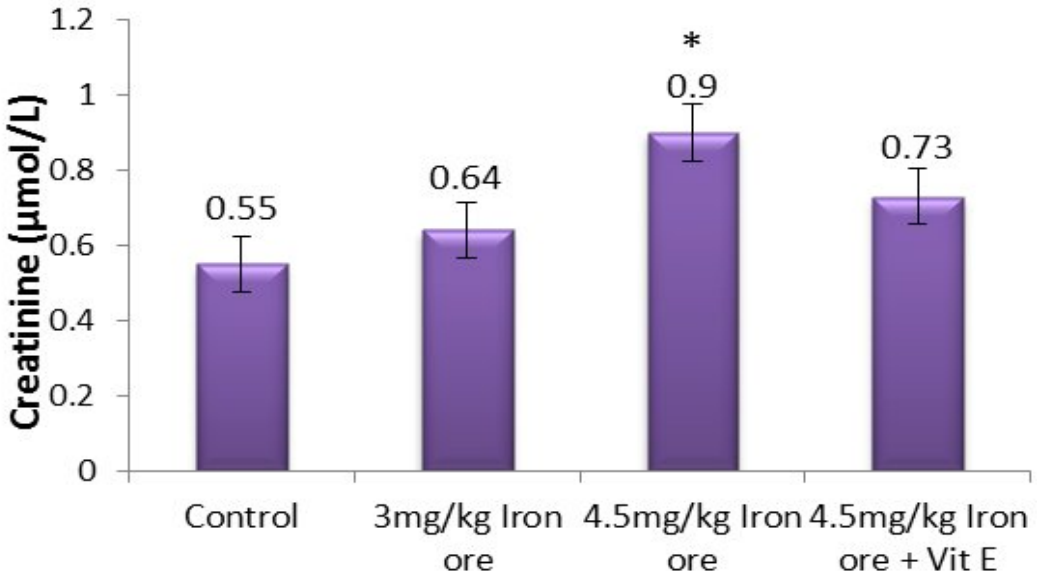

Fig 2 Effect of Iron Ore on the Creatinine Level
*(*p < 0.05 compared with control group)*

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Effect of Iron Ore on Sodium ion

Similar to findings in fig 1 and 2, it was observed that iron ore caused a dose dependent significant ($p<0.05$) increase in the sodium ion level. Subsequent treatment with antioxidant Vitamin E ameliorated the adverse effect of iron ore.

Effect of Iron Ore on Potassium ion

In the determination of effect of iron ore on potassium level, it was found that there was a dose dependent decrease in the level of potassium which was significant ($P<0.05$) only in the 4.5mg/kg group. Reversal effect was also observed in 4.5mg/kg iron ore rats treated Vitamin E.

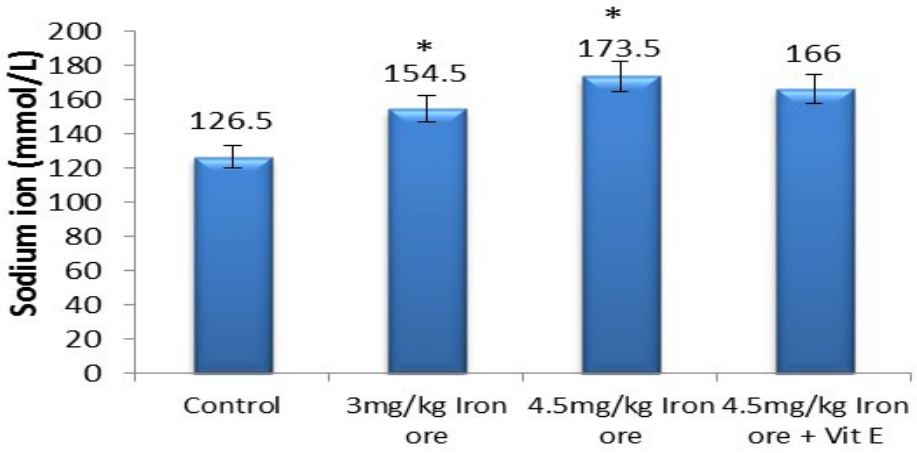

Fig 3 Effect of Iron ore on the Sodium ion level *(n=5); (*p < 0.05 compared with control group)*

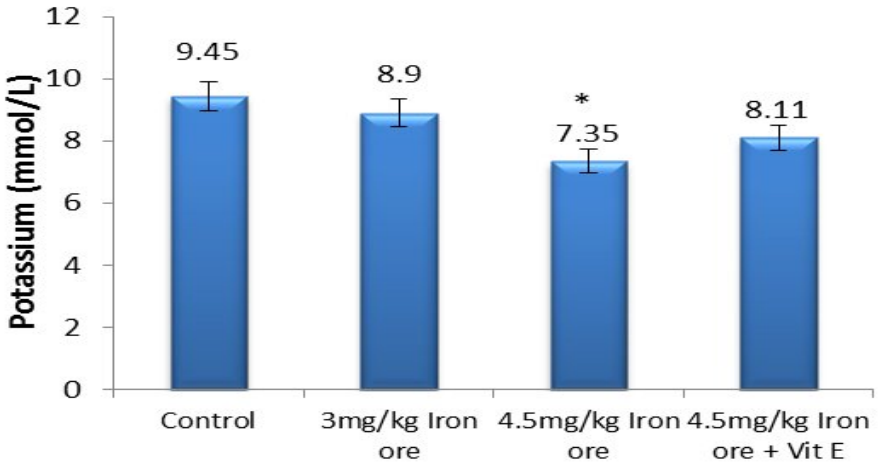

Fig 4 Effect of Iron Ore on the Potassium ion level *(n=5); (*p < 0.05 compared with control group)*

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Effect of Iron Ore on the Chloride ion

In a dose dependent manner, iron ore increased the chloride ion level. Vitamin E administration on rats treated with 4.5mg/kg iron ore did not change the level of Chloride ion. Despite these changes there was no significant difference (see fig 5).

Effect of iron ore on Bicarbonate ion

Fig 6 shows that increase in iron ore dosage significantly ($p<0.05$) decreased the bicarbonate ion level. It was also observed that Vitamin E treatment in rats induced with 450mg/kg iron ore increased the bicarbonate ion level but with no significance

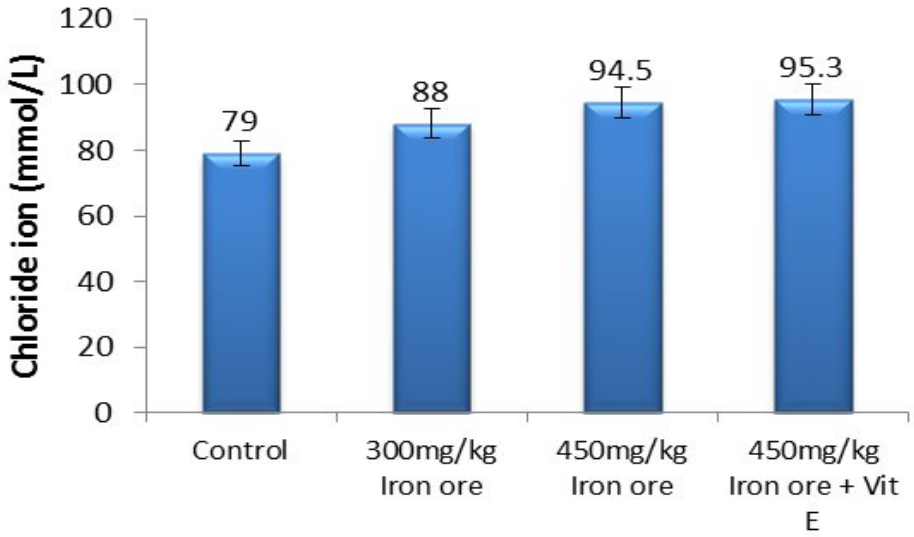

Fig. 5 Effect of Iron ore on the Chloride ion level *(n=5)*

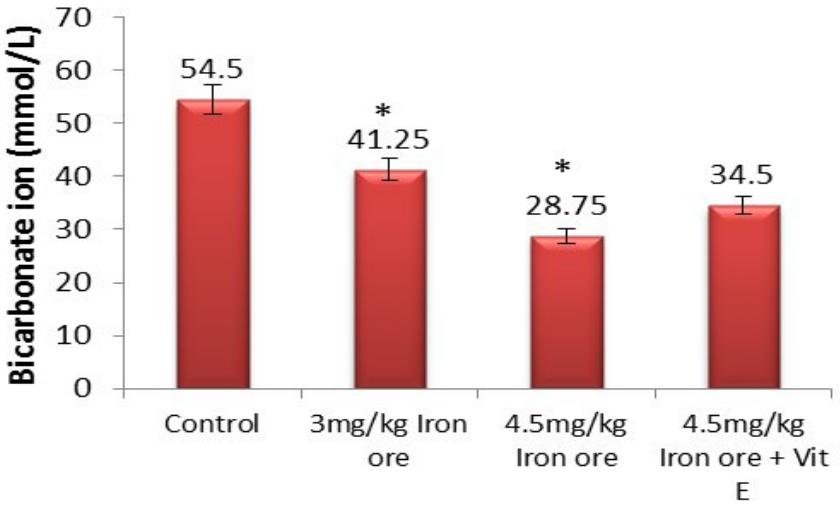

Fig. 6 Effect of Iron ore on the Bicarbonate ion level *(n=5)*
*(*p < 0.05 compared with control group)*

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Histology of the Kidney

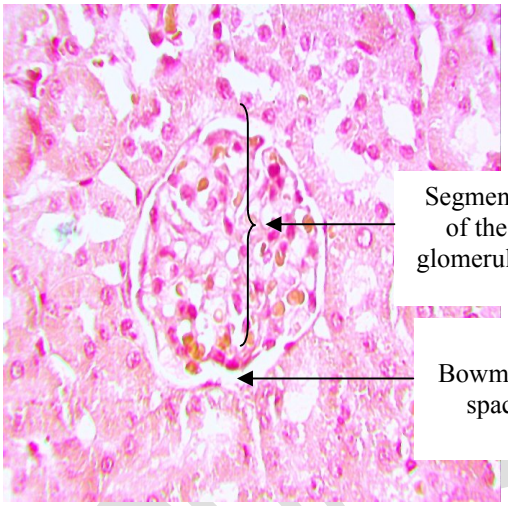

Plate 1: (Control) Section of the kidney showing several glomeruli disposed within a loose connective tissue stroma in which are several tubules. The tubules are lined by cuboidal cells. Also seen are several blood vessels. These features are in keeping with a normal kidney. (H and E x 400).

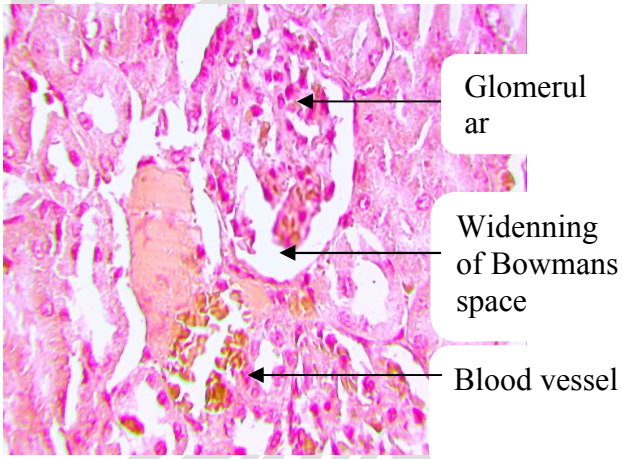

Plate 2: Rats treated with 3mg/kg iron ore body weight. Section of the kidneys showing glomeruli and tubules disposed in a loose connective tissue stroma. There is moderate glomeruli collapse with associated widening of the bowman's space in some nephrons. Features are in keeping with moderate glomeruli collapse (H and E x 400).

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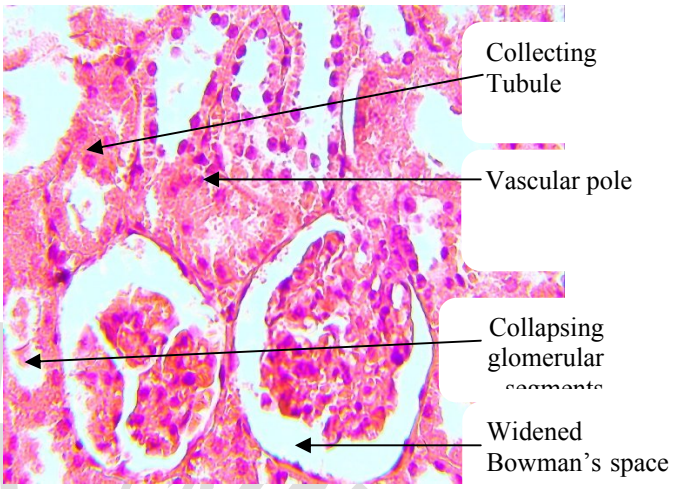

Plate 3: Rats treated with 4.5mg/kg. Section of the kidney showing glomeruli and tubules disposed within a loose connective tissue stroma. There is severe glomerular collapse with obvious bowman's capsular expansion. The tubules are however intact. Features are in keeping with severe glomerulopathy (H and E x 400)

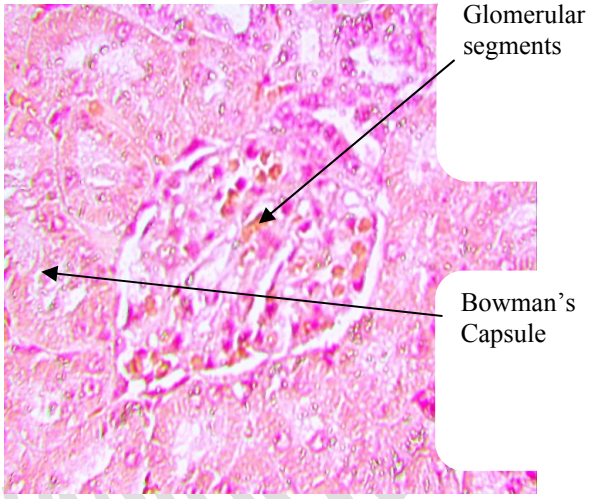

Plate 4: Rats treated with 4.5mg/kg iron ore + Vitamin E. Section of the kidney showing several glomeruli disposed within a loose connective tissue stroma in which are several tubules. The tubules are lined by cuboidal cells. Also seen are several blood vessels. These features are in keeping with mild glomerular collapse (H and E x 400)

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DISCUSSION

Iron ore is one of the agents that cause environmental hazards and are reported to be exceptionally toxic (Ellen et al., 1990). Exposure to iron ore from dust inhalation and water contamination is usually modeled simply as the concentration of a contaminant measured by the quantity of iron or dust inhaled or water ingested (Konz et al., 1989). Iron ore consist of trace elements which include silicon, phosphorus, lead, aluminium and sulfur (Rostoker et al., 1984).

In this research, it was observed that iron ore significantly ($p<0.05$) increased urea, creatinine, sodium and chloride ions level in a dose dependent manner when compared with control group. Conversely, iron ore decreased the levels of potassium and bicarbonate ions at doses of 3mg/kg and 4.5mg/kg. This result suggests that increasing doses of iron ore intake may have deleterious effect on the kidney. The deteriorative effect of iron ore on kidney can be attributed to the actions of manganese, chromium and iron oxides (Gordon 1996). Increase in urea, creatinine levels and sodium and chloride ions level have been reported and possibly attributed to Manganese ingestion which has deteriorative effects on the kidney (Wong, 1996).This was in accordance with the increase in renal biomarker levels in this present study.

In support of the findings of this study is the report by Kaneko (1989) that decrease in potassium and bicarbonate ion levels could be attributed to chromium which is an iron ore constituent with capacity to induce severe kidney damage. Thus, the observed effect of iron ore toxicity on the kidney can be attributed to the action of chromium. Complications resulting from this effect range from renal damage such as glomerular injury with specifications at the proximal convoluted tubules; injury to the brush border membrane is a feature of chromate nephropathy as reported by Kirschbaum and Sprinkel (1981). Severe poisoning can lead to acute tubular necrosis and acute renal failure (Sharma and Singhal (1978). Transient renal effects have been reported to result from low dose chronic chromium (VI) exposure. Acute iron ore exposure in workers at mines show alteration in the electrolytes. The electrolytes reported include sodium and potassium. Chronic iron ore patients may experience low blood concentrations of key electrolytes as well as potentially severe alterations in the body's acid-base balance (Pehoiu et al., 2005).

Vitamin E (α-tocopherol) seems to be a very important agent in providing protection against oxidation of cellular lipids by free radicals that are potentially damaging byproducts of cellular metabolism (Halsted, 2000). Vitamin E supplementation was been shown to present some level of protective effects against deterioration of kidney function in rats (Haidara et al., 2009). Similar result was found in this study with the mild reversal effects of Vitamin E on the iron induce kidney damage. Beytut *et al*. (2003) and Ognjanovic *et al*. (2003) had earlier demonstrated the effectiveness of vitamin E in reducing oxidative stress in heavy metal-treated animals and suggested that reductions in lipid peroxidation due to Cadmium toxicity may be an important factor in the action of vitamin E.

CONCLUSION

This study showed that iron ore has destructive effect on the kidney through glomerular injury and the distortion of the histo-architecture of the kidney. This results to alteration in urea, creatinine and electrolyte level of the kidney. This observation showed that the renal damage may be mediated by the production of reactive oxygen species (ROS) which seemed to be ameliorated by concomitant administration of antioxidant Vitamin E.

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AUTHORS CONTRIBUTIONS

The experiments of this study were carried out by Nwangwa, E.K. and Ekhoye E.I. with supervision, assistance and financial support from Ugorji, A.E. and Nwangwa, E.K . All authors were involved in the preparation of the final draft of this manuscript.

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