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Biochemical changes in tissues of albino rats following subchronic exposure to crude oil

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ABSTRACT:

Biochemical changes in the tissues of thirty-six albino rats (*Rattus rattus*) following subchronic exposure to 1.25% , 2.50% and 5% of crude oil polluted feed was investigated. There were age and sex matched with another twelve albino rats fed without crude oil. The exposure period lasted for thirty days. The concentrations of serum albumin, cholesterol and glucose, liver total protein, ascorbic acid and reduced glutathione (GSH) as well as the activities of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and catalase (CAT) from the rats were determined using standard methods. The results indicate that there were no significant ($P>0.05$) changes in these parameters from the rats fed with 1.25% and 2.50% crude oil mixed feed when compared with the control. However, significant ($P<0.05$) reductions were observed in glucose, ascorbic acid and GSH concentrations as well as in catalase activity, with a concomitant significant ($P>0.05$) increases in serum ALT, AST and ALP activities in the rats fed 5% crude oil polluted feed, in comparison with the control. The results indicate that crude oil ingestion at levels of up to 5% may have serious adverse effect on animal tissues.

Keywords:
Albino rats, crude oil, liver enzymes, pollution.

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INTRODUCTION

The growth of the petroleum industry in Nigeria and the marketing of petroleum products have made oil pollution a serious environmental problem (Amund *et al.,* 1987). Pollution of the environment may result from the exploration, transportation or storage of petroleum products or from accidental events (spills). In some cases it may be a consequence of careless disposal practices of residues like the oily sludge that accumulate in storage tanks (Morgan and Watkinsona, 1989).

Crude oil spill is the release of crude petroleum hydrocarbons into the environment due to human activities and are classified into two main types; the land (on shore) and marine (off-shore) oil spills. Land oil spill occurs when crude oil is released on the land which affects soil ecosystem. The different ways by which crude oil enter the environment are from natural seep (1%), atmospheric input (1%), off-shore production (1%), coastal and estuarine effluents (3%), non-refinery industrial wastes (5%), municipal waste (5%), urban run off (5%), rivers (26%) and oil water discharge from oil industry (53%)(Carla, 2006; Levorsen, 2008). These pollutants (crude oil and their products) are considered recalcitrant to natural biodegradation and persist in the ecosystem due to their hydrophobicity and low volatility (Ademoroti, 1996)

Animals and plants growing in crude oil polluted environment, take in large doses of harmful pollutants over the years. These pollutants and any product of their degradation products can be carcinogenic, mutagenic and are potent immunotoxicants which affect the biological systems of living things. (Boonchan *et al*., 2009; Rao and Panya, 1978). This study sought to investigate the subchronic effect of different percentage concentrations of crude oil ingestion on the tissues of albino rats.

MATERIALS AND METHODS

Experimental Animals:

Forty-eight (48) adult male albino rats (*Rattus rattus*) weighing between 210-380 grammes were used for the experiments. The animals were allowed to acclimatize for 7 days, and were maintained under laboratory condition of humidity, temperature (25± 2° C) and light (12 hr light/ dark cycle) with free access to food and water *ad libitum.* All experimental protocols were in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub. No. 85-23, Revised, 1985). After acclimatization, the animals were divided into four (4) groups (A,B,C,D). Groups A, B and C received 1.25%, 2.50% and 5.0% feed containing-crude oil respectively while group D received feed without crude oil and served as the control. Feeding period lasted for 30 days.

Preparation of Blood and Liver Samples:

After 30 days, each rats was euthanized by anesthesia using chloroform, and 5ml of Blood was collected by cardiac puncture. The blood sample was allowed to stand for 10min for clotting to take place. The serum was separated by centrifugation. The serum obtained was used for the determination of serum albumin, chlorosterol and glucose concentrations and assay of the serum (ALT, AST and ALP). Each rat was then surgically dissected, the liver extracted immediately, washed with ice-cold 1.15% KCl and stored at -10°C. Chilled liver tissues were later homogenized using a homogenizer (Janke and Kunkel, Germany). The homogenate was centrifuged and was used for the determination of liver protein, ascorbic acid and glutathione concentrations, and assay for the activity of catalase (CAT).

Determination of Biochemical Parameters:

Serum and cholesterol concentrations were determined using the methods of Doumas *et al*. (1971) and Allian *et al.* (1974) respectively. Glucose concentration was determined based on glucose oxidase method as described by Trinder (1969). The serum activities of ALT and AST were assayed using the method of Reitman and Frankel (1957), while that of ALP was assayed as described in Tietz (1991). Hepatic Protein concentration was determined by Biuret method as described by Gornall *et al.* (1949). Ascorbic acid concentration was determined using the method of Roe and Kuether (1961). Ascorbic acid is converted to dehydroascorbic acid by shaking with Norit and this was coupled to 2, 4-dinitrophenyl hydrazine in the presence of thiourea (a mild reducing agent). Sulphuric acid was then used to convert the dinitrophenyl hydrazine to a coloured compound whose absorbance was determined spectrophotometrically at 540nm. Reduced glutathione (GSH) was determined by the method of Jollow *et al.* (1974). The method is based on the formation of a relatively stable chromophoric product on reacting a sulphurhydryl compound (GSH) with Ellman's reagent. Catalase activity (CAT, E.C. 1.11.1.1.) was assayed by measuring spectrophotometrically at 570nm the rate of

decomposition of hydrogen peroxide (H_2O_2) over a period of 30 minutes at (1 minute interval) as described by Sinha (1972). The enzyme activity for each tissue was expressed in terms of Katalase feiahigkeit (kat. f) as ks^{-1} mg^{-1} protein where K is the first order rate constant.

Statistical Analysis

Data generated were expressed as means ± standard deviation and analyzed using one way Analysis of Variance (ANOVA) with p £ 0.05 taken to be significant.

RESULTS AND DISCUSSION

There has been an increased incidence of petroleum hydrocarbon pollution from crude oils, gas flaring, diesel oil and motor engine oil over the years (Odu, 1996; Nwaogu *et al.,* 2008; Nwaogu and Onyeze 2010). It has been considered by many researchers that petroleum hydrocarbon-induced oxidant stress is a critical pathologic mechanism that initiates various cascades of metabolic perturbations (Rao and Pandya, 1978; Romieu *et al*., 1999).

Induced changes in liver and blood parameters of albino rats subchronically exposed to different percentage concentrations of crude oil revealed that there were no significant (p>0.05) reductions in the concentrations of serum albumin, cholesterol and liver protein of albino rats fed with feed polluted with crude oil at 1.25% , 2.50% and 5.0% in comparison with the control (**Figures 1-3**). There were also no significant (p > 0.05) reduction in the concentrations of glucose, ascorbic acid and glutathione as well as the activities of hepatic catalase obtained for rats fed with 1.25%, 2.50% and 5% crude oil polluted feed. (**Figures 4, 8-10**). On the other hand, the 1.25% and 2.50% crude oil polluted feed caused non – significant (p>0.05) increases in serum activities of ALT, AST and ALP (**figures 5-7**) . However there were significant (p<0.05) changes in the mean concentrations obtained for glucose, ascorbic acid, glutathione and the activities of ALT, AST and ALP and catalase of rats fed with feed polluted with 5.0% crude oil concentrations in comparison with the control (figures 4-10) indicating that crude oil at that percentage concentration caused changes in the blood and liver parameters in albino rats.

The results for glucose concentration (Figure 4) show that there was a dose-dependent reduction in the blood glucose concentrations as the percentage of crude oil in the feed increased. During hypoglycemia, the brain suffers from substrate deficiency since glucose is the major fuel for the brain cells (Nelson and Cox, 2000). This leads to a decrease in anabolic processes (Patoekora *et al*., 2003). The results indicate that the rats fed with 5% crude oil mixed feed suffered stress as a

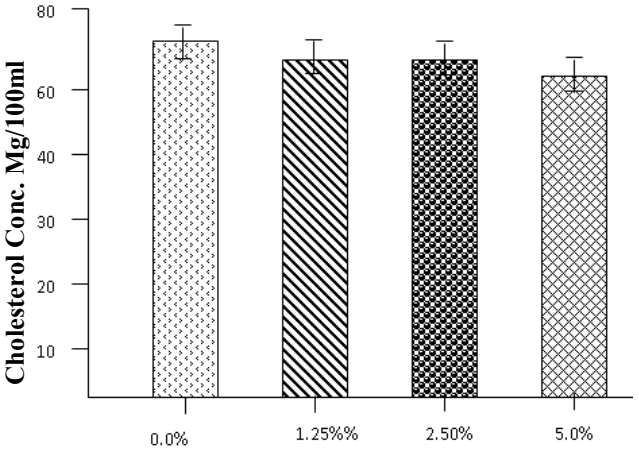

Fig. 2: Serum cholesterol concentrations of Albino rats fed different concentrations of crude oil mixed feed.

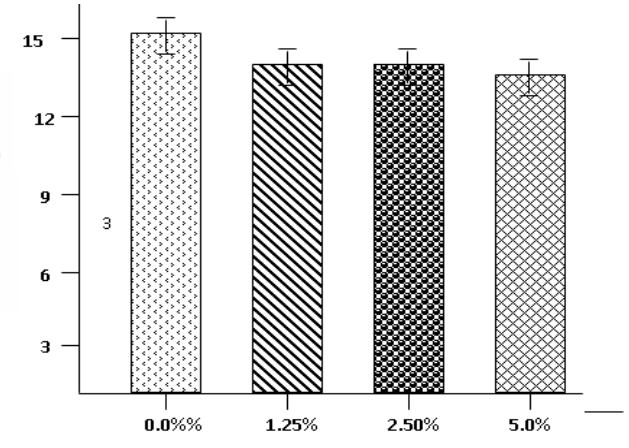

Fig. 1: Serum albumin concentration of albino rats fed different concentrations of crude oil mixed feed.

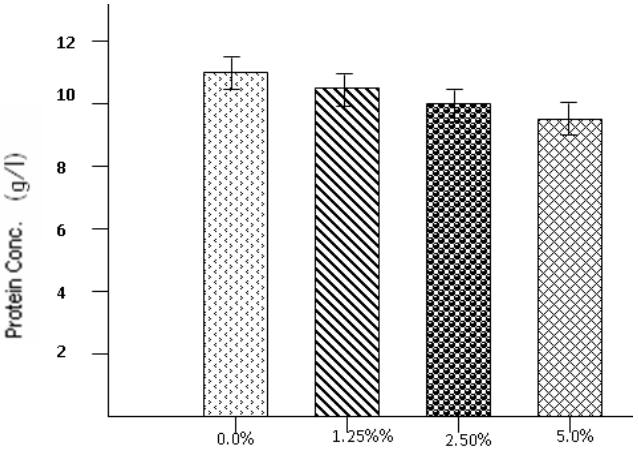

Fig. 3: Liver protein concentration of rats fed different concentration of crude oil mixed feed.

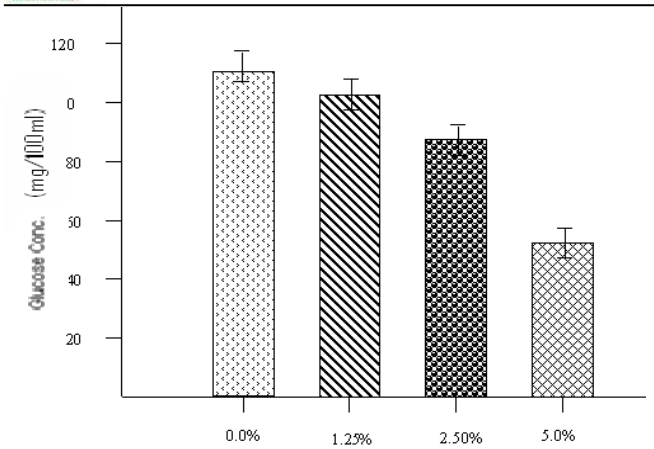

Fig. 4: Serum glucose concentration of rats fed effluent concentrations of crude oil mixed feed.

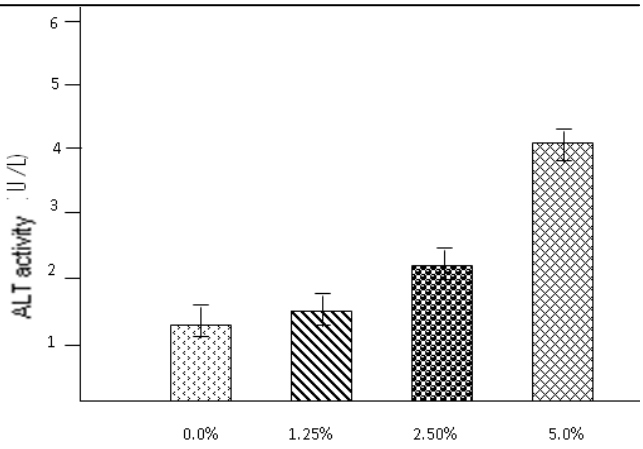

Fig. 5: Serum ALT activities of rats fed different concentration of crude oil mixed feed.

result of the exposure to crude oil.

Serum ALT, AST and ALP activities are used as indicator of chemically induced liver damage (Drotman *et al*., 1978). Hepatotoxicity has been viewed as liver injury associated with impaired liver function caused by exposure to xenobiotics (drugs, petroleum hydrocarbons) and other non-infectious agent (Navarron, 2006). Results of this study revealed that there were significant ($p < 0.05$) increases in the serum ALT, AST and ALP activities in rats fed 5.0% crude oil-mixed feed. These enzymes usually leak out into the blood stream in cases of liver damage or loss of integrity. The observed increase in serum activities of these enzymes indicate a sign of liver dysfunction as a result of ingestion of crude oil in the feed (Delvin, 2006).

Ascorbic acid is a water-soluble antioxidant molecule found in the cytoplasm of the cells, which scavenges free radicals in the cytosol. It readily donates electrons to free radicals thereby quenching their effects in the cellular compartments and is in the process converted to dehydroascorbic acid (DHAA) (Mckee and Mckee, 1999). Our results indicate that there was a significant ($p<0.05$) reduction in the concentration of ascorbic acid as the concentration of crude oil in the feed increased. This significant reduction could be due to the utilization of ascorbic acid in scavenging the reactive intermediates generated in the tissues of the albino rats fed with the animal feed mixed with crude oil.

Glutathione (GSH) is usually located in the cystol, nuclei and mitochondria. It is the major soluble antioxidant in these cell organelles (Masella *et al*., 2005). Reduced glutathione (GSH) and its oxidized form (GSSG) accumulate inside the cells. The ratio of GSH to GSSG is a good indicator of oxidative stress in a living system (Mckee and Mckee, 1999). The reduction in the concentration of glutathione either by conjugation and removal from cell or oxidation to oxidized glutathione could

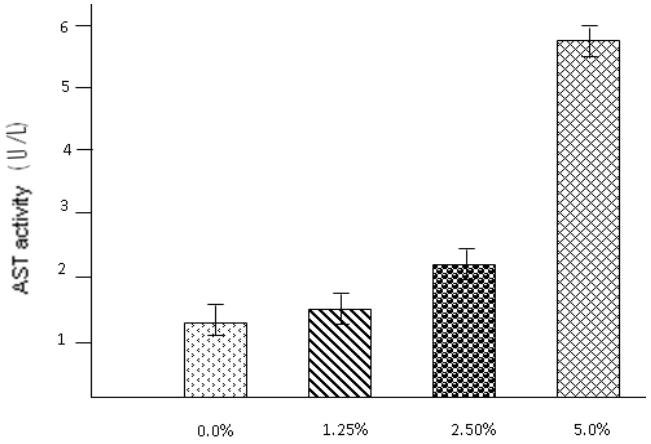

Fig. 6: Serum AST activities of rats fed different-

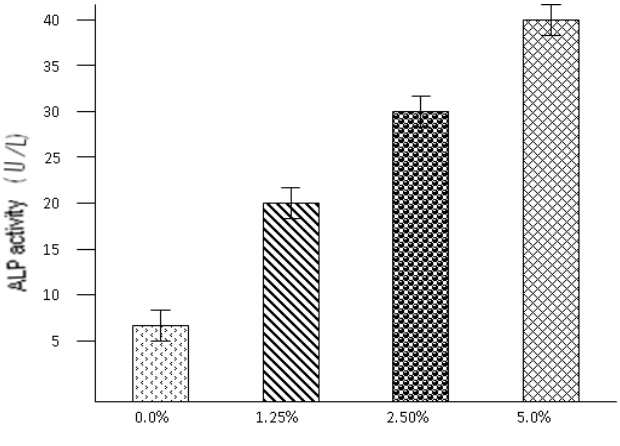

Fig. 7: Serum ALP activities of rats fed different concentrations of crude oil mixed feed.

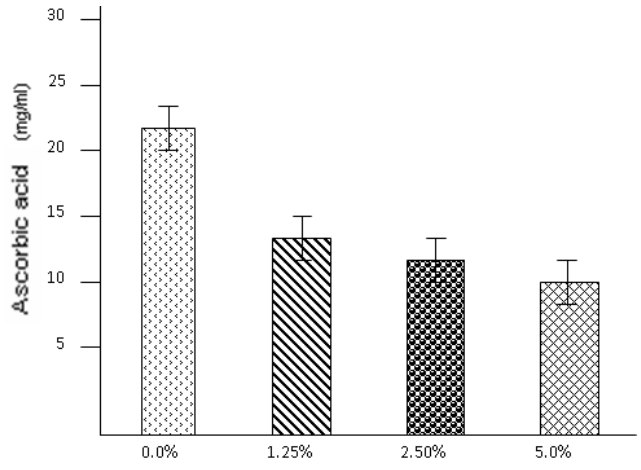

Fig. 8: Liver ascorbic acid concentration activities of rats fed different concentrations of crude oil mixed feed.

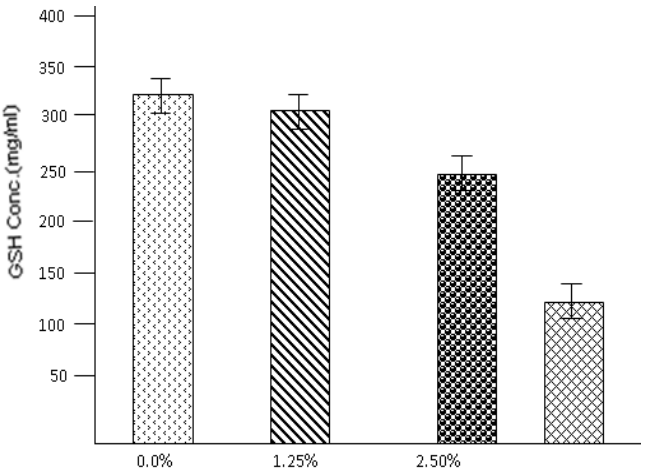

Fig. 9: Liver glutathione (GSH) concentration of albino rats fed different concentrations of crude oil mixed feed.

significantly affect the overall redox potential of the cell (Hansen *et al*., 2001). Our result indicate that glutathione concentration was significantly ($p < 0.05$) reduced in rats fed with 5% crude oil-mixed feed when compared to the control (Figure 9). The reduction could be a compensatory mechanism by the animals fed with feed mixed with crude oil to overcome the effect of the oxidant stress caused by free radicals generated by crude oil. These observations are corroborated by earlier studies on the effects of petroleum hydrocarbon on other animal species (Nwaogu *et al.*, 2008; Ibrahim and Rizk, 2008).

Catalase is an enzyme found in nearly all living organisms (plants and animals) that are exposed to oxygen where it functions to catalyse the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen (Diniz *et al.,* 2004; Pigeolet *et al.,* 1990). A dose-dependent reduction was observed in the activities of catalase from the liver tissues of rats fed with 1.25%, 2.25% and 5.0% crude oil polluted feed. However the reduction in catalase activity was only statistically significant ($p<0.05$) obtained in rats fed with 5.0% crude oil mixed feed when compared to the control indicating a possible attenuation of oxidative stress.

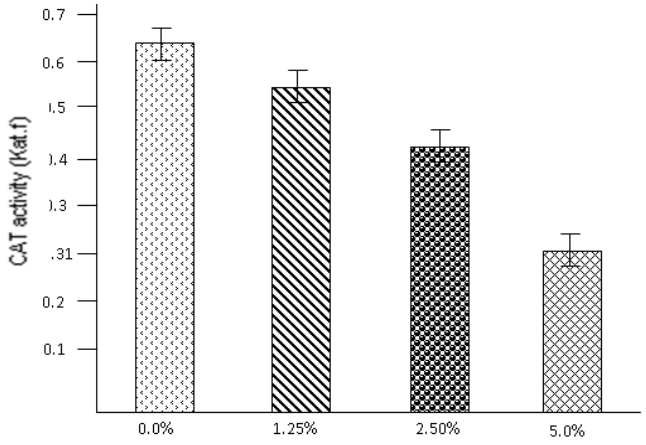

Fig. 10: Liver catalase (CAT) activities of albino rats fed different concentrations of crude oil mixed feed.

CONCLUSION

This study showed that subchronic exposure to crude oil caused adverse changes in the tissues of albino rats. These changes if unchecked could precipitate various disease conditions.

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