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Research Article

Effects of Bonny-light Crude oil Contaminated-diet on Some Antioxidant Enzymes and Body Weight Changes of Wistar Albino Rats

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ABSTRACT

Albino rats were fed on commercial rat feeds contaminated with Bonny-light crude oil of varying concentrations of 1%, 5% and 10% for 28 days. Feed and water were provided *ad libitum* throughout the experimental period. The mean body weights of the rats were taken one week into the treatment and at the end of the treatment. A gradual decrease in body weight was recorded with respect to control. A decrease in feed consumption rate with increasing concentration of the toxicant with respect to the control was recorded. Blood was drawn from ocular cantus vein and analyzed. A high increase in lipid oxidation, which was higher in males than in females, was recorded. There was a significant increase glutathione s- transferase activity in the 1% and 5% groups and a significant decrease in 10% group ($P \leq 0.05$). A significant increase in superoxide dismutase activity was recorded in all the groups with respect to the parallel control group.

Keywords: *Bonny-light crude oil, Contaminated diet, Antioxidant Enzymes, Body Weight changes, Lipid Peroxidation, Rats*

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INTRODUCTION

Bonny-light crude oil (BLCO) was first discovered in commercial quantities in Oloibiri, in the Niger Delta region of Nigeria in 1956. It is the hub of Nigeria economy. It is mainly used as a source of fuel for driving many mechanical engines. Apart from its use as fuel, it has found wide folkloric applications especially in medical treatments: detox, dermal inflammation, digestive disorders anti-poison, anti-convulsion etc. (Raji and Hart, 2012; Azubuike, *et al*, 2013). Bonny-Light Crude Oil just like other crude oils is a complex mixture of over 6000 potentially different hydrocarbons and heavy metals (Orisakwe *et al*, 2004). Bonny-Light Crude Oil is a light volatile oil with an American Petroleum Institute Gravity (API) of 32.9, low sulphur content and good gasoline yields.

Mining activities and sabotage have led to serious environmental pollution arising from incessant oil spillages. This has posed greater risks to inhabitants of these oil rich areas with regards to health and agriculture. The use of crude oil contaminated water for cooking, drinking, recreation and cleaning expose the residents to health hazards such as anxiety, depression, sore eyes and throat, brain damage as well as endocrine and DNA damage (Raji and Hart,2012 and Azubuike, *et al*, 2013). A wide range of symptoms has been reported due to exposure to crude oil either accidentally or for therapeutic purposes (Adedara and Farombi, 2012; Salem *et al*, 2011*;* Ovuru and Ekweozor, 2004; Farombi *et al*, 2010 and Indarato and Izawa, 2001). During normal oxidation of foodstuffs arising from leaks in the electron transport chain in the mitochondria, free radicals are produced; hence metabolism of crude oil contaminated diet may lead to increase in lipid peroxidation and generation of other free radicals. But there is presence of diverse and complex groups of molecules that scavenge and suppress the formation of pro-oxidants as well as opposing their actions, thereby protecting key biological sites from oxidative damage (Vasudevan *et al* 2011). To prevent damage to cellular components, there are numerous enzymatic antioxidant defenses designed to scavenge reactive oxygen species in the cell. Some of these compounds are used as food additives, principally for preventing lipid peroxidation, which can lead to rancidity. Such antioxidants include: Propyl gallate, Butylated hydroxyanisole and Butylated hydroxytoluene. Others may occur naturally such as: Tocopherol, Urate and NADPH, GSH (Roberts *et al* 2003). Antioxidants can be classified into two groups viz: Preventive antioxidants (which reduce the rate of chain initiation e.g Catalase and other Peroxidases) and Chain-breaking antioxidants (which interfere with chain propagation

e.g Superoxide Dismutase, Urate, Vitamin E, etc. (Roberts *et al*, 2003). In a normal cell, there exists pro-oxidant/antioxidant balance. However, fluctuations in this delicate balance may favour pro-oxidant when production of oxygen species is greatly increased. This may result due to drugs, chemicals or diminished levels of antioxidants leading to oxidative stress, hence cellular injury. This study was designed to assess the effect of Bonny-light crude oil contaminated diet on some antioxidant enzymes and body weight changes in Wistar rats.

MATERIALS AND METHODS

Test Sample: Bonny-light crude oil was collected from Nigeria Petroleum Development Commission (NPDC), Ukpoho, Port-Harcourt, Rivers State, Nigeria. Also forty (40) Wistar Albino Rats aged 15-17 weeks were purchased from the faculty of Veterinary Medicine, University of Nigeria, Nsukka.

Chemicals and Biochemicals: All chemicals and biochemical were of analytical grade and were purchased from Merck, Germany; BDH chemicals Ltd, England; May and Baker Ltd, England; Riedel-De-Haen Ag Seilze, Germany and Hopkins and Williams, Essex, England. Deionized water was used in all experiments involving water.

Feed formulation: 1%, 5% and 10% crude oil contaminated diets were prepared by weighing out a definite amount of the crude oil and the feed and mixed in a mixer driven at 100rpm. 5ml of distilled water were added during mixing to facilitate homogenization. The feed for the control contained no crude oil.

Experimental Design: Forty Wistar Albino rats, obtained from the faculty of veterinary medicine, university of Nigeria, Nsukka were fed with normal commercial growers rat feed with water *ad libitum* for one week for them to acclimatize. The rats were then randomly allocated to four groups of ten (10) rats each of five males and five females and labeled A, B, C and D.

Table 1
Experimental design

Groups	Treatment
A (Control)	No crude oil in the diet
B (1%)	1g of crude oil in 99g of feed
C (5%)	5g of crude oil in 95g of feed
D (10%)	10g of crude oil in 90g of feed

Animal treatment: Overnight prior to the administration of the contaminated diet, the test animals were starved of solid food. They were then paired for mating and the varying concentrations of the contaminated diet were fed to the different groups with water *ad libitum.* This lasted for 28 days. Their body weights and feed consumption rates were measured and recorded one week into the treatment and at the end of the

Sample collection: 3ml of blood was collected from the ocular median cantus vein of the rats with the help of capillary tubes and transferred into a test tube containing 1.0Mm. Blood containing EDTA was centrifuged at 2000rpm for 5 minutes and plasma samples collected thereafter.

Lipid Peroxidation determination: Lipid peroxidation was assayed using the method described by Wallin *et al* (1993)

Antioxidant activity: Superoxide dismutase activity was determined by the method described by Misra and Fridovich, (1971), while Glutathione s-transferase activity was determined by the method described by Habig *et al*, (1974).

Statistical Analysis
Mean values (±Standard Deviation) of replicate determination with quadruplet sampling (N=2) were taken for each analysis. Level of significance between results was established using one-way ANOVA. The accepted level of significance was $P \leq 0.05$.

RESULTS

The result showed a significant decrease ($P \leq 0.05$) in feed consumption rate with increasing concentration of the toxicant (Table 1). Similarly, the result showed a significant decrease ($P \leq 0.05$) in body weight with increasing level of toxicant with respect to the parallel control experiment (Table 2).

Table 1:
Mean Feed Consumption Rate of Wistar Albino Rats Fed With Different Concentrations of Bonny-Light Crude Oil Contaminated Diet.

Group	Mean Feed Consumption one week into the Experiment	Mean Feed Consumption at the end of the Experiment	% Feed Consumption
Control	102.11 ± 7.37	123.50 ± 7.37	20.94
1%	87.41 ± 7.10	87.41 ± 7.10	4.18
5%	34.50 ± 5.89	34.50 ± 5.89	-1.85
10%	21.90 ± 5.37	21.90 ± 5.37	-33.15

Table 2:
Mean Body Weights Of Wistar Albino Rats Fed with Different Concentrations Of Crude Oil Contaminated Diet Before And After The Experiment.

Group	Mean weight (G) before experiment	Mean weight (G) after experiment	Difference in weight (G)	% difference in weight (G)
Control	178.0± 10.23	203.90±6.78	+25.82	14.50
1%	181.92± 15.26	175.56±7.32	-6.36	-3.50
5%	164.35 ± 5.84	160.85±5.06	-4.0	-2.44
10%	176.72± 11.75	136.96±11.72	-39.76	-22.50

The lipid peroxidation level showed a significant increase ($P \leq 0.05$) in all the groups and higher in males than in females with respect to the parallel control group (Figure 1). The result showed a significant increase ($P \leq 0.05$) in the Glutathione s-transferase activity in 1% and 5% groups but a significant decrease in the 10% group with respect to the parallel control

group (Figure 2), while a significant increase ($P \leq 0.05$) in Superoxide dismutase activity was obtained in all the groups with respect to the parallel control group (Figure 3).

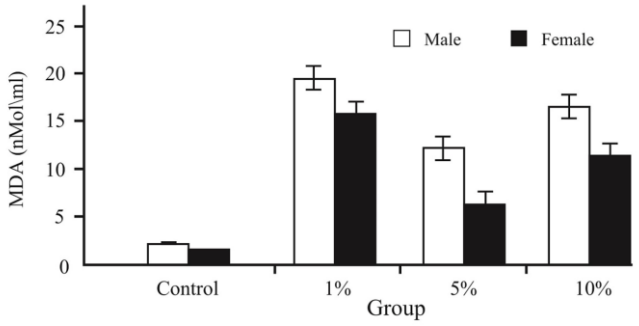

Figure 1
Bar Chart OF Lipid Peroxidation Levels of Wistar Rats fed with Different Concentrations of Crude Oil Contaminated diet.

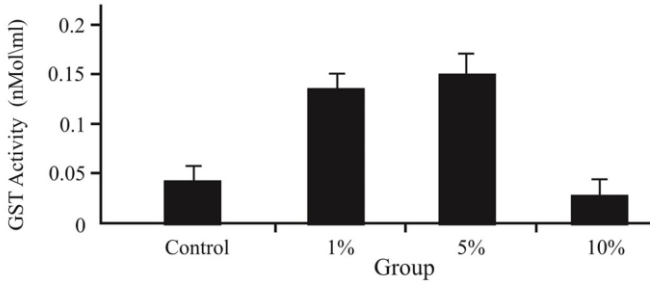

Figure 2
Bar Chart of Glutathione s- transferase activity of Wistar Albino Rats fed with different concentrations of crude oil contaminated diet.

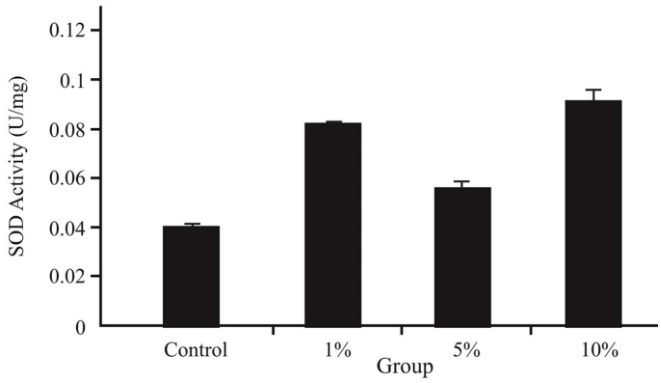

Figure 3
Bar chart showing Superoxide dismutase activity of Wistar Albino Rats fed with different concentrations of crude oil contaminated diet.

DISCUSSION

The reduction in mean food intakes taken before and after the experiment may be attributed to non-palatability of the feeds due to the crude oil contamination. It may be equally attributed to possible protein, DNA and lipid oxidation, which may have taken place in the rats thereby altering their metabolic process (Otitoju and Onwurah, 2007).

The results also showed a gradual decrease in body weight, while an increase in the body weight of the control group was recorded. The decrease in body weight may be attributed to decrease in feed consumption rate. This loss in weight agrees with the work earlier done by Kerr and Kelch, (1998), where they stated that one of the chemical manifestations of crude oil and environmental toxicity is loss of weight.

The results also showed a significant increase in lipid peroxidation levels in both male and female organisms, with males having higher values than females. According to Fridovich, (1986), oxidative stress has been associated with increased lipid peroxidation, protein degradation and degradation of other macromolecules. Also, according to Ben (1999), generator of free radicals is ubiquitous and that free radicals are highly reactive in their tendency to move from their unstable state to a more stable state; hence may be accountable for the observed decrease in weight. Also, the observed increase in lipid peroxidation level may be due to the presence of polyunsaturated fatty acids in crude oil, which act as excellent substrate for lipid peroxidation owing to the presence of Bis-allylic methylene groups (Dos Santos et al, 2018). Lipid peroxidation is a chain reaction providing a continuous supply of free radicals that initiate further peroxidation. Also, the increase in lipid peroxidation level with respect to the control may be due to autoxidation caused by the presence of the toxicant in the diet. This will further cause oxidation of membrane associated proteins, leading to breakdown in membrane integrity and membrane dependent functions. Photo-oxidation of the crude oil in the diet can lead to production of more dangerous metabolites that could act as potent radical inducers (Igwe et al, 2016).

The result showed an increase in Glutathione s-transferase (GST) activity in the 1% and 5% groups with respect to the control and an unexpected non-significant decrease in the 10% group. The conjugation of the various free radicals present in crude oil is a key defense mechanism that protects the test organisms from severe cellular injury; hence in the 1 and 5% groups, they are in a better condition to handle the effects of the toxicant. However, in the 10% group, where an unexpected decrease was observed, it may be due to the overwhelming effect of the high concentration of the toxicant on the cells producing this enzyme and a high cellular injury should be expected.

A non-significant increase in Superoxide Dismutase (SOD) activity was recorded in all the test groups with respect to the control. This increase is to reduce the effects of lipid peroxidation and other auto-oxidation radicals due to the presence of the toxicant. Comparing the activities of the two antioxidant enzymes (GST and SOD), the result showed that the GST activity was highest in the 5% group and lowest in the 10% group , while the SOD activity was highest in the 10% group and lowest in the 5% group. This suggests a compensating effect of the two enzyme systems. This also agrees with the feedback mechanism of SOD and GST (Fridovich,1993). The observed increase in SOD activity agrees with work of Kong and Sang, (1999), where they showed increased level of SOD activity in alga due to increase in the level of pollutants. This means that increase in the level of pollutants will make the cell to readjust in such a way as to support life and prevent damage by these life-threatening compounds.

The results of this study suggest that both target and non-target organisms exposed to crude oil contaminated diet may

be susceptible to cellular injury and the homeostatic response by the organism observed through increase in antioxidant enzymes may be responsible for the survival of the test organisms.

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