Effects of Ingested Crude Oil Contaminated Diets on Antioxidant Enzyme and Lipid Profile in Wistar Albino Rat.

*[1]OGARA, AMAECHI L; [2]JOSHUA, PARKER E; [3]OMEJE, KINGSLEY O [4]ONWURAH, IKECHUKWU NE

Department of Biochemistry, University of Nigeria, Nsukka.
Corresponding Author: Kingsley_omeje@yahoo.com*

ABSTRACT: In this study, we investigated the effect of orally administered crude oil contaminated diet on some biochemical parameters of wistar rat. Twenty four (24) wistar rats weighing between 125-180g were randomly grouped into four (4) of six animals each. Each group was fed with different concentrations of crude oil contaminated diet for 21days. At the end of each week, blood sample (2ml) was drawn from the median cantus vein of the eyes of the rats with the aid of a capillary tube and the plasma samples prepared for the biochemical tests. Also, one animal from each group was sacrificed and dissected every week; their livers were collected, weighed, washed with normal saline and later homogenized in a mortar and the isolate used for biochemical analysis. The results obtained showed the there was an increase in the weight of wet liver, lipid peroxidation and plasma protein concentration. Similarly, a time dependent increase in cholesterol concentration was obtained, with a significant decrease (p<0.05) in the mean body weight and percentage inhibition of superoxide dismutase. These results suggest that there was a negative alteration of the biochemical parameters examined which could have been induced by the contaminated feed they consumed. ©JASEM

Crude oil exploration is the mainstay of the Nigeria's economy and constitutes about 90% of the foreign exchange earnings of the nation (Amadi *et al*., 2000). The exploration of crude oil brings about the pollution of our environment including rivers, streams and exposure to crude oil presents a potential hazard to both aquatic and terrestrial species (Shore and Douben, 2001). Crude oil has been reported to contain several poisonous compounds, which accumulate in the body and induce toxic symptoms that sometimes result in death (Heintz *et al*., 1999). Crude oil in various oil producing areas of Nigeria has caused devastating socio-economic problems and health hazards to communities and explorers, and for this reason, the search for daily bread by oil workers may become a harvest of ill health, diseases and even in extreme cases death (Shertzer *et al*., 2005).

Petroleum hydrocabons or carbon-containing compounds are converted into free radicals or activated metabolites during their oxidation in the cells. These activated metabolites react with some cellular components such as membrane lipids and produce lipid peroxidation products which may lead to membrane damage (Odo *et al*., 2012). The consumption of petroleum hydrocarbon (PHC)-contaminated diets has been reported to cause liver enlargement, growth suppression and histological changes (Onwurah and Eze, 2000).

The toxicity of a petroleum fraction is related to its hydrophobicity because lipid solubility is an important factor in the passage of petroleum components through the plasma membrane of the cells and consequently, in the degree of membrane disruption (Freedman, 2000). There are indications that constant exposure of man and other animals that share common features with man to crude oil could lead to oxidative stress (Odo *et al*., 2012).

Animal species that are not directly in contact with the oil spillage can also be harmed via the food web and predators that consumed contaminated marine preys can be exposed to oil through ingestion of the prey (Sunmonu and Oloyode, 2007). Exposure of humans and animals to these chemicals is increasing in terms of the environmental level and the different usage of crude oil (Patrick-Iwuanyanwu *et al*., 2011). Over the years, local population has used crude oil or its fraction for the treatment of various ailments such as gastrointestinal disorders, burns, foot rot, leg ulcer, poisoning and even witchcraft and is mostly administered orally (Orisakwe *et al*., 2000). Hence, this study was undertaken to investigate the risk effects of crude oil contaminated food on albino rat.

MATERIALS AND METHODS

*Collection of crude oil and preparation of various mixtures***:** Bonny light crude oil was obtained from the Department of Petroleum Resources (DPR),

Corresponding Author Email: Kingsley_omeje@yahoo.com

Nigerian National Petroleum Corporation (NNPC), Port Harcourt, Nigeria.

Formulation of diet: The contaminated diets were formulated by measuring out various volumes (1, 5 and 10ml) of crude oil and the corresponding quantities of animal feed which were mixed thoroughly. The formulated diet was made into pellets to feed the rats.

Experimental design: Twenty-four adult Wistar albino rats (120-200 g) were obtained from the Department of Zoology and Environmental Biology, University of Nigeria and used for this study. They were acclimatized for seven days in Biochemistry department animal house at a temperature of 29 ± 2°C. They were randomly grouped into four with each group containing six rats. Each group was fed with different concentrations of crude oil contaminated diet (0, 1, 5, and 10%) for 21days after acclimatization.

Determination of total plasma protein concentration: The total plasma protein concentration was determined using the method of Lowry (1951). Bovine serum albumin (BSA) was used as the standard. Na2CO3 (2g) was dissolved in 100ml of 0.1N NaOH and labeled solution A. CuSO4.5H20 (0.5%) was added to 1% Na/K tartarate and labeled solution B. Solution A (50ml) was added to 1ml of Solution B and labeled Solution C. Solution C was prepared fresh each day of the experiment. Folin-Ciocalteau reagent (2ml) was diluted to 5ml with distilled water and labeled Solution D. One milliliter (1ml) of the sample was added to Solution C. To the above solution was added 0.5ml of Solution D and the absorbance measured at 750nm.

Determination of superoxide dismutase (SOD) activity: The SOD activity was assayed using the method of Misra and Fridovich (1972). One milliliter (1ml) of Fenton reagent was added to 4ml of epinephrine mixture and 1ml of distilled water. The absorbance was taken at 480nm, every 30 seconds for 5 minutes to obtain the standard curve of the auto-oxidation mixture. One milliliter (1ml) of the sample was then used in place of distilled water and the above procedure repeated. The graphs of absorbance against time were plotted and the slopes of the linear portion determined and used to calculate the percentage inhibition (%I) of superoxide dismutase. The percentage inhibition was calculated using the equation below:

$$\% \text{ I} = \frac{\text{VO} - \text{VSOD}}{\text{VO}} \times 100$$

Where, VO = Slope of the linear portion of the absorbance versus time curve of the auto-oxidation mixture VSOD = Slope of the linear portion of the absorbance versus time curve of sample.

Determination of lipid peroxidation: Lipid peroxidation was measured using the method of Wallin *et al*., (1993). Lipid peroxidation standard curve using 1,1,3,3 – tetra ethoxy propane (TEP) was used. A mixture of 0.2ml of the sample, 0.5ml of 25% TCA and 0.5ml of TBA was made. The reacting mixture was incubated between $90 - 95^0$C in a water bath for 40 minutes and cooled in ice water. To reduce turbidity, 0.2ml of 20% SDS was added to the mixture and shaken thoroughly. The absorbance was taken at 532nm and 600nm and the differences calculated.

Determination of total cholesterol concentration: Total cholesterol concentration was determined by the method of Abell et al., (1952). A quantity of 1.0ml of the standard was mixed with 1.0ml of the blank. The sample (10μl) was later used in place of standard. The mixtures were mixed thoroughly and incubated for 10 minutes at room temperature. Their absorbance was then measured at 500nm. The cholesterol concentration in the sample was then calculated using the following relationship or formula:

$$\frac{Asample}{Astandard} \text{ X } C_{standand} = C_{sample}$$

Where CStandard = Concentration of Standard = 200mg/dl or 5.18mmol/l Sample

Sample collection: At the end of each week during the experiment period, blood sample (2ml) was drawn from the median cantus vein in the eyes of the rats with the aid of a capillary tube and transferred into EDTA plastic bottles. These were centrifuged at 2000 x g for 5 minutes and the plasma samples decanted into clean test tubes and used for further analysis. Also, one animal from each group was sacrificed and dissected every week. Their livers were removed, weighed, washed with normal saline and later homogenized in a mortar and the isolate used for biochemical analysis.

Statistical analysis: The results were expressed as mean ±SD and test of statistical significance was carried out using one–way analysis of variance (ANOVA). The means were separated using Duncan multiple Test. Differences were considered significant at p < 0.05. The statistical packaged used

was the statistical package for social sciences (SPSS), version 17.

RESULTS AND DISCUSSION

The mean body weights of albino rats at various weeks of exposure to different concentrations of crude oil contaminated diets are shown in Fig. 1. It shows that there was a progressive increase in the mean body weights of the control group that were fed the normal uncontaminated diet. The result also shows a concentration-related significant decrease (P<0.05) in the mean body weights of the groups of animals fed crude oil contaminated diets. Therefore, the duration of exposure and the amount of crude oil in the diet (degree or extent of contamination) decreased the body weights of the rats within the three week period of study. The growth of an organism integrates a range of physiological, biochemical and cellular processes, thus, loss in body weight is a sensitive indicator of a toxic impact. This is in line with the observation by Donkin and Widdows (1986) that body weight of exposed organisms declined in a predictable way with respect to the concentration of the pollutant and duration of exposure. Also, organisms found on oiled shores had lower body mass and elevated levels of biomarkers than those living in non-oiled areas (Taylor *et al*., 2000).
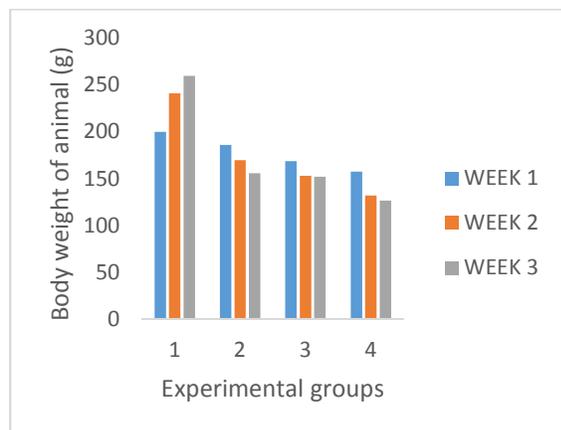

Fig 1: The mean body weights of rat after exposure to contaminated food (g)

The changes in wet liver weights of rats fed crude oil-contaminated diets are shown in Fig. 2. The control group reveals that the wet liver weights were relatively stable over the 3-week exposure period. The figure shows that increase in organ weights in the first weeks of exposure was not significantly different from that of the control. There were increases observed in the wet liver weights of the rats exposed to 1%, 5% and 10% crude oil contaminated

diets. These increases were found to be statistically significant (P<0.05) in weeks 2 and 3 compared to the 0% group. Within the treated groups, the increases were statistically significant (P<0.05) in week 3 compared to week 2 and in week 1 compared to week 3. The increases observed among groups were time-dependent and not substantially concentration dependent. Exposure of rats to contaminated food resulted in fatty liver, hence, the increase in the wet liver weights, relative to the control experimental animals. This is in line with the observation of Warry *et al*., (1993) and Lewis *et al*., (1998) where it was reported that increase in liver weight without corresponding increase in the body weights of the animal exposed to toxicants are common signs of mutation induced toxicity as a result of an increase in the induction of liver enzymes.. This could be as a result of proliferation of the smooth endoplasmic reticulum (Hansell and Ecobicon, 1974; Norback and Allen, 1972).
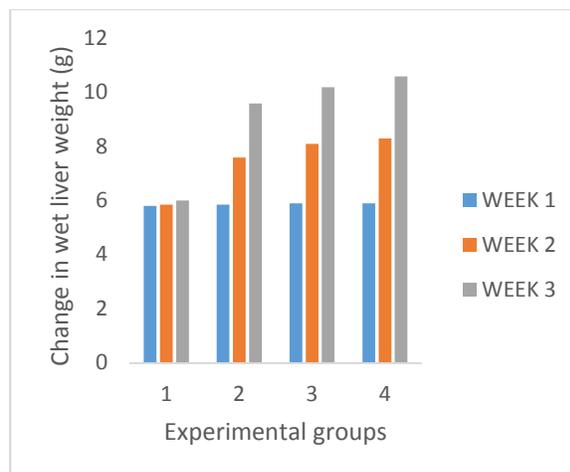

Fig. 2: Changes in wet liver weights of rat after exposure to contaminated food (g)

The plasma lipid peroxidation products of rats exposed to different concentrations of crude oil contaminated diets are shown in Fig. 4. Results from this study show increase that were statistically significant (P<0.05) in the plasma lipid peroxidation products in the groups fed the crude oil contaminated diets compared to the control. No statistically significant difference was observed during the first weeks of exposure in all the groups. The increases observed in the subsequent weeks in all the treated groups were time dependent. Within the groups, group 1 (0%) showed increases which were not statistically significant (P>0.05) during the 21 day of exposure. This was also observed in group 2 (1%) except in week 3 when the increase was substantially high (P<0.05). The pattern of increase also observed

in groups 3 (5%) and 4 (10%) indicate concentration and time dependence. Increase in lipid peroxidation has been reported in several cases of toxicity induced by hydrocarbon and transition metals (Reid *et al*., 1994), fenton reagent and crude oil (Onwurah, 1999). It has been reported that high lipid peroxidation products observed in liver and brain homogenates may be as a result of damage to the cell membrane caused by free radical attack (Onwurah, 1999). Similarly, Achuba (2005), also reported increase in the peroxidation product in his work on petroleum contaminated diet in rabbit.
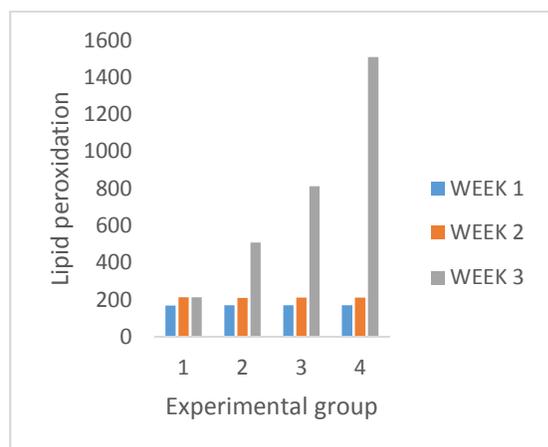

Fig. 3: Plasma lipid peroxidation products of rats

The mean plasma total protein concentrations in rats exposed to different concentrations of crude oil-contaminated diets for a period of 21 days are shown in Fig. 5. The mean plasma total protein concentration decreased significantly (P<0.05) in control group when compared to the treated groups in weeks 2 and 3. The increases in protein concentrations observed in the weeks 2 and 3 in the treated groups increased significantly (P<0.05) when compared to the control. The observed increases in protein concentration could be a direct consequence of damage to cell membrane and lysis (Uboh *et al*., 2005).
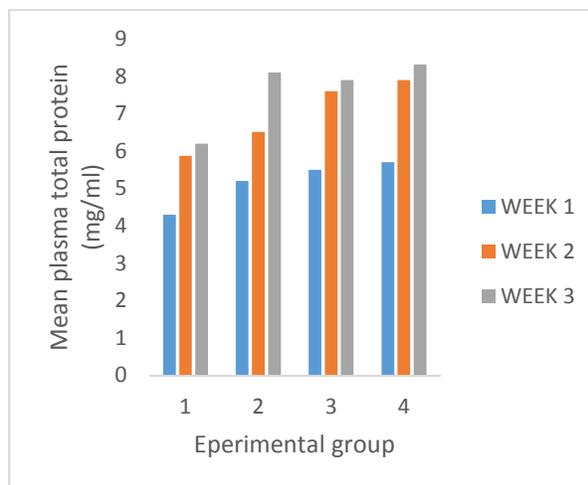

Fig. 4: The mean plasma total protein concentrations in rats

The mean plasma total cholesterol concentrations in rats exposed to different concentrations of crude oil-contaminated diets for a period of 21 days is shown in Fig. 5. An increase was observed in the plasma total cholesterol level in the rats exposed to crude oil contaminated diet. Though, the increase was not statistically significant (p>0.05). The increase on the 3rd week was statistically significant (p<0.05). This is an indication that exposure to crude oil affects lipid metabolism. The result of the 3rd week implies that exposure to crude oil affects lipid metabolism. It has been reported that ingestion or exposure of crude oil fractions affect lipid metabolism causing the tissue to compromise its effectiveness in lipid metabolism (Uboh *et al*., 2005).
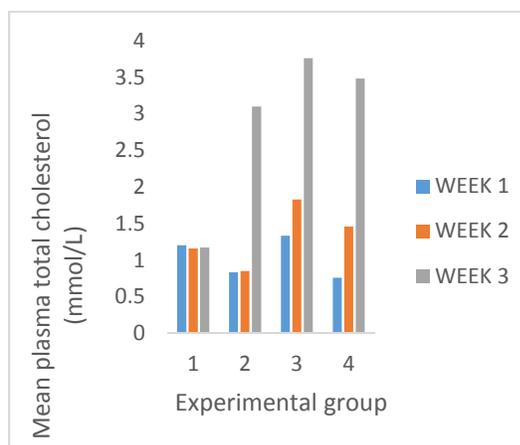

Fig.5: The mean plasma total cholesterol concentrations

The mean (I%) activity of Superoxide dismutase (SOD) in rats fed different concentrations of crude oil contaminated diets is shown in Fig. 6. Results from

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this study show increases that are significant (P<0.05) in 3rd week in the treated groups when compared to control. A progressive increase in the percentage inhibition was observed in the control group. The level of inhibition observed in group 2 (1%) drastically decreased in week 3. This pattern was also applicable to groups 3 (5%) and 4 (10%). For week 1 in groups 1, 2 and 3, the level of inhibition was fairly stable, but in group 4, it reduced substantially (P<0.05). The increases in week 2 was biphasic, increasing statistically (P<0.05) in group 2 and thereafter decreasing gradually through group 3 to group 4. The result presented above reveals both concentration and time dependence. The result of this work showed a significant increase in the percentage inhibition of SOD in the control group throughout the period. There was significant increase observed in the 1st and 2nd week of exposure. The result agrees with the work of Ledig *et al*., (1988), who reported an increase in the activity of SOD in the erythrocyte of alcoholic patients and microorganisms exposed to crude oil and fenton reagent (Onwurah, 1999). But, a prolonged exposure to the 3rd week decreased the activity of the enzyme. This could be attributed to the over production of reactive oxygen species (ROS) that overwhelmed the concentration of antioxidant system of the organism. Kurata *et al*., (1993) is of the opinion that excess concentration of ROS would cause cellular damage in a living system.
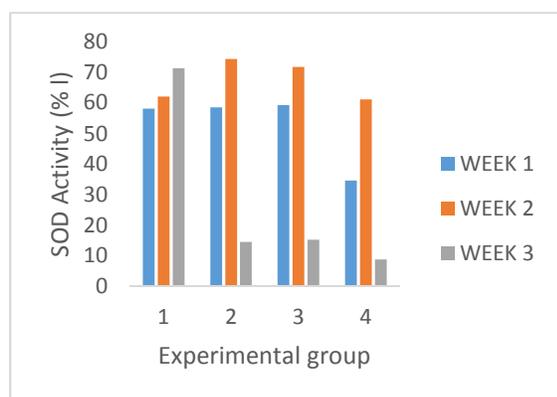

Fig. 6: The mean (I %) activity of Superoxide dismutase (SOD) in rats

The findings of this work and correlations with other works show that hydrocarbons of crude oil origin negatively affect the physiological as well as biochemical statues of the living systems as is evident in the results of this study. Similarly, the contaminated feeds were found to have oxidative stress-inducing potential which are pointers to the toxicity of crude oil

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