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Nephrotoxic potentials of *Oxytenanthera abyssinca* (Rhizomes) from crude oil polluted areas and non-polluted areas of South Eastern Nigeria

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ABSTRACT: *Oxytenanthera abyssinica* is a tropical drought resistant plant. Its rhizome had been wildly used in ethenomedicine for the treatment of dysentry, rheumatism, oedema and polyuria as well as for the management of diabetes. Rhizomes of O. *abyssinica* used for this study were sourced from Owerezukala Anambra State that has not experienced crude oil pollution and from Akirika community in Abia State that had experienced crude oil pollution. The aim of this study is to compare the nephrotoxic potentials of methanol extract of the rhizomes in order to ascertain the possible effects of crude oil spillage on bio lives of the two areas. After the extraction of the rhizomes of O. *abyssinica* with 80% methanol, the extracts from non- crude oil polluted area of Owerezukala (NCOPOAE) and crude oil polluted area of Akirika (COPOAE) were dried and stored in a freezer for further studies. Sub-chronic toxicity profile was evaluated using the effects of 100, 200, and 400 mg/kg b.w of the extracts on the albino rats for 28days. Kidney function tests were used for the evaluation of the integrity of the nephrocytes. Histopathology was done using standard method on kidney cells. Results showed LD_{50} of 5000 mg/kg and 3800mg/kg for NCOPOAE and COPOAE respectively. Exposure of rats to different doses of the extracts for 28days resulted to significant increases in kidney function test parameters with COPOAE showing more damage than NCOPOAE particularly at 200 and 400 mg/kg. This result could indicate kidney impairment. These results were confirmed by histoparthological assay, which revealed more damages in rats fed 200 and 400 mg/kg COPOAE than NCOPOAE. Histopathology of the kidney cells revealed different stages of necrotic and morphological damages at different concentrations of both extracts, changes being more in rats' organs treated with COPOAE than NCOPAE. These results could indicate that NCOPOAE was less toxic than COPOAE from crude oil contaminated community and hence could be more beneficial in ethnomedicine.

Keywords: Nephrotoxic, *O. abyssinica*, Histopathology and Electrolytes. NCOPOAE. COPOAE.

Introduction

Pollution is one of the greatest problems faced by living organisms, ranging from human beings, aquatic and wildlife. Pollution has introduced a lot of toxic chemicals to the environments which have been shown by researchers to be detrimental to the ecosystem. Crude oil being one major pollutant is a compound

mixture of over 6000 potentially different hydrocarbons and metals [1]. Exposure of workers to oil spill in their places of work and some cultural practices which involves the use of crude oil, does not only contaminate the environment but also endanger lives [2]. The regular forms of contact are dermal contact, inhalation, and ingestion of petroleum-contaminated food and water. Studies have documented the adverse environmental and health effects of petroleum hydrocarbons over the years

The kidney is a vital organ needed by the body to carry out numerous key functions including the maintenance of homeostasis, excretion and detoxification of toxic metabolites and drugs [3]. Acute kidney injury is the deterioration of the renal function over hours or days, resulting in the accumulation of toxic wastes and the loss of internal homeostasis. It can be caused by numerous etiologies [4, 5], this study is therefore aimed at studying the effect of crude-oil polluted *O. abyssinica* rhizome extract (COPOAE) and non crude-oil polluted *O. abyssinica* extract (NCOPOAE) on the kidney functions of albino rats.

Materials and Methods

Plant Materials

The rhizomes of *Oxytenanthera abyssinica* were collected from Owerezukala in Orumba Local Government Area of Anambra State Nigeria. The Oil polluted *Oxytenanthera abyssinica* rhizome samples were collected from Akirika Ndoki in Ukwa East Local Government Area of Abia State Nigeria. The rhizomes were authenticated in Nnamdi Azikiwe University Awka by the taxonomist.

Experimental Animal Models

Fifty six (56) male albino wistar rats weighing 140 – 200g were purchased from animal unit of Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were kept for two weeks in the animal house of Department of Biochemistry for acclimatization.

Chemicals

The chemicals and reagents used for this study include, methanol, chloroform, and ethanol are from BDH England; the commercial kits for urea and creatinine were products of Randox 4QY (United Kingdom) while kits for electrolytes were of Teco diagonistic kit. All other reagents used were of analytical grade.

Method of Extraction

The rhizomes of *oxythenantera abyssinica* were air dried at room temperature and by milling it was reduced to powder. The powder was extracted with 80 % methanol and concentrated with rotary eveaporator and stored at 4^0C until being used.

Experimental Design

Fifty six (56) male healthy albino rats were weighed and divided into seven groups of eight rats each according to their body weights, they were given standard animal feed and access to drinking water was allowed *ad libitum* for the period of the study. Group one served as the control and received normal saline while group two (2) to four (4) received NCOPOAE at doses of 100, 200 and 400 mg/kg. bw, groups five (5) to seven (7) received COPOAE at doses of 100, 200 and 400 mg/kg. b.w. respectively. At the end of every seven days two rats from each group were sacrificed. Blood samples were collected and allowed to clot, after which they were centrifuged at 3,000 rpm and the supernatant sera samples were used for urea, creatinine and electrolytes assay to determine the kidney functionality. At the end of the twenty eighth day of the study, the kidney of the rats were removed and used for histological assay.

Acute toxicity study

The LD_{50} of the extract was determined in mice using the Lorke method [6]. The animals were administered with the extracts and monitored for 24 hours for signs and symptoms such as excitation, paw licking,increased respiratory rate, writhing, convulsion and death. LD_{50} was calculated.

Assay method

Serum urea concentration, serum creatinine concentration, serum sodium ion (Na^+), potassium ion (K^+) , chloride ion (Cl^-) and biocarbonate ion (HCO_3^-) was determined following the method of Tietz [7] as outlined in Teco Diagonistic Kit. Histological examination of tissue was carried out by the method of Raghuramulu [8]. Tissue fixation was carried out immediately after removal of the liver from the rats with 10 % neutral buffered formaldehyde solution (7.0).

Statistical analysis

Data were reported as mean ± standard deviation of triplicate determination, where a. One – way analysis of variance (ANOVA) and student T-test were used to analyze the data results using statistical package for social science (SPSS) version 20. Group mean obtained after each treatment were compared with controls and difference considered significant when the results is $p<0.05$.

Results

Effects of NCOPOAE and COPOAE ON mean serum urea concentration of albino rats

The result showed that, at day 7, groups 6 (200mg/kg COPOAE) and 7 (400mg/kg 400mg/kg) significantly increased ($p<0.05$) serum urea concentration while group 4 (400mg/kg NCOPOAE) significantly reduced ($p<0.05$) serum urea concentration compared to group 1 control, all the groups administered COPOAE also significantly increased ($p<0.05$) serum urea concentration compared to groups administered NCOPOAE at day 7 of the study. At day 14, group 7 significantly increased ($p<0.05$) serum urea concentration compared to group 1 control while there was no significant change ($p>0.05$) in urea concentration in groups administered COPOAE compared to groups administered NCOPOAE. At day 21 and 28 of this study, there was no significant change ($p>0.05$) in urea concentration in all groups administered COPOAE compared to group 1 control and groups administered NCOPOAE as shown in Figure 1.

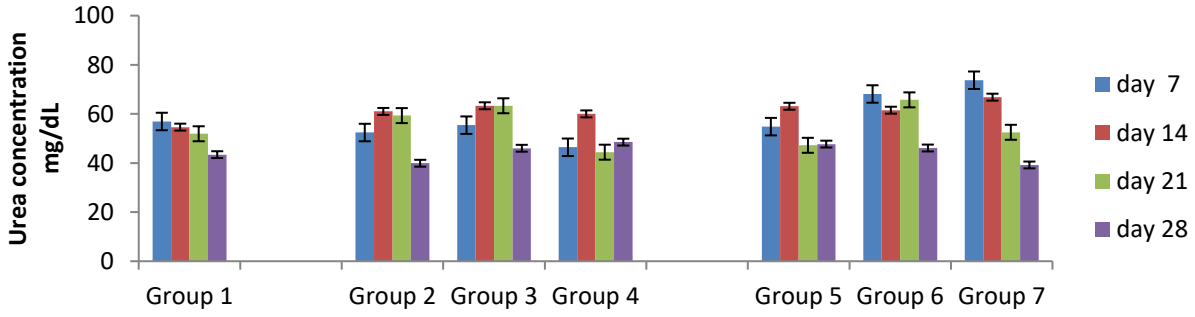

Figure 1: Mean Urea concentration of test and rats fed NCOPOAE and COPOAE.
Values are mean ± Standard deviation of triplicate determinations.

Effects of NCOPOA and COPOAE ON mean serum creatinine of albino rats

As shown in Fig 2, at day 7 of the study, groups administered COPOAE showed no significant change ($p>0.05$) in serum creatinine concentration compared to groups administered NCOPOAE and group 1 control. Group 6 administered (200mg/kg b.w COPOAE) at day 14 significantly increased ($p<0.05$) serum creatinine concentration compared to group 1 control while groups administered COPOAE had no significantly increased ($p>0.05$) in serum creatinine concentration compared to groups administered NCOPOAE. At day 21 and 28 of the study, there was no significant change ($p>0.05$) in serum creatinine concentration in groups administered COPOAE compared to group 1 control and groups administered NCOPOAE.

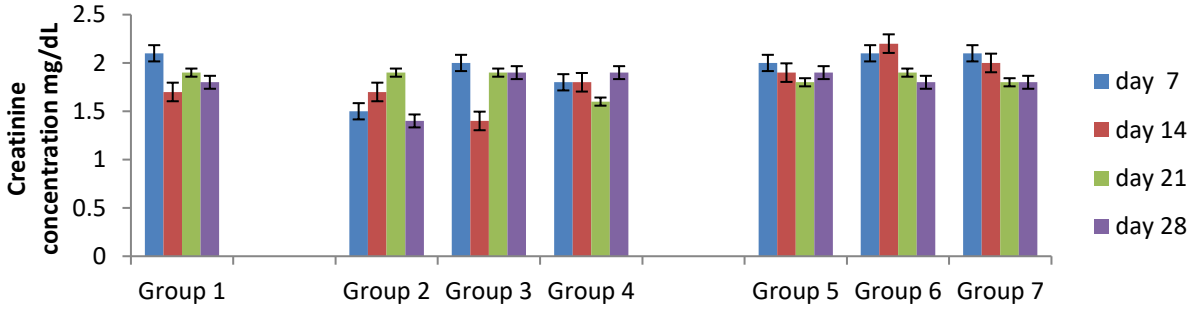

Figure 2: Mean Creatinine concentration of test and rats fed NCOPOAE and COPOAE
Values are mean ± Standard deviation of triplicate determinations.

Effects of NCOPOAE and COPOAE on mean Na^+ of albino rat

The result shows that, there was no significant difference of all the test groups compared to control at day 7 while group 6 (200mg/kg b.w. COPOAE) significantly reduced ($p>0.05$) Na^+ concentration compared to group 2 (100mg/kg b.w. NCOPOAE). Group 3 administered (200mg/kg b.w NCOPOAE) significantly increased ($p<0.05$) Na^+ concentration compared to group 1 control, while groups administered COPOAE showed no significant change ($p<0.05$) in Na^+ concentration compared to groups administered NCOPOAE at day 14 of the study , while at day 21, group 6 and 7 administered (200 and 400mg/kg b.w COPOAE) significantly reduced ($P<0.05$) Na^+ concentration compared to group 1 control, while there was no significant change ($p>0.05$) in Na^+ concentration in groups administered COPOAE compared to groups administered NCOPOAE. There was no significant change ($p>0.05$) in Na^+ concentration on the tested groups compared to group 1 control. While group 6 (200mg/kg b.w COPOAE) significantly increased ($p<0.05$) in Na^+ concentration compared to groups administered NCOPOAE at day 28 of this study. (Fig 3).

Effects OF NCOPOAE and COPOAE on mean serum K^+ concentration of albino rat

From the Figure 4, there was no significant change ($p>0.05$) in K^+ concentration in all groups administered COPOAE compared to group 1 control and groups administered NCOPOAE at day 7. 14 and 21 of the study, while at day 28 of the study, all the groups apart from group 7 administered (400mg/kg b.w COPOAE) non significantly increased ($p<0.05$) K^+ concentration compared to group 1. There was no significant change ($p>0.05$) in K^+ concentration in groups administered COPOAE compared to groups administered NCOPOAE at day 28 of the study.

Effects of NCOPOAE and COPOAE on mean Cl^- concentration of albino rat

There was significant decrease ($p<0.05$) in Cl^- concentration in all the tested groups compared to control, at day 7 of the study. While the groups administered COPOAE showed no significant change ($p>0.05$) in Cl^- concentration compared to groups administered NCOPOAE at the same day. At day 14, groups 2 and 7 administered with (100mg/kg NCOPOAE and 400mg/kg COPOAE) significantly reduced

($p<0.05$) Cl^- concentration compared to group 1 control. At day 21, there was no significant change ($p>0.05$) in Cl^- concentration in groups administered COPOAE compared to group 1 control and groups administered NCOPOAE. At day 28 of this study, all the tested groups significantly reduced ($p<0.05$) Cl^- concentration compared to group 1 control, while groups administered COPOAE showed no significant change ($p>0.05$) compared to groups administered NCOPOAE. (Fig 5).

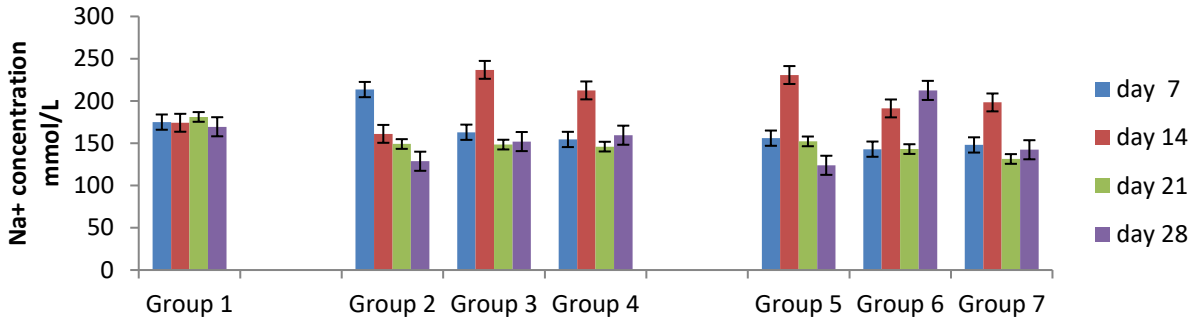

Figure 3: Mean Na^+ concentration of test and rats fed NCOPOAE and COPOAE
Values are mean ± Standard deviation of triplicate determinations.

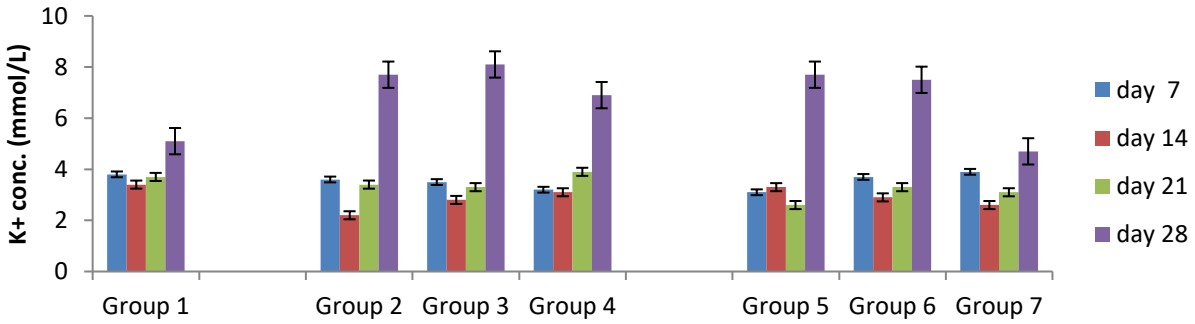

Figure 4: Mean K^+ concentration of test and rats fed NCOPOAE and COPOAE
Values are mean ± Standard deviation of triplicate determinations.

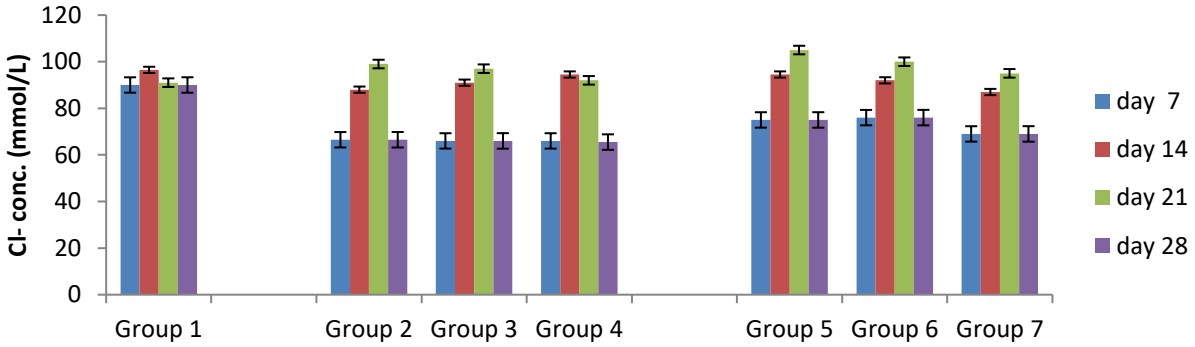

Figure 5: Mean Cl^- concentration of test and rats fed NCOPOAE and COPOAE
Values are mean ± Standard deviation of triplicate determinations.

Effects of NCOPOAE and COPOAE on mean serum HCO_3^{2-} concentration of albino rat

At day 7 and 14 of the study, there was no significant change ($p>0.05$) in HCO_3^{2-} concentration in all the tested groups compared to group 1 control, at day 21, group 3 and 7 administered (200mg/kg b.w NCOPOAE) and (400mg/kg b,w COPOAE) significantly reduced ($p<0.05$) HCO_3^{2-} concentration compared to group 1, while there was no significant change ($p>0.05$) in HCO_3^{2-} concentration at day 28 compared to group1 control. Within the groups, there were significant changes ($p<0.05$) in HCO_3^{2-} concentration with COPOAE having more effect. Fig 6.

Histopathology of the rats kidney fed NCOPOAE and COPOAE

The histomorphological changes in the kidney sections of the different groups of rats dosed with NCOPOAE and COPOAE (Plate 1 – 7), showed atrophy of glomerular tufts, minor vacuolation, increased lumen diameter and interstical nephritis seen in groups 2 and 3 fed with 100 and 200 mg/kg NCOPOAE. Groups 5 to 7 fed with 100, 200 and 400 mg/kg COPOAE showed degenerated glomerulus, renal fibrosis, slightly congested blood vessels, vacuolation and swollen glomerulus. These changes indicate kidney impairment by the extracts. Group 1 showed normal typical kidney cortex with intact glomerulus.

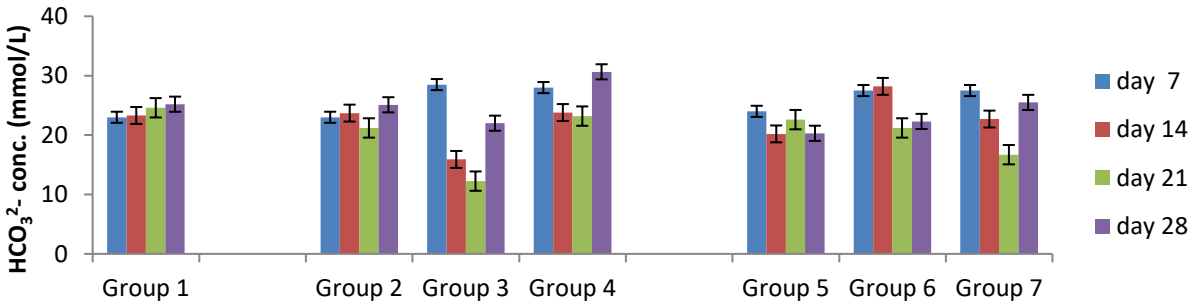

Figure 6: Mean HCO_3^{2-} concentration of test and rats fed NCOPOAE and COPOAE
Values are mean ± Standard deviation of triplicate determinations.

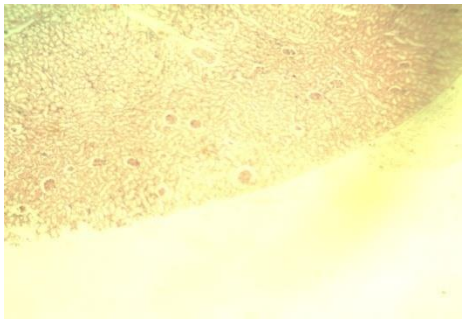

Plate 1: Photomicrograph of kidney of albino rat administered with normal saline + feed. This shows typical kidney cortex with intact glomerulus. (H&E. mag. 100X).

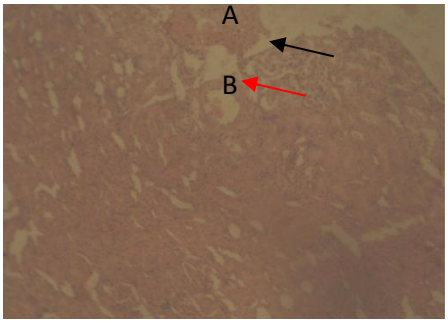

Plate 2: Photomicrograph of kidney of test rat fed with 100 mg/kg NCOPOAE. This shows,(1) minor enlarged urinary space with loss of glomerular contents (atrophy of glomerular tufts) (black arrow) (A) and minor vacuolations (red arrow) (B). (H&E. mag. 100X).

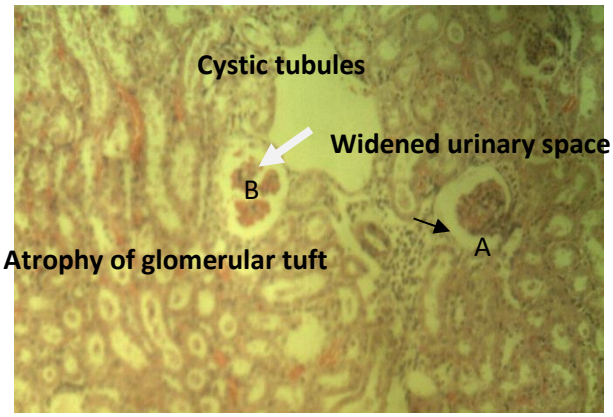

Plate 3: Photomicrograph of kidney of albino rat administerd 200 mg/kg NCOPOAE. The photomicrograph showed (1). Minorenlarged urinary space (black arrow) (A) with loss of glomerular contents (atrophy of glomerular tufts) (white arrow) (B). This condition is usually seen in advanced stage of chronic nephropathy and chronic infarctions. (H&E.mag.400X.)

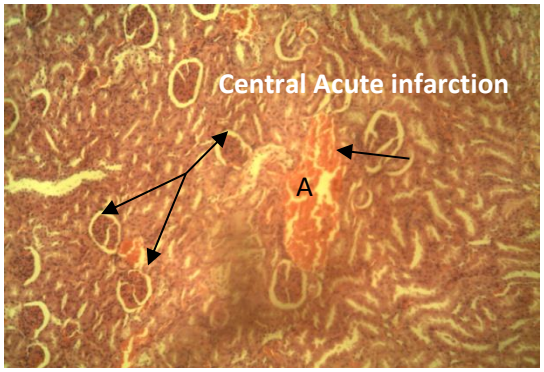

Plate 4: Photomicrograph of kidney of albino rat dosed with 400 mg/kg NCOPOAE and feed. This shows (1). Vascular changes which consisted of acute infarction (A)(black arrow) due to congestion in the central zone of the tissue, (2), intact tubules and glomeruli (B) (black arrow). (H&E. mag.100X).

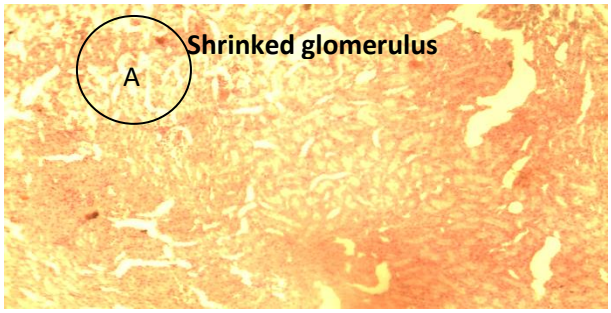

Plate 5: Photomicrograph of kidney of rat fed with 100 mg/kg COPOAE showing kidney cortex with intact tubules and degenerated glomerulus characterized by the widening of the urinary space, shrinked glomerulus (A) (circles). (H&E. mag. 100X).

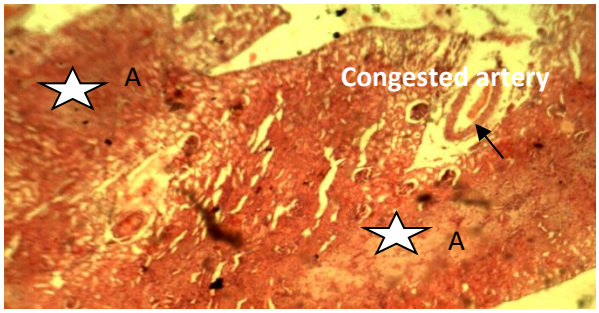

Plate 6: Photomicrograph of kidney of rat fed with 200 mg/kg COPOAE and water.The photomicrograph shows. Slightly congested blood vessel and shrinked glomeruli. Renal fibrosis(A) (star) with patchy deposits of collagens in the interstitium. (H&E. mag. 100X).

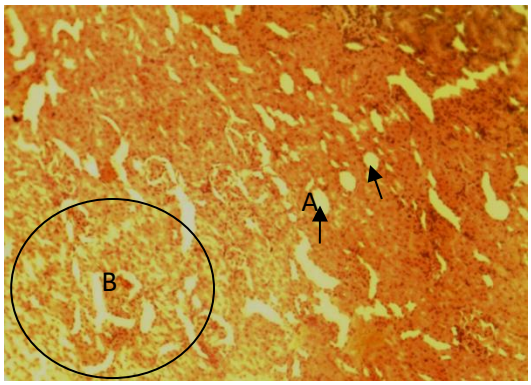

Plate 7: Photomicrograph of kidney of albino rat administered with 400 mg/kg COPOAE and water showing (1). Vacuolations (A) (black arrow) indicating degenerative changes in the kidney with discrete clear or transluscent spaces of variable size and swollen glomerulus (B) (circles) caused by congestion of glomerular capillaries and thickening of the glomerular basement membrane. (H&E. mag. 100X).

Discussion

Serum and urinary concentrations of creatinine and urea are indices of renal condition, and reduced urinary clearance of urea or creatinine evident by their reduced urinary concentrations and consequent rise in their serum concentrations are indicators of impaired renal function[9]. It was also equally shown that chemically induced nephrotoxicity caused by halogenated hydrocarbons, injured the proximal tubule monolayer, resulting in gaps in the epithelial lining leading to back leak of filtrate and reduced glomerular filtration rate [10]. Similarly, the increased urea and creatinine concentrations in rabbits exposed to crude oil contaminated food was also established [11]. The significant increase in urea serum concentration ($p<0.05$) and non significant increase in serum creatinine concentration ($p>0.05$) in the test rats, in which COPOAE have more increasing effect than NCOPOAE (Figure 1 and 2) could indicate kidney impairment. This observation is in agreement with the assertion that urea and creatinine concentrations were increased in Wistar albino rats fed crude oil contaminated diet [12]. This implies that the kidney was not able to excrete these metabolites and there was decrease in glomerular filtration rate which may have been induced by hydrocarbon fractions present in crude oil.

The significant decrease of serum sodium ion (Na^+) concentration ($p<0.05$) by group 6 and 7 test rats and non-significant increase of potassium ion (K^+) concentration ($p>0.05$) in all the tested groups of rats at day 28. (Figure 3 and 4) could indicate that NCOPOAE and COPOAE affected the Na^+/K^+ ATPase activity which pumps the transmembrane movement of Na^+ and K^+ ions against their concentration gradients [13]. The disruption of this enzyme activity could compromise the integrity of kidney and liver; thereby disturbing their normal functions adversely. Chloride – biocarbonate exchanger protein of the erythrocyte membrane is a co transport system that allows the entry into and exit of HCO_3^{2-} out of the red blood cells without change in transmembrane electrical potential[14]. Its role is to increase the CO_2 carrying capacity of the blood [15]. The significant decrease in Cl^- concentration ($p<0.05$) observed on days 7 and 28 in all the tested groups and the non-significant increase in HCO_3^{2-} concentration ($p>0.05$) observed in this study, could suggest that NCOPOAE and COPOAE affected the chloride-bicarbonate exchanger of the erythrocyte. This is in affirmation with the work reported by Achuba [12] that crude oil contaminated diet affected the concentration of serum electrolytes in albino rats.

The significant increase in serum urea, creatinine and potassium ion as well as the decrease in serum sodium and chloride ions observed in this study, seem to indicate compromised kidney functions, in which COPOAE compromised the kidney functions more than NCOPOAE. The crude oil components of COPOAE could contribute to kidney impairment [16]. This result correlates the histopathology results of the kidney functions in this study (Plate1 -7). It's also in tandem with the observation of Achuba and Nwokogba, [16] on crude oil toxicity and diesel toxicity.

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