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Full Length Research Paper

Evaluating Biochemical Changes in Rats Induced by Crude Oil Contaminated Catfish (Clarias gariepinus) Consumption

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Biochemical investigations were carried out to assess the effect of consumption of crude oil contami-nated catfish on the hepatocytes and performance of rat. Catfish (*Clarias gariepinus*) (n = 120) were grouped into 6 of 20 catfish each and held for 30 h in 5 different mixtures of crude oil polluted water (0.1, 0.25, 0.5, 0.75 and 1% v/v). Catfish in the control group were held in borehole water. At the expira-tion of 30 h, the catfish were harvested and used to formulate diet. Albino rats (n = 60) were grouped into 6 of 10 rats each and fed on the formulated diet for a period of 30 days. Compared with the control, the result showed no significant difference (p>0.05) in the average daily feed intake in all the groups of rats. However, a significant reduction (p<0.05) was observed in the overall body weight, the liver-to-body weight ratio as well as the serum concentrations of albumin and globulin of rat as the amount of crude oil in the diet increased. A significant increase (p<0.05) was also observed in the serum bilirubin concentration of rats fed on diet formulated with crude oil contaminated catfish when compared with the control. Histological analysis also revealed that the architectural arrangement of the liver was alte-red following the consumption of diet containing catfish exposed to crude oil polluted water. Overall, the data obtained indicate a possible adverse effect on the performance and impaired liver function in the rats fed on diet containing catfish exposed to crude oil polluted water.

Key words: Crude oil, hepatocytes, catfish, albumin, globulin, bilirubin.

INTRODUCTION

In Nigeria, crude oil was discovered at Oloibiri in 1956, and it has generated so much revenue for the country (Akpofure et al., 2000). However, this is not without its attendant problem of spillage into adjoining water bodies and land. Each year, according to the United States Envi-ronmental Protection Agency, an average of 14 million gallons of oil from more than 10,000 accidental spills is discharged into water bodies worldwide particularly thro-ugh the leakage of pipes carrying oil and from under-ground reserves (USEPA, 1999).

The devastating consequences of oil spill especially in the Niger Delta region of Nigeria together with its even-tual hazards on both aerial and terrestrial environs mani-fest as an irreversible chain effect on both the biodiver-sity and human safety. As this occurs, the oil threatens surface water and a wide range of subsurface marine or-ganisms which are linked in a complex food chain (Kat-wijk Van et al., 1999). Oil spillage has caused destruction of food resources (Percival and Evans, 1997). Animal species that are not directly in contact with the oil spillage can also be harmed via the food web. Predators that consumed contaminated marine preys can be exposed to oil through ingestion of the prey.

Aquatic environments are made up of complex inter-relations between plant and animal species and their physical environment. Harm to the physical environment will often lead to the death of one or more species in a food chain, which may lead to damage for other species further up the chain. Whether an organism spends most of its time in open water, near coastal areas or on the shoreline will determine the effects an oil spill is likely to have on that organism (Brown and Weiss, 1978).

In open water, fish have the ability to swim away from a spill by going deeper in the water or further out to sea, reducing the likelihood that they will be harmed by even a major spill. Aquatic animals that generally live closer to

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shore, such as turtles, seals and dolphins risk contamination by oil that washes onto beaches or by consuming oil contaminated prey. In shallow waters, oil may harm sea grasses and kelp beds which are used for food, shelter and nesting sites by many different species (Carls et al., 1999).

The effects of oil spill on aquatic lives are caused by either the physical nature of the oil (physical contamination and smothering) or by its chemical components (toxic effects and accumulation leading to tainting). Aquatic lives may also be affected by clean up operations or indirectly through physical damage to the habitats in which plants and animals live (Cooney et al., 2001). The main threat posed to living resources by the persistent residues of spilled oils and water-in-oil emulsions ("mousse") is one of physical smothering. The animals and plants most at risk are those that could come into contact with a contaminated sea surface. These include aquatic mammals and reptiles; birds that feed by diving or form flocks on the sea as well as aquatic lives on shorelines (Suchanek, 1993).

Whenever oil spills, it spreads almost immediately and some of the components get dissolved in water while others may become oxidized or undergo bacterial degradation. The resulting products eventually sink to the bottom of the water body by gravitational action and constitute threat to aquatic lives. The inhabitants of the oil-polluted areas, who are mostly fishermen, harvest the fish from the polluted water for human consumption. The effect of crude oil pollution on aquatic lives is therefore the concern of many scientists, since most of the world's population is dependent upon aquatic animals for food, and this dependency increases as the demand for food increases. Thus, research studies have shown increase in physical and psychological symptoms attributed to exposure of crude oil.

Acute, chronic and long term effects of chemical compounds (including crude oil) on living systems could be studied by evaluating the biochemical and morphological changes in various organs especially the liver. The basic structural component of the liver is the liver cells or heaptocytes. The liver is the principal organ of metabolism and has a role to play in many body processes most especially detoxification of chemical compounds. Research studies have shown a variety of adverse effects on the hepatocytes of rats and catfish following exposure to environmentally toxic compounds (Oloyede et al., 2003, Sunmonu and Oloyede, 2006). In the present study, an attempt is made to assess the effect of crude oil contaminated catfish on the hepatocytes and performance of 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), Nigerian National Petroleum Corporation (NNPC), Port Harcourt, Nigeria and diluted with borehole water to obtain mixtures of 0.1, 0.25, 0.5, 0.75 and 1% by volume.

Experimental fish and treatments

One hundred and twenty apparently healthy juvenile catfish (*Clarias gariepinus*) were obtained from a commercial fish pond at Unity Road in Ilorin, Kwara State, Nigeria and acclimatized for ten days prior to the commencement of the experiment. The catfish were grouped into six of twenty catfish and were kept in 30L plastic aquaria. Group 1 served as control and the catfish here were cultured in borehole water while those in Groups 2 to 6 were exposed to the different mixtures (0.1, 0.25, 0.5, 0.75 and 1% v/v) of crude oil. The catfish were fed *ad libitum* with commercial fish meal for 30 h during which the experiment lasted.

Formulation of diet

At the end of the 30 h experimental period, the catfish were harvested, oven dried at 40^{O}C and used as a source of protein (25%) to formulate diet for albino rats. The diet for each group was formulated by mixing known quantities of sources of each food class comprising corn starch (52%), oil (4%), maize cob (4%), sucrose (10%) and vitamin/mineral mixture (5%). The food items were mixed together and manually made into pellets to feed albino rats.

Experimental rats and treatments

Sixty albino rats (*Rattus norvegicus*) with an average weight of 50.20±4.24g were obtained from the Small Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were grouped into six with each group containing ten rats. The rats in Group 1 served as the control and they were fed on the control diet, which was formulated with catfish cultured in borehole water. Animals in Groups 2 to 6 were fed on diet formulated with catfish exposed to the different mixtures of crude oil (0.1, 0.25, 0.5, 0.75 and 1.0% v/v respectively). The feeding lasted for a period of thirty (30) days (after an acclimatization period of ten days) during which the weight and feed intake were monitored.

Collection of blood sample and isolation of liver

The rats were anaesthetized and blood samples were collected by cutting the jugular vein with a sharp sterile blade. The blood sample collected was spun using a centrifuge at 4000 rpm for 35 min and the serum was collected using a Pasteur's pipette for analyses. The rats were thereafter dissected and the whole liver was excised into a beaker containing ice-cold 0.25 M sucrose solution. The liver-to-body weight ratio was determined by taking the weight of the whole liver and comparing it with the final body weight of each rat.

Determination of serum metabolite concentrations

The method described by Doumas et al. (1971) was employed for the determination of serum albumin concentration. The determination of serum globulin concentration was carried out using the method described by Tietz (1995) by subtracting the concentration serum albumin from the total protein content determined using Biuret method as described by Henry et al. (1974). The total serum bilirubin concentration was determined using the method outlined in AACC (1984).

Table 1. Performance of rats fed on diet containing catfish exposed to crude oil polluted water.

Mixtures of crude oil (%v/v)	Average daily wt. gain/loss (g/rat/day)	Average daily feed intake (g/rat/day)	Feed Conversion Ratio
Control	0.84±0.02[a]	13.21±0.26[a]	15.73±0.65[a]
0.10	0.48±0.01[b]	13.27±0.27[a]	27.65±0.55[b]
0.25	0.30±0.01[c]	13.35±0.34[a]	44.50±0.58[c]
0.50	0.27±0.01[d]	13.38±0.29[a]	49.56±0.50[d]
0.75	0.21±0.01[e]	13.28±0.34[a]	63.24±0.85[e]
00	0.15±0.01[f]	13.19±0.29[a]	87.93±0.80[f]

Values are means ± SEM for 10 rats**.** [a,b,c,d,e,f] Column values with different superscripts are significantly different ($p<0.05$).

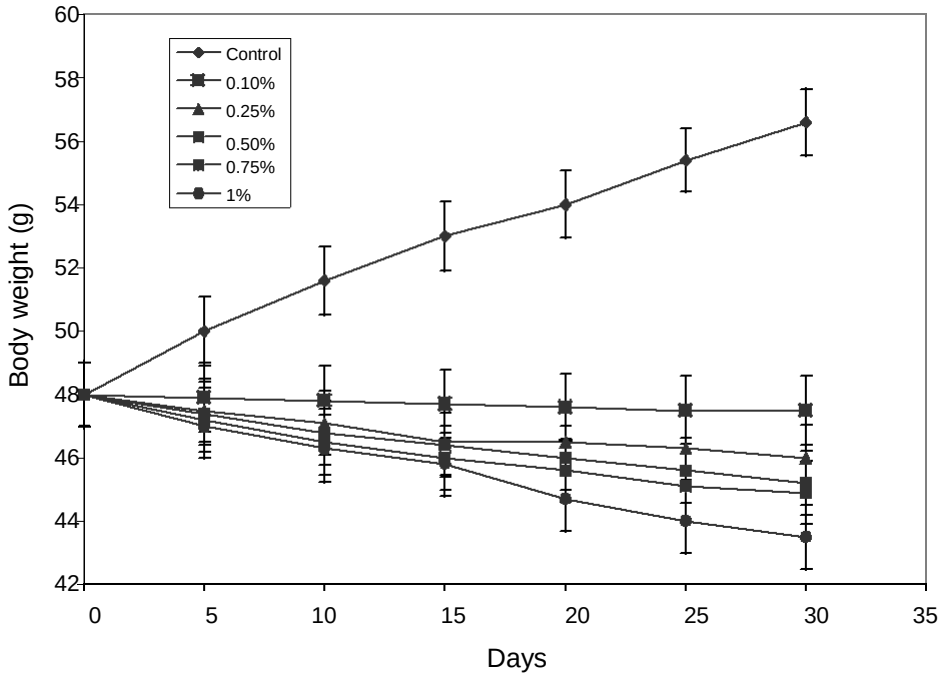


Figure 1. Growth response curve of rats fed on crude oil contaminated catfish over a period of 30 days.

Histological examination of the liver

A portion of the liver was fixed immediately on removal from the animal in 10% Buffered Neutral Formalin (BNF) for 72 h at room temperature for histological analysis using the method described by Krause (2001).

Statistical analysis

All data were analysed using Analysis of Variance (ANOVA) by employing the method of Steel and Torrie (1960). Significant difference between the treatment means was determined at 5% confidence level using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS

The performance of rats fed on diet containing catfish exposed to crude oil polluted water is as presented in Table 1. Compared with the control, there was no significant difference ($p>0.05$) in the average daily feed intake in all the experimental groups of rats. However, the feed conversion ratio of rats fed on the polluted diet was significantly higher ($p<0.05$) than that of the control animals; ranging between 2 to almost 6 folds of the control value.

The growth response curve of rats fed on diet containing catfish exposed to crude oil is presented in Figure 1. The data revealed that rats fed on the control diet experienced a significant increase ($p<0.05$) in weight while those fed on diet containing contaminated catfish experienced a significant ($p<0.05$) weight reduction over the period of study.

The data obtained with respect to the liver-to-body weight ratio of rats fed on diets containing catfish exposed to crude oil polluted water is as presented in Figure 2. Com-

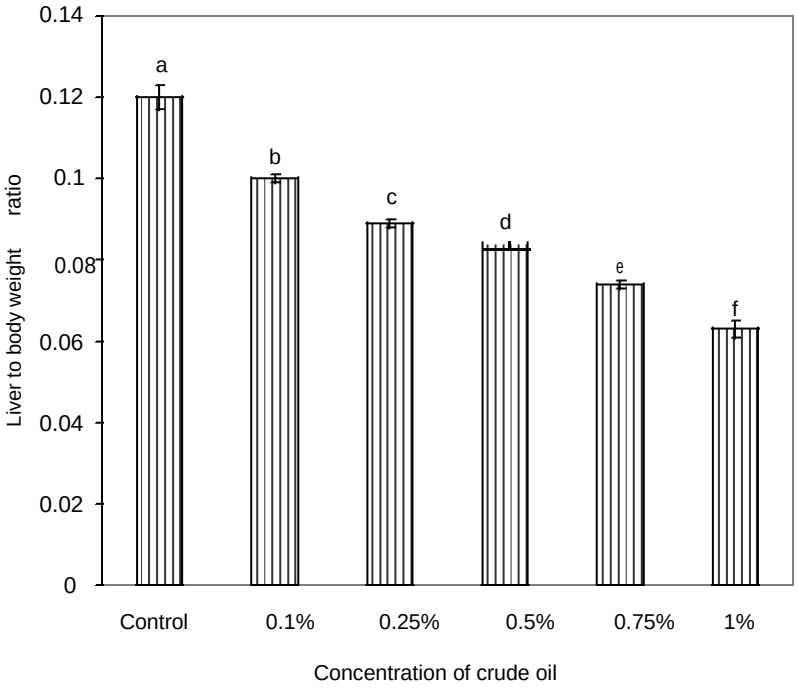


Figure 2. Liver to body weight ratio of rats fed on crude oil contaminated catfish over a period of 30 days.

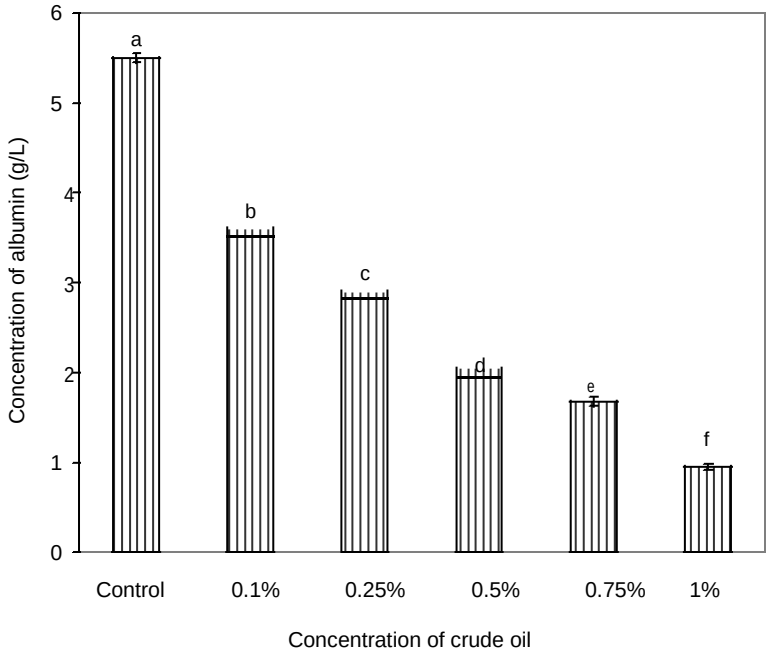


Figure 3. Concentration of albumin in the serum of rats fed on crude oil contaminated catfish over a period of 30 days.

pared with the control, the rats fed on diet formulated with catfish exposed to crude oil experienced a significant reduction ($p<0.05$) in the liver-to-body weight ratio dropping to about half of the control value for the highest crude oil mixture.

Figures 3 and 4 show the concentration of albumin and globulins in the serum of rats fed on diet formulated with catfish exposed to various mixtures of crude oil polluted water. Compared with the control, a significant reduction ($p < 0.05$) was obtained in the concentration of serum in

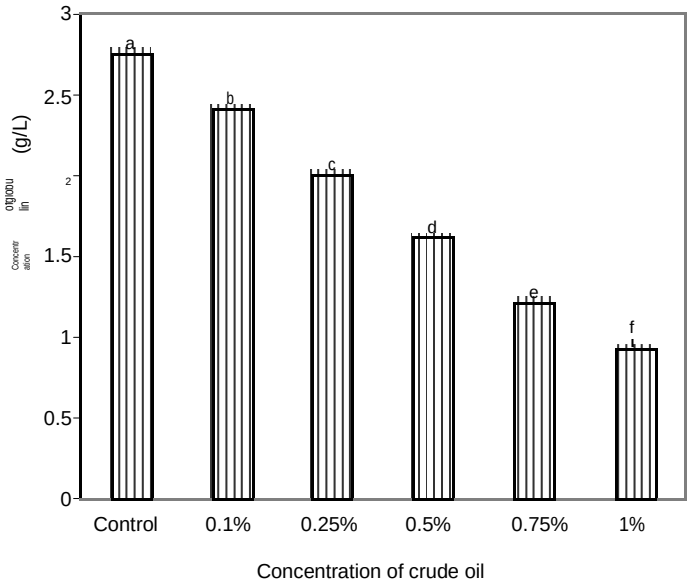


Figure 4. Concentration of globulins in the serum of rats fed on crude oil contaminated catfish over a period of 30 days.

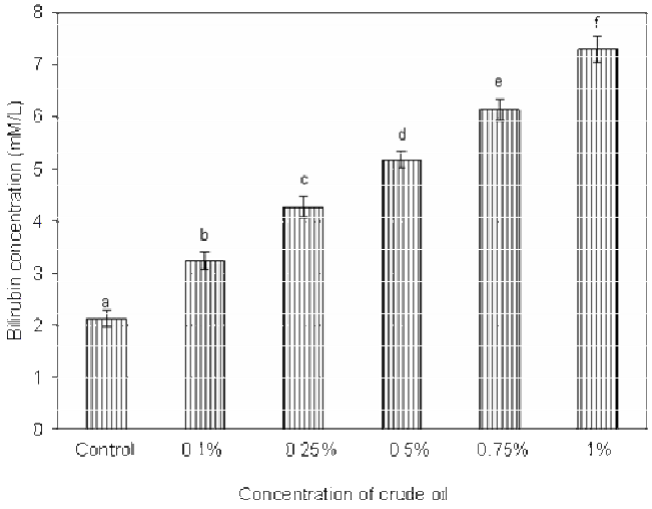


Figure 5. Serum bilirubin concentration of rats fed on crude oil contaminated catfish over a period of 30 days.

albumin and globulin in the rats as the amount of crude oil in the mixture increased.

The serum bilirubin concentration of rats fed on diet formulated with catfish exposed to crude oil is presented in Figure 5. The data indicate a significant increase ($p<0.05$) in the serum bilirubin concentration as the concentration of crude oil in the diet increased. Indeed, the highest concentration of bilirubin was obtained in rats fed on diet containing catfish exposed to 1% crude oil.

The light micrographs showing the histological appearance of the liver of rats fed on diet containing catfish cultured in various mixtures of crude oil polluted water are presented in Figures 6a – 6f. The liver of rats fed on diet containing catfish cultured in 0.1% concentrated crude oil polluted water (Figure 6b) showed no observa-ble morphological changes when compared with the control (Figure 6a). However, marked cellular disintegra-tion manifested in the other experimental groups which is more pronounced in rats fed on diet containing catfish cultured in 1% crude oil polluted water (Figure 6f).

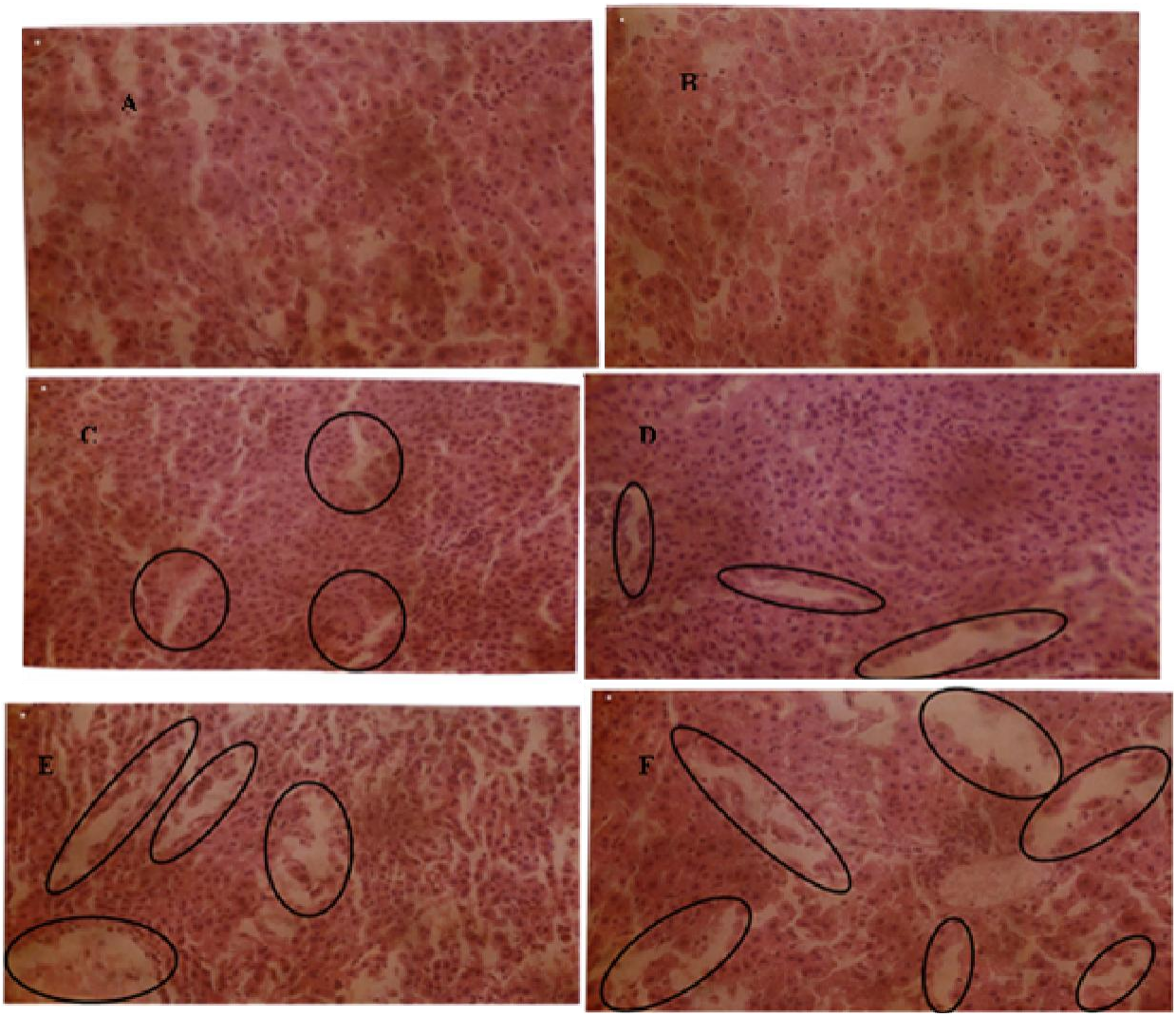
DISCUSSION

The consumption of crude oil contaminated catfish may manifest in various forms including changes in physical performance and/or biochemical changes. In the present study, evidences are presented to address the possible effects that can emanate following the consumption of crude oil contaminated catfish by rats.

The observation with respect to increase in the feed conversion ratio of experimental animals (Table 1) may be an indication that the animals could not convert the feed consumed into useful nutrients required by the body, thus accounting for the reduced daily weight gain when compared with the control. This factor may also be responsible for the reduction in weight experienced by rats fed on diet containing catfish exposed to crude oil (Figure 1).

Reduction in the liver-to-body weight ratio of the experimental animals as observed in the present study (Figure 2) is in agreement with earlier report by Berepubo et al. (1994) who observed a significant reduction in the liver-to-body weight of weanling rabbits fed on crude oil contaminated diet. Therefore, crude oil portends serious adverse effects on the growth potentials of the experimental rats. The significant reduction ($p<0.05$) in the liver-to-body weight ratio may also be attributed to abnormality in nutrient absorption by the liver from the crude oil contaminated diet since the animals were feeding quite well as observed in the present study (Table 1).

Two important biochemical indices that can be used to assess the health status of the liver are the serum levels of albumin and globulin. Albumin, which is manufactured by the liver, is a major protein that circulates in the bloodstream (Tietz, 1986). The significant reduction ($p<0.05$) in serum albumin concentration with increase in the amount of crude oil in the mixture (Figure 3) may be a consequence of poor diet or an indication of liver dysfunction amongst others. The fish meal may have been contaminated with the crude oil and consequently contaminating the fish that consume it. Thus, it is possible that the contaminated diet consumed by the rats contains toxic compounds like polycyclic aromatic hydrocarbons (an important constituent of crude oil), which may affect the liver thereby preventing it from manufacturing enough albumins for release into the serum. Globulins are a larger protein than albumin and they are important for its immunologic responses (Tietz, 1986) . Reduced serum globulin concentration with increase in the amount of crude oil in



Figures 6a – 6f. Light micrographs (x400) of the liver of rats fed on diet containing catfish cultured in various concentrations (control, 0.1%, 0.25%, 0.5%, 0.75% and 1% respectively) of crude oil polluted water for 30 days. Gross tissue disintegration and breakage is typified by large open spaces (see black circle).

the diet as observed in the present study (Figure 4) may be an indication of reduced immunity in the rats since the liver will not be able to synthesize enough globulins for immunologic action.

Bilirubin is the major breakdown product that results from the destruction of old red blood cells. It is removed from the blood by the liver; hence it is a good indication of the function of the liver. Bilirubin concentration is elevated in the blood either by increased production of bilirubin or decreased liver uptake (as a result of liver disease). The observed increase in bilirubin concentration (Figure 5) corroborates the fact that there may be evidence of liver dysfunction, which may be as a result of the effect of the toxic components of crude oil in the formulated diets. This assertion is supported by the fact that the water-soluble components of crude oil are toxic and could affect the survival and metabolism of aquatic animals including fish (Cote, 1976). In a similar manner, the effect may also occur in other animals like rats if exposed to crude oil contamination as observed in this study. This position is also supported by the work of Ovuru et al. (2004) who reported an increase in total serum bilirubin concentration in semi adult rabbits exposed to crude oil contaminated diet and attributed this to a metabolic disturbance in the liver arising from defective conjugation and/or excretion of bilirubin. The present finding is further supported by Cheeseborough (1992) who reported that a rise in the concentration of serum bilirubin indicates or suggests liver damage since the liver serves as an excretory unit rather than a distributing unit for bilirubin.

Histological analysis can also be used to examine the morphological changes in rat liver to reflect possible effect of the crude oil on the hepatocytes. Analysis of the light micrographs (Figures 6a to 6f) revealed gross struc-

tural disintegration in the hepatocytes when compared with the control. The disintegration is more pronounced in rats fed on diet containing catfish cultured in 0.75 and 1% mixtures of crude oil polluted water (Figures 6e and 6f respectively) as evidenced by the presence of large open spaces. Loekle et al. (1983) described these open spaces as areas of tissue disintegration. These further supports the fact that consumption of crude oil contaminated catfish portends serious damaging effects on the hepatocytes.

Conclusion

1. Diet formulated with catfish exposed to crude oil polluted water possibly has adverse effect on the performance of rats as manifested by the increased feed conversion ratio and reduced body weight gain.
2. The reduction in the liver-to-body weight ratio of rats may be as a result of malabsorption of the required nutrient from the contaminated diet or dysfunction in the metabolism of rats.
3. Possible damaging effect was also manifested on the hepatocytes as evidenced by reduced serum concentrations of albumin and globulins.
4. Deviation from the normal serum bilirubin concentration as observed in the control rats may be attributed to hepatic dysfunction causing the release of its metabolites into the blood rather than being excreted.
5. Extensive structural damage to the hepatic cells may result following the consumption of crude oil contaminated catfish as revealed by the histological study.

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