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Full Length Research Paper

Biochemical blood parameters in semi-adult rabbits experimentally fed crude oil contaminated diets

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Biochemical parameters in blood specimens obtained from semi- adult rabbits of both sexes fed crude oil contaminated diets were examined. The diets had crude oil inclusions of 0, 0.05, 0.10, 0.15 and 0.20%. Blood samples were obtained from the marginal ear vein of representatives in each treatment group and assayed for urea, creatinine, total bilirubin, conjugated bilirubin and cholesterol. There were significant differences (P < 0.05) in biochemical parameters between control and crude oil treated animals. The biochemical changes observed indicate that ingestion of crude oil fractions cause disturbances in kidney and hepatocytes.

Keywords: Biochemical parameters, rabbits, crude oil contaminated diets.

INTRODUCTION

Various environmental pollutants, particularly those associated with crude oil cause many biochemical and toxic effects in marine and terrestrial animals (Berepubo et al., 1994, Carls et al., 1999, Ngodigha et al., 1999). Many halogenated aromatic hydrocarbons and polycyclic aromatic hydrocarbon do enhance mono-oxygenase activities. Different fractions of crude oil, differing in their substrate specificities cause different toxicities both in marine and terrestrial animals. A hand full of biochemical blood parameters are routinely used to assess health status and also aid in the diagnosis of diseases in man and animals. These tools in most cases have less frequently been applied in studies dealing with ecotoxicology or related disciplines. In this study, an attempt was made to investigate biochemical blood parameters in semi adult rabbits fed crude oil contaminated diets. Owing to the differences in crude oil components, the stable components may differ in toxicity. The main objectives were to assess the overall toxicity and relate the biochemical changes to the specific weathered fraction as this fraction simulates the portion that remains in the ecosystem after a spillage.

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MATERIALS AND METHODS

Thirty rabbits aged 20-22 weeks and weighing 1.10-1.42 kg were procured from the Rivers State Ministry of Agriculture and Natural Resource, Port Harcourt. They were acclimatized for two weeks at the Rivers State University of Science and Technology Teaching and Research Farm. The animals were housed in 3-tier conventional bamboo hutches in the rabbitary section and administered prophylactic coccidiostat and broad – spectrum antibiotics. Their feed consisted mainly of forage prepared from grass and centro and further supplemented with concentrate (growers mash). All drinking and feeding troughs were cleaned and hygienically kept throughout the experimental period. The thirty rabbits were randomly allocated to five dietary groups. Each dietary group consisted of six animals in two blocks. The groups were:

A:	0.00%	No contamination (control)
B:	0.05%	Crude oil contamination
C:	0.01%	Crude oil contamination
D:	0.15%	Crude oil contamination
E:	0.20%	Crude oil contamination

Crude oil was obtained from the Brass Terminal of the Nigerian Agip Oil Company Ltd. The oil was exposed to sunlight for 24 h in

Table 1. Mean squares from analysis of variance in biochemical parameters in rabbits exposed to varying levels of crude oil contaminated diets

Source	DF	Urea (U)	Creatinine (Cre)	Total bilirubin	Conjugated bilirubin	Cholesterol
Treatment (T)	4	2.56*	1992.88**	16.42***	0.26***	0.28*
Sex (S)	1	1.15^{ns}	0.80^{ns}	3.79**	0.69***	0.00^{ns}
T x S	4	6.86***	650.18^{ns}	18.41***	0.26***	0.74**
Error	10	0.58	287.30	0.20	0.00	0.07
Total	19					

* = P < 0.05
** = P < 0.01
*** = P < 0.001
ns = not significant

Table 2. Biochemical responses of rabbits exposed to varying levels of crude oil contaminated diets.

Treatment	Urea (mmol/l)	Creatinine (µmol/l)	Total bilirubin (µmol/l)	Conjugated bilirubin (µmol/l)	Cholesterol (mmo/l)
Grp A: 0.00%	5.40 ± 1.13^{b}	134.50 ± 11.67^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	1.80 ± 0.08^{b}
Grp B: 0.05%	6.45 ± 0.36^{ab}	152.25 ± 5.11^{bc}	3.98 ± 0.49^{b}	0.00 ± 0.00^{c}	2.15 ± 0.07^{ab}
Grp C: 0.01%	6.45 ± 0.19^{ab}	158.50 ± 6.56^{bc}	4.08 ± 2.35^{b}	0.00 ± 0.00^{c}	2.35 ± 0.41^{a}
Grp D: 0.15%	7.43 ± 0.52^{a}	178.25 ± 12.59^{b}	4.40 ± 0.87^{b}	0.43 ± 0.25^{b}	2.50 ± 0.09^{a}
Grp E: 0.20%	7.43 ± 1.08^{a}	191.50 ± 9.61^{a}	5.18 ± 0.28^{a}	0.50 ± 0.29^{a}	2.25 ± 0.34^{a}

Within column, Mean ± SEM with different superscript(s) differ significantly at (P < 0.05)

shallow pans measuring 30 cm x 30 cm x 5 cm in order to allow the extremely light and volatile fraction to vaporise thus leaving behind a stable component (Neff et al., 2000). Measured amount of the stable component of crude oil was incorporated into the hay/forage mix and concentrate by simple mixing and homogenization with a manual mixer. Animals were starved for 24 h before being introduced to the experimental feed. Feeding of the experimental diet continued for 12 weeks. Water was served *ad libitum*.

At the end of the experimental period, blood samples were collected from two bucks and two does from each treatment group through the marginal ear vein using a sterile disposible syringe and needle. The blood was transferred into plain vacutainer tubes and centrifuged at 1500 rpm. Serum was harvested into plastic tubes and stored in a refrigerator at -20^{0}C until required for analysis. All analysis was completed within 14 days after collection. Serum urea was quantified with the urease – glutamic dehydrogenase reaction (Eisenweiner, 1976). Serum creatinine was measured by the Jaffe reaction at 500 nm (Fabiny and Ertinghausen, 1971). Total bilirubin and conjugated bilirubin was measured by the Jendrassik and Grof method (AACC, 1984) and read at 600 nm mavelength in a spectrophotometer. Cholesterol was determined by the enzymatic endpoint method (Allain et al., 1974).

Data collected were subjected to the analysis of variance (ANOVA) and where differences existed, results were further subjected to Duncan Multiple Range Test (DMRT) for means separation according to the procedures of SAS (1999).

RESULTS

The biochemical parameters investigated showed significant (P < 0.05) differences when compared to the control groups. Serum urea, creatinine, total bilirubim, conjugated bilirubin and cholesterol were altered. Effects of treatment on urea and cholesterol were significant (P < 0.05), and highly significant (P < 0.001) in total bilirubin and conjugated bilirubin. Effects of interaction between treatment and sex (TS) was also highly significant (P < 0.001) in urea, total bilirubin and conjugated bilirubin. These are presented in Table 1. Serial determination of biochemical parameters revealed a progressive increase with increasing concentration of crude oil in the diet. The mean urea level was 5.40±1.13 mmo/l in the control and increased from 6.45±0.36 to 7.43±0.52 mmo/l in the crude oil treated animals. Mean level of creatinine was 135.50±11.67 mmo/l in the control. The value increased (152.24±5.11 to 191.50±9.61 mmo/l) with increasing dietary crude oil concentration. The values of total bilirubin, conjugated bilirubin and cholesterol also increase with increasing concentration of crude oil contamination in the diet. The biochemical parameters in rabbits experimentally fed crude oil contaminated diets is presented in Table 2.

DISCUSSION

The biochemical parameters investigated showed significant (P < 0.05) differences and were altered. Animals given the experimental diets all had elevated

serum urea, creatinine, total bilirubin, conjugated bilirubin and cholesterol (Table 2). The long duration of crude oil treatment seem to impose stress on the animals. Stress is here defined to mean any stimulus, physical or emotional that interferes with the homeostasis of the organism. Increased serum urea and creatinine have been used as important indices for the evaluation of the effects of chemicals on the kidney (Davis and Berdt, 1994). The presence of increasing urea and creatinine concentration in the blood suggest the inability of the kidney to excrete these products, which further suggest a decrease in glomerular filteration rate (GFR). A common manifestation of nephritic damage is acute renal failure characterised by decline in glomerular filteration rate, which may have been induced by the hydrocarbon fractions present in the diet. This assertion is supported by Counts et al. (1995) who reported that chemically induced nephrotoxicity by halogentated hydrocarbons, injure the proximal tubule monolayer, resulting in gaps in the epithelial lining, leading to back leak of filterate and diminished glomerular filteration rate.

This study has demonstrated that total bilirubin and conjugated bilirubin increased with increasing dietary crude oil concentration (Table 2). A rise in serum level of these metabolites suggest liver cell damage in which, usually, there is an increase in both metabolites (Cheesborough, 1992). Another possible reason may be a metabolic disturbance in liver involving defective conjugation and/or excretion of bilirubin. The bilirubin route of elimination is perhaps most important contributing source to the excretion of xenobiotics, but is of primary importance for the excretion of the animal's metabolites. Since the liver encounters nutrients, environmental toxicants and waste products, within this framework, it extracts the environmental toxicants and waste products to prevent their circulation to other parts of the body.

A linear increase in plasma concentration of cholesterol was observed with increasing dietary concentration of crude oil in this study (Table 2). This rise in cholesterol is an indication and probably suggests a general increase in lipid mobilization and perhaps ingestion of food containing saturated fatty acid. Serum cholesterol may also tend to rise with renal retention disease resulting in diminished removal of lipoproteins from the plasma, thus causing the concentration to increase markedly. Previous studies with dietary additions of PCB in rats have resulted in increase cholesterol, triglycerides and liver lipids (Kato et al., 1982; Quazi et al., 1983). In separate studies in which cholesterol was measured in blood of subjects, Oruwari et al. (1998) reported that palm oil, a saturated fatty acid was implicated in increased level of cholesterol in roosters and rabbits. Their findings corroborates well with the observation in this study and further supports the fact that increased cholesterol level is an index of stress in animals. Therefore, crude oil is an environment stressor.

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