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Changes in rat liver enzyme activities following consumption of crude oil contaminated catfish (*Clarias gariepinus*)

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ABSTRACT
Objective: To study changes in selected liver enzymes of albino rats fed on diet formulated with catfish that were exposed for 30 days to water polluted with crude oil.
Methodology and results: One hundred and twenty (120) catfish (*Clarias gariepinus*) in six groups of 20 catfish each were held for 30 h in water having five different concentrations of crude oil (0.1, 0.25, 0.5, 0.75 and 1% v/v). The catfish in the control group were held in un-polluted borehole water. After 30 h, the catfish were harvested and used to formulate diet. Albino rats (n = 60) in six groups of 10 rats each were fed on the formulated diet for a period of 30 days. The control rats were fed on diet containing catfish cultured in borehole water while those in groups two to six were fed on diets containing catfish exposed to the various concentrations of crude oil. In comparison with the control, a significant reduction ($p<0.05$) in the activities of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) was observed as the concentration of crude oil in the diet increased. Conversely, the activities of these enzymes in the serum of treated albino rats increased significantly ($p<0.05$) when compared with the control.
Conclusion and application of findings: The data obtained suggests a possible adverse effect of crude oil on albino rats, as manifested by changes in liver enzymes. This demonstrates the adverse impact of crude oil intoxication during oil spillage and pollution, a common occurrence on aquatic life and animals.

Key words: Crude oil, enzymes, pollution, serum, liver, catfish

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INTRODUCTION

Water pollution may result from discharge of untreated or partially treated human waste and biologically degradable industrial waste into watercourses. Pollutants introduce a significant organic load into river water, which increases the biological oxygen demand (Terry, 1996). Managing the concentration of pollutants in drinking water is very important since adult human beings drink much water per day and are therefore likely to be affected by pollutant in water than from diet (Alloway, 1992).

The effects of water pollution are varied and may include food poisoning such as through fish accumulating toxic chemicals from the environment. Water pollution can cause imbalances resulting in river and lake ecosystems that cannot adequately support full biological diversity (Ken, 2005). For humans, the consequences of drinking contaminated water include

gastro-intestinal disorders, several chronic ailments and possibly death. The Love Canal incident (Niagara Falls, New York State), which occurred between 1942 and 1953 is a classical example of danger to water posed by chemical waste (Alloway, 1992).

The characteristics of industrial wastewater differ considerably both within and among industries. The impact of industrial discharges depends not only on their collective characteristics, such as biochemical oxygen demand and the amount of suspended solids but also on their content of specific inorganic and organic substances (Tibbetts & Anon, 1996; Richman, 1997). Industrial waste belongs to the group of undesirable waste referred to as "hazardous substances" that are injurious to the welfare of man. Hazardous wastes are characterized by their properties of ignitability (presents a fire hazard), corrosivity (attacks other materials by destructive chemical action), reactivity (undergoes violent, spontaneous chemical action including explosion) and toxicity (acts as an acute or chronic poison to living organisms) (Alloway, 1992).

Crude oil is made up of petroleum and natural gas. It is a complex mixture of hydrocarbons from which various petroleum products such as gasoline, kerosene, fuel oil, lubricating oil, wax and asphalt are derived (USEPA, 1999). In Nigeria, inadequate technological expertise on crude oil exploration often leads to spillage into water bodies and on land, with most incidences occurring in the Niger Delta area. Economic activities in this area are negatively affected due to inadequate water and oil effects limiting land for fishing and farming (Akpofure *et al*., 2000). A majority of the people rely on consumption of fish caught downstream where the concentration of crude oil is assumed to be less.

Previously, research studies have been carried out to determine the effects of crude oil on rats and catfish (Sunmonu & Oloyede, 2006; 2007). The study presented in this paper aimed to use enzymes as biochemical indicators to assess the effect of consuming catfish grown in water that is contaminated with crude oil. Due to their high sensitivity, enzymes are suitable and rapid indicators of effects at the cellular level, and could reduce waiting periods to observe long-term effects that may take several months or years to manifest.

MATERIALS AND METHODS

Preparation of crude oil concentrations: 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 concentrations of 0.1, 0.25, 0.5, 0.75 and 1% by volume (Table 1).

Experimental fish: One hundred and twenty apparently healthy juvenile catfish (*Clarias gariepinus*) were obtained from a commercial fishpond at Unity Road in Ilorin, Kwara State, Nigeria and acclimatized for ten days prior to the commencement of the experiment. The catfish were divided into six groups of twenty catfish each, and kept in 30L plastic aquaria. Group 1 served as control with the catfish cultured in borehole water while Groups 2 to 6 were exposed to the different concentrations of crude oil. The catfish were fed ad libitum on commercial fishmeal for 30 hours during which the experiment lasted.

Formulation of diet: After 30 hours in treated water, the catfish were harvested, oven dried at 40°C and used as a source of protein to formulate diet for albino rats. The diet for each group was formulated by mixing known quantities of sources of each food class (Table 2). The food items were mixed and manually made into pellets to feed albino rats.

Experimental rats: 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 *unpolluted* borehole water. Animals in groups 2 to 6 were fed on diet formulated with catfish exposed to varying concentrations 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.

Table 1: Preparation of various concentrations of crude oil.

Concentration (%v/v)	Crude oil (cm^3)	Borehole (cm^3)	Total Vol.(cm^3)
0.10	0.10	99.90	100
0.25	0.25	99.75	100
0.50	0.50	99.50	100
0.75	0.75	99.25	100
1.00	1.00	99.00	100

Table 2: Composition of formulated diet fed to albino rats.

Feed component	% Composition
Contaminated catfish*	25
Corn starch	52
Oil	4
Cellulose (maize cob)	4
Sucrose	10
**Mineral/vitamin mixture	5
Total	100

* Catfish cultured in water with varying concentrations of crude oil (0.1 - 1.0%). Control catfish were cultured in unpolluted borehole water. ; ** Vit A 15,000,000i.u, Vit. D, 32,000i.u, Vit E, 12,000i.u, Vit K, 2i.u, thiamine 1.5g, riboflavin 25g, pyridoxine 5g, folic acid 0.5g. For the mineral mixture, manganese 75g, zinc 45g, iron 20g, copper 5g, iodine 1g and selenium 100mg.

Collection of blood samples and liver: The rats were anaesthetized by dropping them in a jar containing cotton wool soaked in chloroform. Blood samples were collected by cutting the jugular vein with a sharp sterile blade. The blood sample collected was spinned using a centrifuge at 4000rpm for 35 minutes and the serum was collected using a Pasteur's pipette for enzyme assay. The rats were thereafter dissected and the liver was excised and placed in a beaker containing ice-cold 0.25M sucrose solution. Known weight of the liver was cut, chopped into small pieces and then homogenized using pre–cooled pestle and mortar in a bowl of ice chips. The homogenized tissue was diluted with 0.25M sucrose solution to obtain a 1 in 5 dilution for enzyme assay.

Enzyme assay: The protein content of the liver homogenate was determined using the Biuret method of Henry *et al*. (1974). The activities of alanine and aspartate transaminases were assayed using the procedure described by Schmidt and Schmidt (1963), alkaline phosphatase activity was determined as described by Wright *et al*. (1972) while the activity of gamma glutamyl transferase was determined using the method described by Szasz *et al*. (1974).

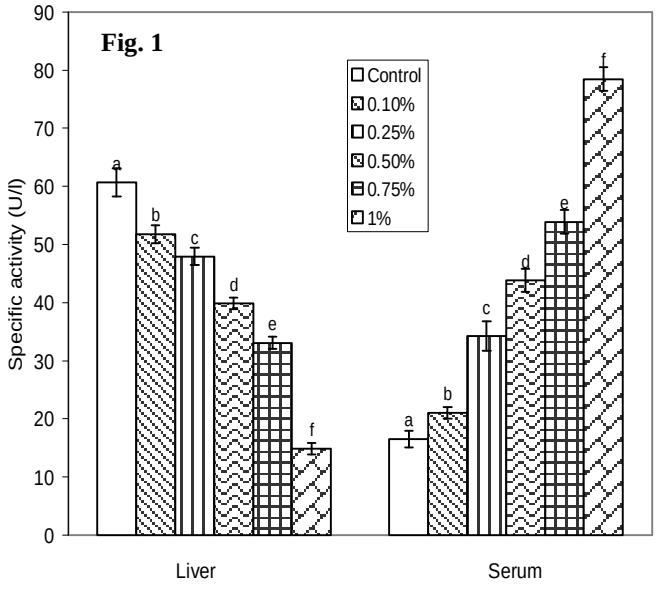
Data analysis: All data were subjected to Analysis of Variance (ANOVA) as described by Steel and Torrie (1960). Significant differences between the treatment means were detected at at 5% confidence level using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS

The specific activity of gamma glutamyl transferase (GGT) in the liver of rats fed on diet formulated with catfish exposed to crude oil polluted water is presented in Figure 1. Compared to the control, the result revealed that there was a significant reduction ($p<0.05$) in the specific activity of GGT in the liver while the activity of the enzyme increased significantly ($p<0.05$) in the serum of rats. A similar pattern was observed in the activities of AST, ALT and ALP (Figures 2, 3 and 4) in the liver and serum of rats fed on diet formulated with catfish exposed to water polluted with crude oil.

DISCUSSION

Damages to biological tissues can be assessed by changes in their enzyme activities, which indicate the catalytic influence of various factors, e.g. inhibitors and activators, during pathological conditions. With respect to the activity of GGT, the data obtained in this study agrees with earlier reports (Kulhanek & Dimod, 1966; Ruppin *et al*., 1982) showing that any blockage to the flow of bile inside or outside the liver would lead to induction gamma glutamyl transferase and its increased levels in the blood.



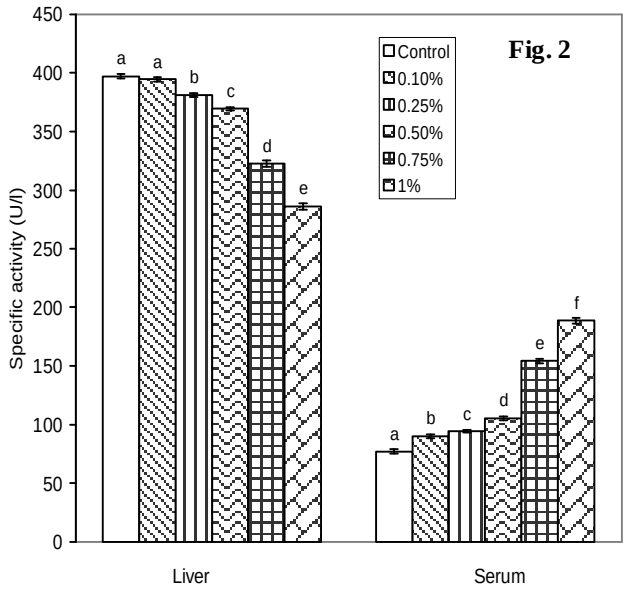
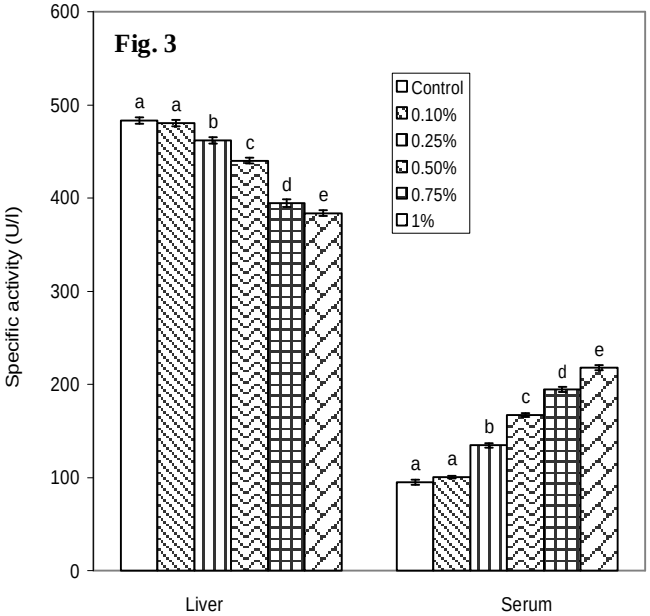


Fig. 1: Specific activity of gamma glutamyl transferase in the liver and serum of rat fed on diet containing catfish exposed to crude oil. Fig. 2: Specific activity of aspartate transaminase in the liver and serum of rat fed on diet containing catfish exposed to crude oil. Data are mean ± SE for 10 rats. Bars with different letters are significantly different.



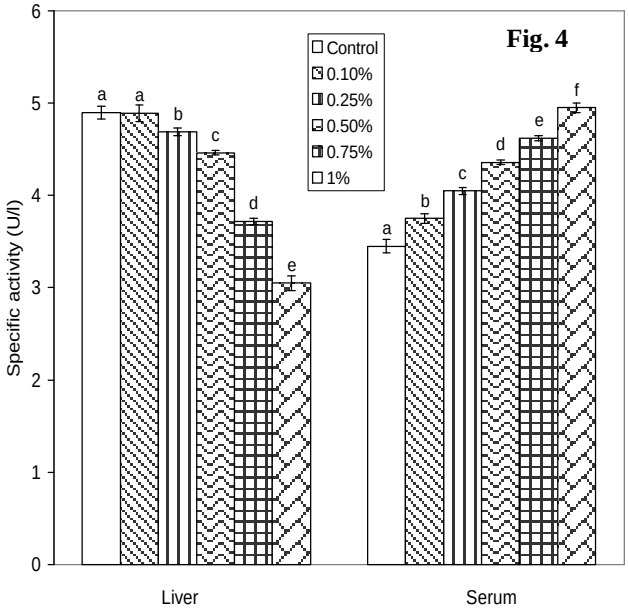


Fig. 3: Specific activity of alanine transaminase in the liver and serum of rat fed on diet containing catfish exposed to crude oil. Fig. 4: Specific activity of alkaline phosphatase in the liver and serum of rat fed on diet containing catfish exposed to crude oil. Data are mean ± SE for 10 rats. Bars with different letters are significantly different.

It can therefore be inferred that the observed increase in serum activity of gamma glutamyl transferase in the experimental rats may be as a result of an obstruction to the flow of bile in the bile ducts probably caused by the composition of the crude oil contaminated fish. In a related submission, Aslan *et al*. (2000) stated that similar reductions in activity of gamma glutamyl transferase in rat liver (as observed in this study) may lead to an increase in the level of antioxidants particularly glutathione (GSH) arising from free radicals generated from the unsaturated chemical

components of crude oil ingested through the contaminated diet.

In another study, Deoras *et al.* (2003) found that the activity of gamma glutamyl transferase reduced significantly in the liver of Sprague-Dawley rats treated with gossypol, possibly due to the presence of toxicants, e.g. polycyclic aromatic hydrocarbons in the system of the rats. This may be likened to the effect produced by crude oil, which contains toxic components or compounds which directly or indirectly inhibit the activity of the enzyme or cause hepatic damage. This conclusion is supported by the fact that GGT is the most sensitive indicator of hepatobiliary disease, which is evident in altered specific activity of GGT (Arnold, 1976). Therefore, the significant increase ($p<0.05$) in the activity of GGT in the serum coupled with the reduced activity in the liver of albino rats may be an indication of possible leakage of the enzyme from the liver into the serum; hence suggesting liver dysfunction caused by the ingestion of crude oil contaminated diet.

Similarly, reduction in the activities of AST and ALT in the liver of rat observed in the present study supports earlier studies by Mousa and Khattab (2003) where the activity of transaminases in the liver of catfish were inhibited after intoxication with ochratoxin in diet. Abdel Tawwab *et al.* (2001) also observed a similar result in liver AST of Nile tilapia after exposure to mercury. These workers ascribed the reduction in enzyme activity to liver necrosis caused by the toxicants and a possible damage to the hepatocytes. The reduction in the activities of ALT and AST in the liver may be due to the interference with protein metabolism in the cells or inhibition of the enzyme (Karmen *et al*., 1995). There may also be leakage of the enzyme from the liver into the serum accounting for the significant increase in enzyme activity in the serum. All these are evidences of possible damage to the liver of rat by the crude oil.

The significant reduction ($p<0.05$) in the specific activity of ALP in the liver as the concentration of crude oil increased may in part be due to damage to the plasma membrane of the hepatocytes. It could also be attributed to inhibition of the enzyme by the crude oil component of the diet or inactivation of the enzyme molecules *in situ* (Umezawa & Hooper, 1982). In contrast, ALP activity in the serum increased as the level of crude oil in the diet increased. This may be associated with possible leakage of the enzyme from the hepatocytes into the serum as a result of the damage done to the plasma membrane. Normally, increased enzyme activity would not be observed in serum except when there is damage to one or more organs or tissues of the body. Therefore, enzymes from diseased tissues or organs and from drug assault or other xenobiotics may become manifested in the serum resulting in increased activity since they must have leaked from the diseased tissue. Increased activities of serum enzymes have been reported in conditions of tissue damage due to such disease conditions and from the use of several chemicals and drugs (Hanley *et al*., 1986). This is often accompanied by a corresponding decrease in enzyme activity in the affected tissue or organ.

This study has shown that crude oil portends serious damaging effects on the hepatocytes as evidenced by reduced activities of GGT, AST, ALT and ALP in the liver of rat fed on crude oil contaminated catfish. Hence, hepatic functions may be impaired. Therefore, it is advisable that people should avoid consuming fish from water that is potentially contaminated with crude oil especially at concentrations above 0.25% v/v.

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