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Original Research

Effects of excravos light crude oil on liver enzyme markers activity and malondialdehyde levels of rats

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Abstract

Crude oil has been implicated in causing many physiological effects when it is ingested directly or indirectly. The effects of ingesting varying concentrations of Excravos light crude oil on the activity of some liver marker enzymes and lipid peroxidation was studied in rats. The experiment was carried out for two weeks. Albino rats (n-24) were grouped into six of four rats per group in five different concentrations of the crude oil (0.1%, 0.25%, 0.50%, 0.75% and 1.00%) per body kg, which were administered by oral intubation, leaving out the last group as the control. The rats were fed on normal diet and water *ad libitum.* From the results, the malondialdehyde (MDA) level increased significantly ($p<0.05$) as the concentration of the crude oil increased in both days 7 and 14; indicating increased risks of lipid peroxidation and consequent rise in oxidative stress at high concentration of ingested crude oil. The effect of crude oil on liver function was also ascertained as indicated by a significant increase in the activities of some liver marker enzymes (AST and ALT) at low concentration of crude oil (0.10%) per body weight (Kg). Observed difference in the activities of ALP across the groups for days 7 and 14 was not statistically significant ($p>0.05$). These results are indicative of cases of increased lipid peroxidation during long term accumulation of ingested crude oil and a consequent emergence of serious hepatotoxic effects amongst other haematological effects.

INTRODUCTION

Crude oil is a naturally occurring complex mixture of flammable liquid hydrocarbons of various molecular weights often found together with natural gas formed by organic substances (especially those of plants and fossils origin) which were buried under the earth's surface thousands of years ago. This mixture of complex hydrocarbons contain various petroleum products such as Gasoline, Kerosene, Fuel oil, Lubricating oil, Gas Oil, Wax Distillate, Cylinder Stock or Bottoms and Asphalt. Moreover, among the hydrocarbons described so far, the Polyclyclic Aromatic Hydrocarbons (PAHs), present in crude oil, are amongst the most dangerous environmental contaminants due to their toxic, carcinogenic, and mutagenic effects [1]. Crude oil spillage is known to have constituted several environmental and physiological toxicity effects such as the pollution of the aquatic and terrestrial ecosystems; either due to the physical nature of the crude oil (chemical contamination and smothering) or by its chemical components which cause toxicity effects leading to tainting, thereby, affecting aquatic lives by the various clean-up operations or indirectly through physical damage to the habitats in which plants and animals live [2]. Consequently, terrestrial lives are hampered by the pollution of the aquatic habitat as many animals (including humans) and plants are at the risk of getting in contact with the polluted water and contaminated soil. Effect of consuming fish (*Clarias gariepinus)* cultivated in water contaminated with equal concentrations (10µg/ml) of phthalate, benzene and cyclohexane respectively on the liver of rats has been investigated. Standard enzyme assays conducted for selected liver enzymes followed by histological

examination of liver section shows that Serum albumin and globulin concentrations are found to be significantly lower in rats fed with contaminated *C. gariepinus* than control [3]. Growing evidence also revealed that animals placed on water contaminated with leachate from waste gain considerable body weight [4. 5, 6, 7, and 8]. Moreover, various environmental pollutants, particularly those associated with crude oil cause many biochemical and toxic effects in marine and terrestrial animals [9]. The liver is the principal organ of metabolism and has a role to play in many body processes most especially detoxification of chemical compounds [6, 10]. The effects of crude oil pollution are varied and may lead to various levels of liver damage. Increased ALT/AST ratio may be indicative of the extent of liver cell damage. Loss of AST and ALT activity in tissues may be interpreted as a compromise of the tissues integrity [11]. Crude oil consumption has also been found to inhibit protein metabolism in the liver [11]. Ovuru *et al*., [12] observed that ingestion of crude oil or crude oil contaminated fish leads to a decrease in liver – body weight ratio in rat. Other research studies have shown a variety of adverse effects on the hepatocytes of rats and catfish following exposure to environmentally toxic compounds [6, 10]. However, little or no attention has been given to effect of components of crude oil on public health. This work aims at divulging the belief (which lacks basic scientific backing) that crude oil ingestion and bath confers some therapeutic effects such as anti-poison, anti-convulsion or anti-fever effects on individuals; thus, ascertaining the toxicological effects associated with crude oil ingestion and re-establishing the above claims on proper findings within the limits of scientific experimental evidences.

MATERIALS AND METHODS

Experimental Protocol/Design

A total of twenty (24) Wistar rats were divided into six (6) groups of four (4) rats. The animals were grouped as:

Group 1: Rats administered 0.1% Escravos light crude oil

Group 2: Rats administered 0.25% Escravos light crude oil

Group 3: Rats administered 0.50% Escravos light crude oil

Group 4: Rats administered 0.75% Escravos light crude oil

Group 5: Rats administered 1.00% Escravos light crude oil

Reference/Normal Control: Rats fed normal diet and water *ad libitum.*

Collection of test sample

The test sample for the study was Warri Excravos light crude oil, obtained from the Department of Petroleum Resources (DPR), Nigeria National Petroleum Cooperation (NNPC), Port Harcourt in Rivers state of Nigeria and reported to have been extracted from Warri, in Delta state; also in Nigeria.

Determination of relative density of the crude oil

The relative density bottle was first weighed empty, and then 10ml of the crude oil sample was added into the bottle and weighed. The weight of oil was obtained by taking the difference between the weight of the empty bottle and the weight of the bottle containing 10ml of the crude oil. The weight of 10ml of water was also obtained using similar procedure.

The relative density was determined as follows:

$$\textit{Relative density} = \frac{\textit{Weight of crude oil}}{\textit{Weight of equal volume of water}}$$

The density of the Excravos light crude oil was determined to be *0.988.*

Mode of administration and collection of the samples

The crude oil was administered to the rats in the specified concentrations by oral intubation while the blood needed was collected by ocular bleeding.

Lipid Peroxidation (Malondialdehyde) Assay

Lipid peroxidation was determined spectrophotometrically by measuring the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin *et al*. [13].

Assay of Alkaline Phosphatase Activity

This was done using the QCA Commercial Enzyme Kit which is based on the phenolphthalein monophosphate method of Klein *et al.*[14]; Babson [15].

Assay of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST)Activities

A Randox Commercial Enzyme Kit based on the methods of Reitman and Frankel [16] and Schmidt and Schmidt [17] was used to test the activities of ALT and

AST.

Statistical Analysis

Data were reported as means ± SEM, where appropriate. Two-way analysis of variance (ANOVA) was used to analyse the experimental data. Students' t-test was used to analyse the differences between the two durations (Days 7 and 14) of the experiment. Differences were considered significant when $p \leq 0.05$.

RESULTS

Effect of Excravos light crude oil on Malondialdehyde (MDA) level

Figure 1 shows a significant decrease($p<0.05$) in the percentage of MDA in groups 2,3 and 5 when compared to the control in day 7 and the difference between groups 1 and 4 is not statistically significant ($p>0.05$) when compared to the control at day 7. Also there is a significant increase ($p<0.05$) in group 1,2,3,4 when compared to the reference control in day 14 and the observed difference in group 5 when compared to the control in day 14 is not significant ($p>0.05$).

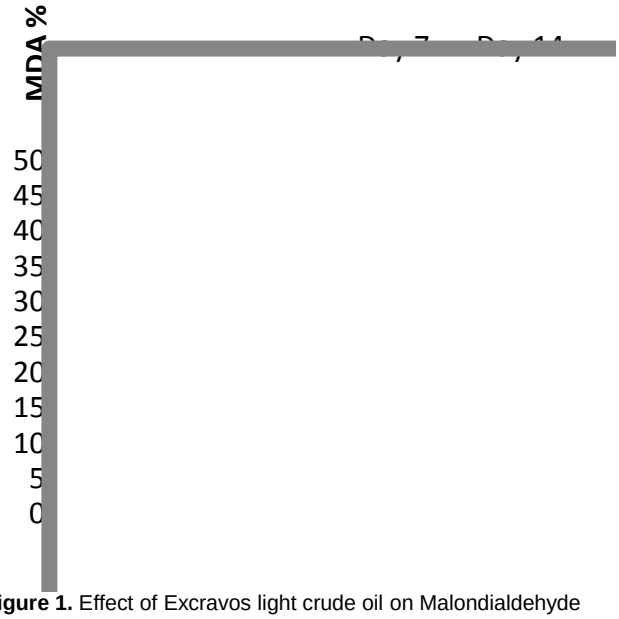

Figure 1. Effect of Excravos light crude oil on Malondialdehyde (MDA) level

Effect of Excravos light crude oil on the alanine aminotransferase (ALT) activity in rats.

Figure 3 depicts a significant increase ($p< 0.05$) in alanine amonotransferase (ALT) or Serum Glutamate-Pyruvate transaminase (SGPT) activity in group 1 for both days 7 and 14 when compared to the control and a subsequent decrease ($p>0.05$) in groups 2 and 3 at day 7 while the observed difference in ALT activity between days 7 and 14 in groupd 4 and 5 are not statistically significant when compared to the control. Also, the difference in ALT activity across the groups were not significant ($p>0.05$).

Effects of Excravos light crude oil on the alkaline phosphatase (ALP) activity in rats.

Figure 2 shows a relative decrease ($p> 0.05$) in the serum alkaline phosphatase (ALP) activity in groups 2 and 3 at day 7 and not in day 14; while the observed difference in the activity of alkaline phosphatase (ALP) in groups 1, 4 and 5 were not statistically significant at day 7. Also, at day 14, there was no significant difference in ALP activity across the groups.

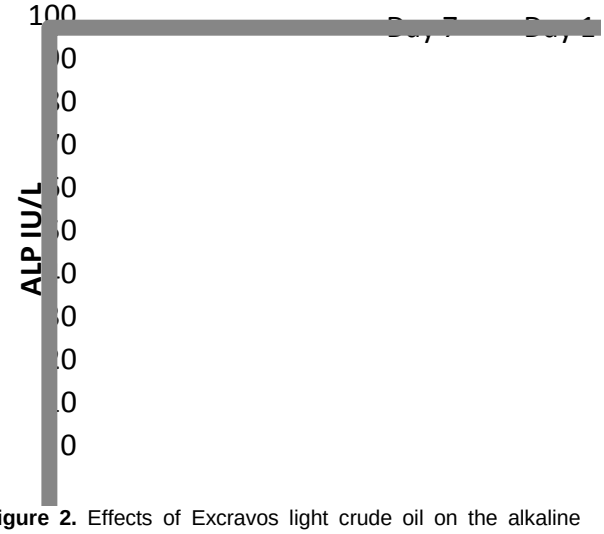

Figure 2. Effects of Excravos light crude oil on the alkaline phosphatase (ALP) activity in rats.

Effect of Excravos light crude oil on the aspartate transaminase (AST) activity in rats.

Figure 4 shows a significant increase ($p<0.05$) in aspartate transaminase (AST) activity in group 1 and a decrease in groups 2 and 3 while the observed difference between groups 4 and 5 was not significant for both day 7 and 14 when compared to the control. Day 14 also shows a relative increase ($p>0.05$) in groups 1, 2 and 3 when compared to the reference control.

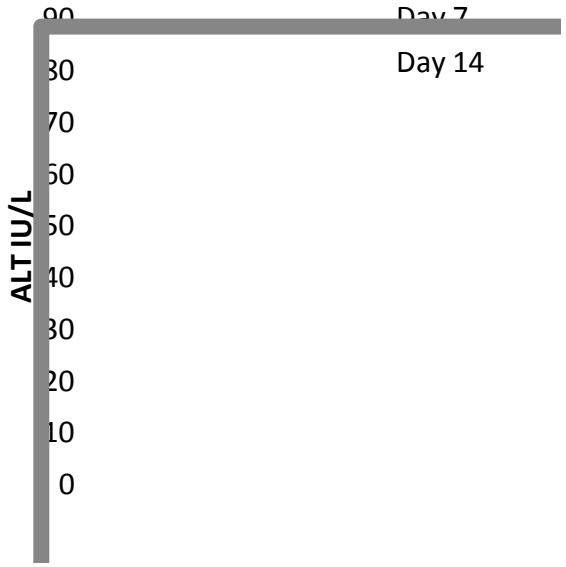

Figure 3. Effect of Excravos light crude oil on the alanine aminotransferase (ALT) activity in rats.

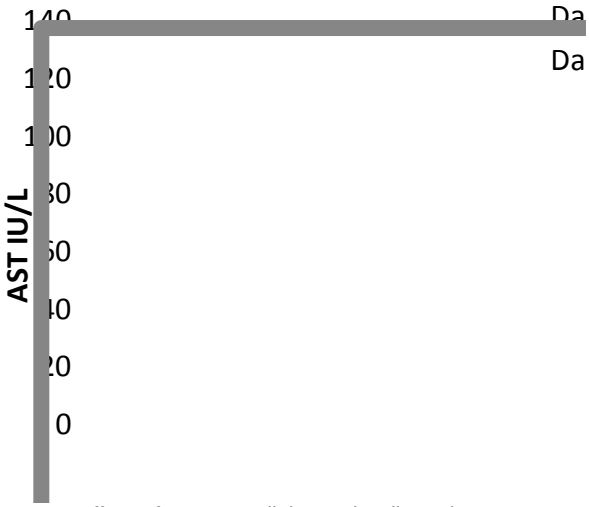

Figure 4. Effect of Excravos light crude oil on the aspartate transaminase (AST) activity in rats.

DISCUSSION

Crude oil is believed to elicit some therapeutic effects and functions in the treatment of various skin diseases, rheumatism, arthritis, fever and even constitutes an antidote in detoxifying (attenuating) ingested poisons [18]. Also, majority of the people in the Warri and Bonny communities ingest crude oil either directly as curative agents for anti-poisoning (snake venom antidotes), anti-convulsion, treatment of skin infection e.g. scabies or indirectly by eating marine animals found in surrounding coastal waters as source of protein [18]. However, the adverse effects of crude oil spillage on the environment, health and ecological biodiversity is overwhelming. In the present study, hepatotoxicity model in wistar rats was successfully produced by administering varying concentrations of Excravos light crude oil (0.1%, 0.25%, 0.50%, 0.75% and 1.00%). Evidences are presented in this study to further buttress the fact that the ingestion of crude oil, whether directly or indirectly, poses great health risks. The alanine aminotranferase (ALT) activity was found to increase significantly ($p<0.05$) in group 1 (0.01%) for both days 7 and 14. There was also a significant increase ($p<0.05$) in aspartate transaminase (AST) activity in group 1 as was the case with ALT. This marked rise above the normal upper limits in the measured transaminases in group 1 on both day 7 and 14 of the experiment was as a result of crude oil administration and thus, a biochemical indication of liver injury [19]. Also from this experimental investigation, it was found that there was a relative decrease ($p>0.05$) in the alkaline phosphatase (ALP) activity in groups 2 and 3. However, the decrease in ALP activity was not statistically different. Therefore, other environmental, genetic or nutritional factors may be responsible for the changes in the concentration of the enzyme marker. A progressive significant decrease ($p<0.05$) in the MDA levels across groups 2, 3 and 5; is indicative of increased possibilities of lipid peroxidation and a consequent increase in oxidative stress. Crude oil ingestion has also been reported to result in higher rate of inhibition of biliary secretion and an increase in liver cell lipid peroxidation. Also, oxidative stress is one of the mechanisms for crude oil induced hepatic injury. Metabolism of chemicals takes place in the liver, which accounts for the organ's susceptibility to metabolism-dependent, substance induced hepatotoxicity. A previous study on the bioaccumulation index of Bonny light crude oil in cat fish [20] had revealed that incessant consumption of sea foods exposed to crude oil pollution could lead to disease conditions caused by the carcinogenic, mutagenic or even teratogenic properties of crude oil and its derivatives. The fact that 0.1% crude oil contamination caused remarkable increase in the levels of the liver marker enzymes as suggested in the report of Ubani and Joshua [21] who observed a dose dependent effect of kerosene mixed with feed in rats and the same 0.1% contaminants of crude oil showed no significant difference ($p>0.05$) when compared with the control, suggests that the route of administration also play an important role in the levels of toxicity of crude oil to rats. Hence, in this study, 0.1% crude oil administered by oral intubation, gives

no significant toxicological effect. The crude oil ingested or its free radicals generated, undergo or promote a variety of chemical reactions, such as depletion of reduced glutathiones or inducing lipid peroxidation. Increased levels of lipid peroxidation is indicative of severe liver, kidney and heart damage such as acute myocardial infarction, artherosclerotic plaque stability [22]. Haematological studies of the effects of crude oil on African cat fish (*Clarias gariepinus*) and rats [3] had revealed significant decrease ($p<0.05$) in both RBC and PCV counts and a consequent increase in methaemoglobin formed.

CONCLUSION

From the aforementioned toxicological effects of crude oil on various physiological parameters of fishes, aves and mammals, it should be noted that ingestion of crude oil elicit more adverse health hazards than the assumed therapeutic benefits within the limits of available experimental evidences outlined herein. Therefore, there is need to employ working health and environmentally friendly techniques to adequately reduce the escalating global pollution of the environment and ecological biodiversity.

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