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Comparative studies of hepatotoxic potentials of *Oxythenantera abyssinca* (Rhizomes) sourced from crude oil polluted areas and non crude oil polluted areas in South Eastern Nigeria using male albino rats as model

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ABSTRACT: Pollution has been one of the major problems faced by organisms and crude oil being one of the major pollutants especially to countries it serves as major component of their economy, living organisms could be directly or indirectly affected negatively by crude oil pollution. A comparative study of the effect of non-crude oil polluted *Oxythenantera abyssinca* rhizomes extract (NCOPOAE) and crude oil polluted *Oxythenantera abyssinca* rhizomes extract (COPOAE) in some biochemical parameters using albino rats. Serum liver enzyme activities and histopathology of the albino rats' liver was assayed after a four- week treatment using standard methods. Findings showed that, mice fed with NCOPOAE had no death at 5000 mg/kg .bw making it safe while the once fed with COPOAE had a lethal toxicity dose calculated to be 3807.8 mg/kg. bw. At different doses of 100, 200 and 400 mg/kg .bw., the extracts caused significant ($p<0.05$) and non-significant ($p>0.05$) changes on the liver enzyme activity which reflected in histopathological result compared to the control fed with feed and normal saline, indicating liver impairment with COPOAE having more of the negative effect compared to NCOPOAE and the control.

Keywords: NCOPOAE, COPOAE. *Oxythenantera abyssinica*, Hepatotoxicity.

Introduction

Pollution by crude oil is widespread and a universal problem, and mainly endemic in countries whose economies are dependent on the oil industry. Such pollution arises either accidentally or operationally wherever oil is produced, transported, stored, processed or used [1]. The constituents of crude oil are complex. It contains aliphatic, alicyclic, polyaromatic hydrocarbons, oxygen, nitrogen and sulphur containing substances with most of them being poisonous and accumulates in the body and induces toxic symptoms that sometimes result in death [2]. Plants serve as phytoextractors by taking up toxicants from their roots and accumulate them in their biomass [3]. Animal species that are not directly in contact with the oil spillage can also be affected negatively through the food web and predators that consumed contaminated marine preys can be exposed to oil through ingestion of the prey [4]. Residents within the areas polluted by crude oil use plants that have constantly experienced crude oil pollution as food source and in the treatment

of diseases. Therefore, this study compared the effect of crude oil polluted *O. abyssinica* sourced from Ukwa East in Abia State and non-crude oil polluted *O. abyssinica* sourced from Anambra, both in Nigeria in some biochemical parameters using wistar albino rats as model.

Materials and Methods

Plant Materials

The rhizomes of *O. abyssinica* were collected from Owerezukala of Orumba Local Government Area of Anambra State. The Oil polluted *O. 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 – 200 g 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 aspartate aminotransferase, alanine amino transferase, urea and creatine were products of Randox 4QY (United Kingdom) while kits for alkaline phosphatase and electrolytes were of Teco Diagnostic kit. All other reagents used were of analytical grade.

Method of Extraction

The rhizomes of *O. abyssinica* were air dried at room temperature and by milling it was reduced to powder. The powder was extracted with 80 % methanol and concentrated using rotary evaporator, 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 group and received normal saline while group two (2) to four (4) received NCOPOAE at doses of 100, 200 and 400 mg/kg. bw respectively. Group's 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 cloth, after clothing they were centrifuged at 3,000 rpm and the supernatant sera samples were used for liver enzyme activity assay. At the end of the twenty eighth days of the study, the liver of the rats were removed and used for histological studies.

Acute toxicity study

The LD_{50} of the extract was determined in mice using the Lorke method [5]. 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, after which the LD_{50} was calculated.

Assay method

Activity of aspartate aminotransferase (AST), and alanine aminotransferase (ALT) was assayed using the method described by Reitman and Franklel [6] as shown in Randox kit. While, Alkaline phosphatase (ALP) activity 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 *et al*. [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

Effect of NCOPOAE and COPOAE on mean serum aspartate aminotransferase (AST) activity of albino rats

The result (Figure 1), showed no significant change ($p>0.05$) in serum AST activity in all the tested groups compared to group 1 control at day 7. At day 14 of the study groups 2 rats fed 100 mg/kg b.w of NCOPOE significantly increased ($p<0.05$) serum AST activity compared to group 1 control while all the groups administered COPOAE significantly increased serum AST activity compared to group 3 fed (200mg/kg b.w) NCOPOAE at the same day . At day 21, groups 2 and 3 rats fed 100 and 200mg/kg NCOPOAE significantly increased ($p<0.05$) ALT activity compared to group 1 control while, groups 5 and 6 rats fed 100 and 200mg/kg b.w of COPOAE significantly reduced ($p<0.05$) serum AST activity compared to group 3 fed 200mg/kg NCOPOAE. At day 28 of this study groups 3 and 6 rats fed 200 and 200mg/kg b.w of NCOPOAE and COPOAE respectively significantly increased ($p<0.05$) AST activity compared to group 1 control. Groups fed COPOAE significantly increased ($p<0.05$) serum AST activities compared to group 4 (400mg/kg b.w NCOPOAE) at the same day. Generally the groups fed with COPOAE increased the activity of AST more than those fed NCOPOAE indicating liver impairment.

Effects of NCOPOAE and COPOAE on mean serum alanine aminotransferase (ALT) activity of albino rats

As shown in Figure 2, the result showed that, groups administered COPOAE non significantly increased ($p>0.05$) serum ALT activities compared to group 1 control and groups administered NCOPOAE at day 7, at day 14, groups administered COPOAE significantly increased ($p<0.05$) serum ALT activities compared to group 3 administered (200mg/kg b.w NCOPOAE). Also all the groups administered COPOAE non significantly increased ($p>0.05$) serum ALT activities compared to group 2 and 4 administered (100 and 400mg/kg b.w NCOPOAE) at day 14. All the tested groups showed non significantly increased ($p>0.05$) in serum ALT activities except group 4 administered (400mg/kg b.w of NCOPOAE) having significant reduction ($p<0.05$) compared to group 1 control at day 21 of the study, also all the groups administered COPOAE significantly increased ($p<0.05$) serum ALT activity compared to group 4 administered (400mg/kg NCOPOAE). At day 28 of the study, group 3 administered (200mg/kg b.w NCOPOAE) significantly reduced ($p<0.05$) serum ALT activity compared to group 1 control and the groups fed COPOAE. The significant increases observed in groups fed COPOAE compared to control and groups fed NCOPOAE imply liver damage.

Effects of NCOPOAE and COPOAE on mean serum alkaline phosphatase (ALP) activity of albino rats

As shown in Figure 3, the result showed that, at day 7, groups 2 and 7 administered (100mg/kg b.w NCOPOAE) and (400mg/kg b.w COPOAE) significantly increased ($p<0.05$) serum ALP activity compared to group 1 control while group 7 administered (400mg/kg b.w COPOAE) significantly increased ($p<0.05$)

serum ALP activities compared to group 5 administered (400mg/kg b.w NCOPOAE). At day 14 of the study, no significant change was observed in serum ALP activity in groups administered COPOAE, compared to group 1 control and groups administered NCOPOAE. Group 3 administered (200mg/kg b.w NCOPOAE) at day 21 significantly increased ($p<0.05$) serum ALP activity compared to group 1, while all the groups administered COPOAE significantly increased ($p<0.05$) serum ALP activity compared to group 4 administered (400mg/kg b.w NCOPOAE). Compared to group 1 and groups administered NCOPOAE, no significant change ($p>0.05$) in serum ALP activities was observed in groups administered COPOAE at day 28 of the study.

Histoparthological result of the liver of the rats fed NCOPOAE and COPOAE

The histomorphological changes in the liver sections of the different groups of rats dosed with NCOPOAE and COPOAE (fig 4 - 10), revealed that groups 2 – 4 fed with 100, 200 and 400 mg/kg NCOPOAE showed minor necrosis of the hepatocytes, hepatocyte cytoplasmic vacuolation, liver cholestasis ,intact hepatocytes with passive haemorrhage observed in the liver tissues, while groups 5 – 7 fed with 100, 200 and 400mg/kg COPOAE presented proliferated focal inflammatory cells, enlarged sinusoid, massive liver degeneration , chronic proliferation of predominance dilated inflammatory cells, Kupffer cells hyperplasia, enlarged and clogged sinusoids indicating liver impairment. The severity of liver damage is more in the groups dosed with COPOAE than NCOPOAE.

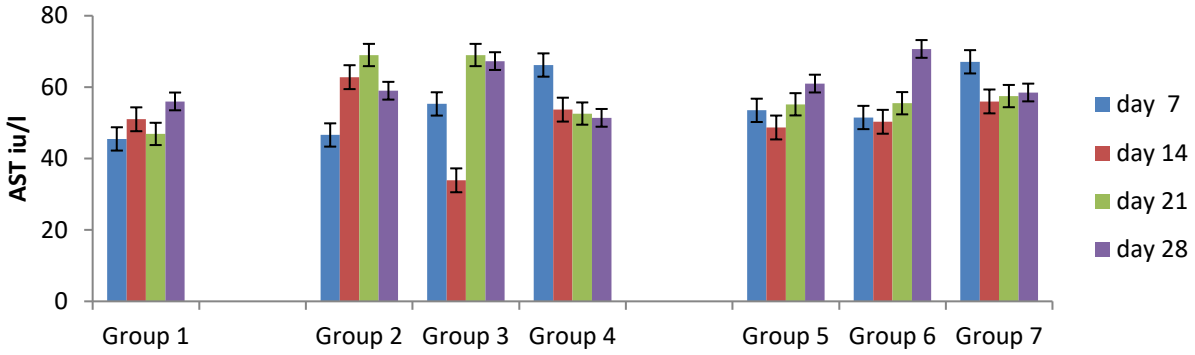

Figure 1: Mean aspartate aminotransferase activity of test and control groups
Values are mean ± Standard deviation of triplicate determinations.

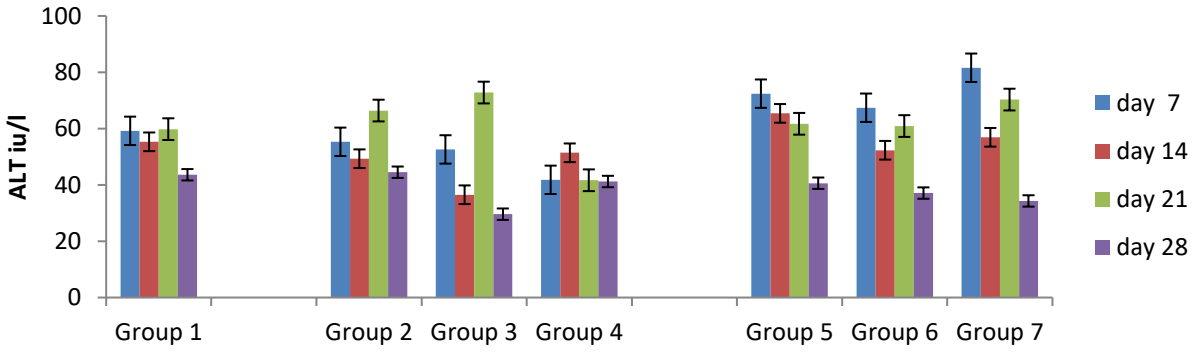

Figure 2: Mean alanine aminotransferase activity of test and control groups
Values are mean ± Standard deviation of triplicate determinations.

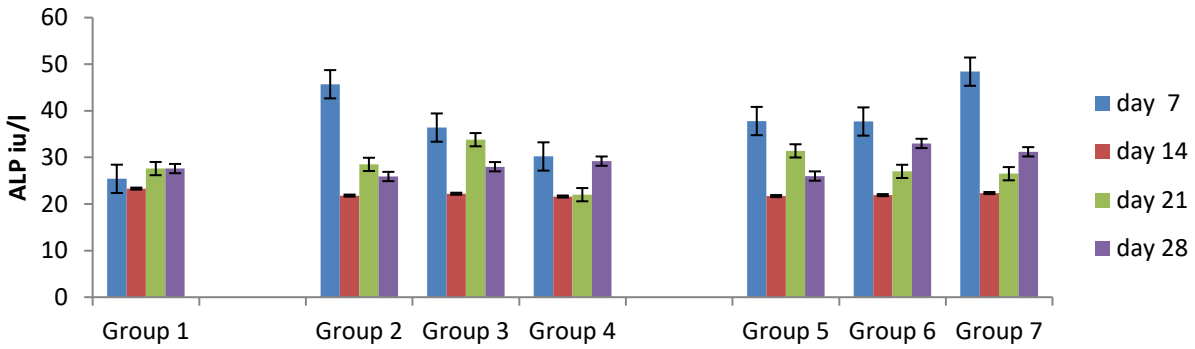

Figure 3: Mean alkaline phosphatase activity of test and control groups.
Values are mean ± Standard deviation of triplicate determinations.

Histopathology Results of rats liver administered with NCOPOAE and COPOAE

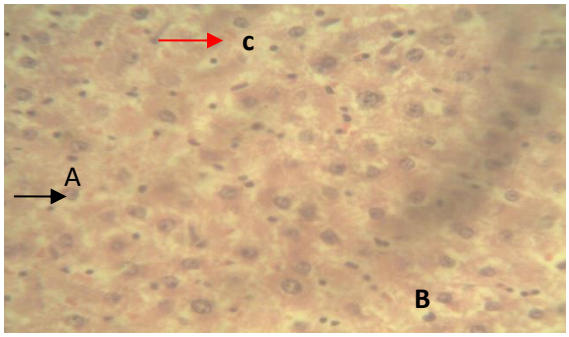

Figure 4: Photomicrograph of group 1 rat fed, normal saline and feed showing, **A**, Hepatocyte. **B**, Sinusoid. **C**, Kuffer cells. H&E.mag. 100x

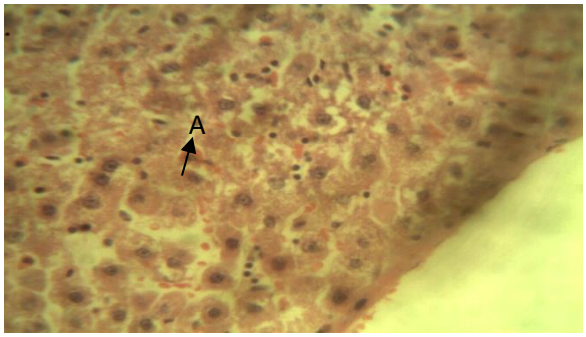

Figure 5: Photomicrograph of group 2 rat fed 100mg/kg NCOPOAE **A**, kuffer cells attached to the walls of the Sinusoid. H&E.mag. 100x.

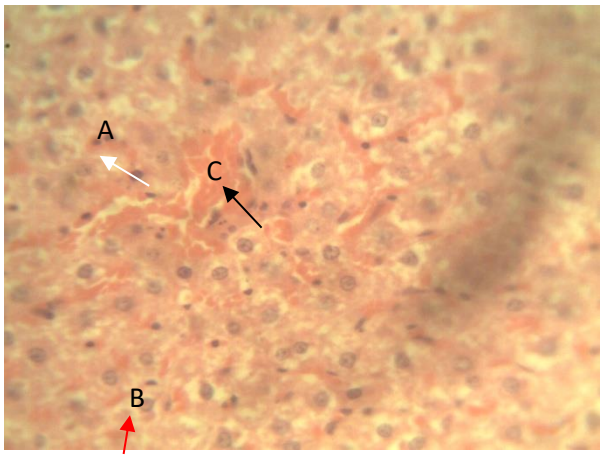

Figure 6: Photomicrograph of group 3, rat liver fed 200mg/kg NCOPOAE showing **A**, Pyknotic nuclei. **B**, Cytoplasmic vaculation. **C,** Red dense patches, signifying liver cholestais. H&E.mag. 100x

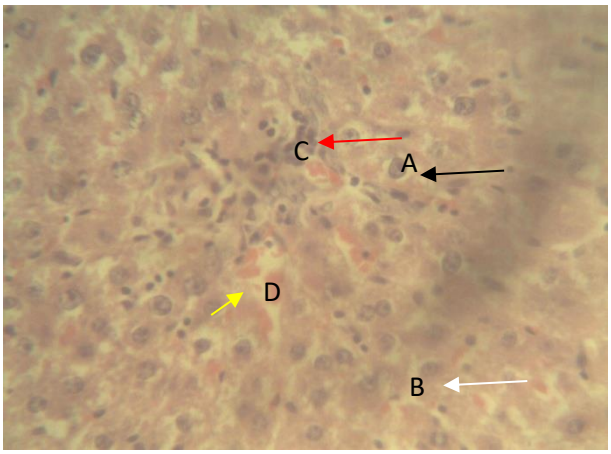

Figure 7: Photomicrograph of group 4 rat liver fed 400mg/kg NCOPOAE showing **A**, Intact hepatocyte **B**, Sinusoid, **C**,Kuffer cells **D**, Passive heamorrage. H&E.mag. 100x.

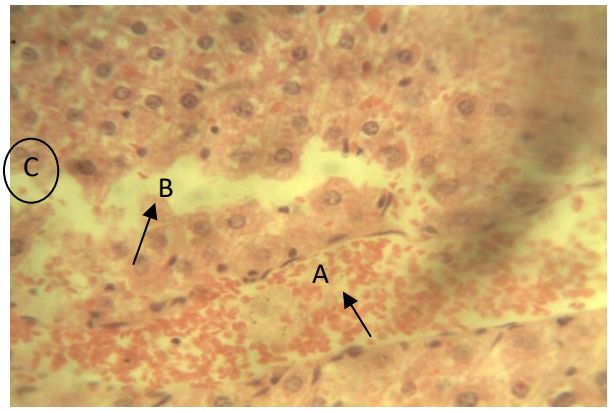

Figure 8: photomicrograph of group 5 rat liver fed 100mg/kg COPOAE showing, **A**, Proliferated inflammatory cells. **B**, Enlarged sinusoid. **C**, Binucleation of hepatocytes. H&E.mag. 100x

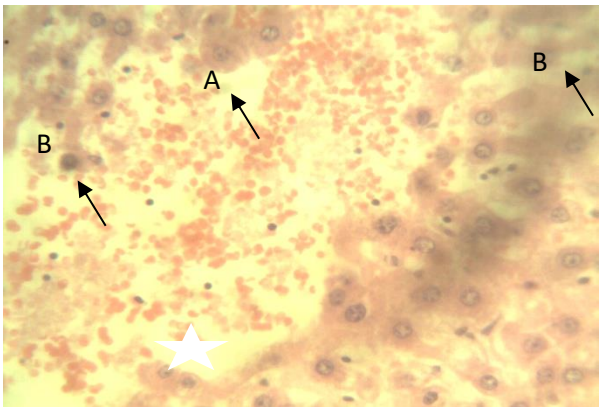

Figure 9: Photomicrograph of group 6 rat liver fed 200mg/kg COPOAE showing, **A**, Liver degeneration. **B**, Necrosis of the hepatocytes. H&E.mag. 100x

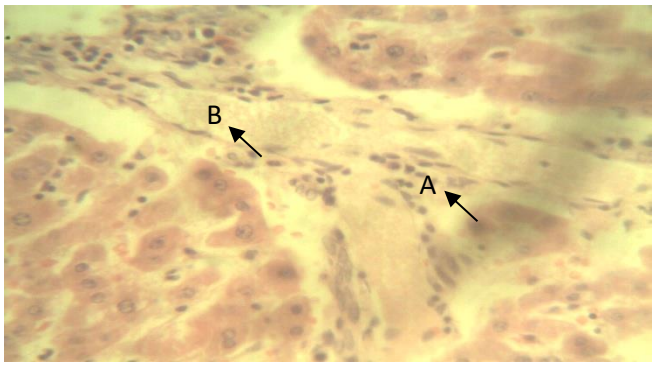

Figure 10: Photomicrograph of group 7 rat liver fed 400mg/kg COPOAE showing, **A**, Kuffer cells. **B**, Clogged and enlarged sinusoids. H&E.mag. 100x

Discussion

Crude oil has been described as a complex mixture of over 6000 potentially different hydrocarbons and metals [9] and its pollution, directly or indirectly has been shown to cause toxicological effects in animals [10]. The toxicity of crude oil on living systems could be investigated by evaluation of the biochemical and / or functional changes in the liver and kidney as they are involved in the metabolism of xenobiotics [11].The elevation of serum liver marker enzymes indicate cellular leakage due to damage of the structural integrity of the liver [12].The significant increase in serum AST, ALT and ALP by COPOAE and non-significant increase by NCOPOAE (Figure 1, 2 and 3) observed in this study, which correlates with the histopathology result (Figure 4 - 10) in which some of the organs damaged, indicates liver impairment. Lipophilic xenobiotics may have the characteristics of both electron uncoupler and energy inhibition [13]. A large proportion of crude oil component is lipophilic in nature therefore biological membrane may be the target site where adverse effect occurs [13]. The observed difference in the elevation of serum liver enzymes activity between NCOPOAE and COPOAE could be as a result of the presence of lipophilic hydrocarbons present in COPOAE and their direct consequences of damage to hepatocytes, which is in tandem with the assertion that serum liver enzyme markers were increased in albino rats fed with crude oil contaminated diets [14].

Conclusion

The finding of this study shows that, the use of *O. abyssinica* rhizomes sourced from non-crude oil polluted area and crude oil polluted area, negatively affected the biochemical statues of rats, with COPOAE having more of the effect, these suggests that the use of *O. abyssinica* rhizomes and plants that have been affected by crude oil pollution in ethanomedicine and as source of food by organisms should be monitored. Its continuous use may result to toxicity and destruction of important organs such as the liver and kidney.

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