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NEPHROTOXİCİTY İN RABBİTS FOLLOWİNG SUBCHRONİC EXPOSURE TO NİGERİAN CRUDE OİL

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SUMMARY

Nephrotoxicity in rabbits following subchronic exposure to crude oil was studied. The exposure levels were w/w 0.00%, 0.05%, 0.10%, 0.15% and 0.20%. The graded doses were included in their diets. Serum from experimental animals was assayed for indicators of kidney function. The parameters were alkaline phosphatase, urea, creatinine, glucose, total protein and albumin. All the biochemical indicators increased significantly (P<0.05) with increasing concentration of crude oil. Morphologically, a marked reduction in size of the kidneys was observed. The mean weight of the kidneys in the control was 11.41g ± 0.84 and decreased from 9.31g ± 0.25 to 6.47g + 0.55 with increasing dietary concentration of crude oil. Histological examination revealed that there were eosinophilic casts in the lumen of the distal convoluted tubules. The Bowmans capsule was dilated and contain some loose eosinophilic casts. Eosinophilia was also observed in some sections of the kidneys. It is concluded that crude oil is nephrotoxic in rabbits and may probably result to kidney failure.

INTRODUCTİON

Environmental exposure of both marine and terrestrial animals to xenobiotics can result in sublethal effects (Carls, *et al.*, 1999; Ngodigha *et al.*, 1999; Carls *et al.*, 2000). These may include biochemical alterations, immunological and indeed an array of physiological impairment. Within the Niger Delta region, the absence of any systematic long-term research on the content and effects of crude oil in organisms is a serious lacuna in that it is now impossible to judge the actual state of pollution of the environment with petroleum hydrocarbons. Yet, this region is the richest in oil mineral resources, shellfish, finfish and has an extensive mangrove swamp and other forest reserves as well as human population (Imevbore, 1979). This valuable ecosystem is, however vulnerable to destruction by petroleum and petroleum related products due to the fact that the oil industry operational activities are concentrated within this zone (Imevbore and Adeyemi, 1981, Snowden and Ekweozor, 1987).

Various components of crude oil are taken up and bioaccu mulate in tissues with concentrations much higher than the surrounding environment (Payne *et al.*, 1988; Thomas *et al*; 1989). Most marine and terrestrial animals receive hydrocarbons from their food and water sources and this represent the major route of contamination and uptake.

Pollutants such as petroleum hydrocarbons alter the growth of pelagic fish (Vignier *et al.*, 1992). The same petroleum hydrocarbons in polluted urban sediments have been closely correlated with alterations in detoxification enzymes (Vignier *et al.*, 1992), tissue abnormalities (Mc Cain *et al.*, 1978) and reproductive hormones (Truscott *et al.*, 1992). Unfortunately, the correlation of many of these cellular biomarkers with changes in kidney tissues is unknown.

The main objective of this work was to determine if twelve weeks of exposure to crude oil would significantly affect kidney functions and alterations in rabbits. The choice of rabbits for this study was predicated upon the fact that they are known to feed on forage in the wild just like their counterparts, the grasscutter. These animals could therefore be prone to crude oil contamination should any spillage occur within the vicinity of their habitat. The results can be used to determine the potential pathological effects of hydrocarbon exposure under field conditions.

MATERİALS AND METHODS

Thirty-two (32) rabbits of known pedigree aged 20-22 weeks, mean weight 1.26kg were procured from Rivers State Ministry of Agriculture and Natural Resources. They were housed in conventional hutches made of local materials (bamboo) at the Teaching and Research Farm, Rivers State University of Science and Technology, Port Harcourt and pre-conditioned for two weeks. During pre-conditioning, they were given coccidiostat (25% of embazin) and broad-spectrum antibiotics (teramycin). Their feed was prepared from grass and legumes and supplemented with concentrate (growers mash) from Pfizer (Nig.) Plc. Drinking and feeding troughs were cleaned and kept hygienically from preconditioning to the end of the study period.

Crude oil was obtained from the Bonny Terminal and allowed to weather after being exposed in shallow pans to sunlight for 24hrs in order to allow the volatile fractions to vaporize thus simulating the naturally occurring fraction during spillage (Neff, *et al.*, 2000). Measured (w/w) amount of weathered crude oil was incorporated into the forage/hay mix and poultry feed by standard methods according to Sastry and Thomas (1981).

Thirty rabbits of both sexes were randomly assigned into batches of six to five dietary groups in two blocks of varying dose levels as follows:

Group I: 0.00% w/w (No contamination control)
Group II: 0.05% w/w Crude Oil Contamination
Group III: 0.10% w/w " " "
Group IV: 0.15% w/w " " "
Group V: 0.20% w/w " " "

The animals were starved for 24hrs before being introduced to the experimental diets. The experiment lasted 90 days. Feed and water were served ad-libitum.

At the end of the 90day period, blood samples were obtained from two bucks and two does from each of the treatment groups by cardiac puncture with a sterile disposable syringe and needle. About 10ml blood was transferred into EDTA fortified tubes, labeled and centrifuged. Serum was then harvested, into plastic tubes and stored at –20°C until ready for analysis. Analyses were completed within 7days of collection. Alkaline phosphatase (ALP) was determined according to the procedures of the German Society of Clinical Chemistry (Hafkensheid and Kohler, 1986). Serum urea was quantified with the urease-glutamic dehydrogenase reaction (Eisenweiner, 1976), creatinine was measured by the Jaffe reaction at 500nm (Fabiny and Ertinghausen, 1971). Glucose was determined according to Bendar and Mead (1974). Total protein concentration in serum was measured using the biuret reaction (Doumas, 1975) while Albumin was determined with the Bromocresol Green (BCG) method.

Furthermore, the animals whose blood samples were taken were slaughtered and autopsied immediately. The kidneys were obtained and fixed in 10% neutral buffered formalin and embedded in paraffin wax. The fixed tissues were sectioned to about 6μm thin layers and stained with haematoxylin and eosin (H&E). The stained tissue was examined and photographed with a Zeiss photomicroscope III.

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

RESULTS

Means squares from the analysis of variance (ANOVA) of biochemical indicators of nephrotoxicity in rabbits exposed to subchronic levels of crude oil are presented in Table I. Effects of treatment (T) was highly significant (P<0.001) in alkaline phosphatase, significant (P<0.01) in creatinine and glucose. Effects of sex (S) was highly significant (P<0.001) in alkaline phosphatase while the interaction between treatment and sex (T x S) was highly significant (P<0.001) in urea and alkaline phosphatase (P<0.01).

TABLE I: Mean squares from Analysis of Variance in biochemical repsonses in rabbits fed subchronic levels of crude oil

Source	DF	Alkaline Phosphatase	Urea	Creatanine	Glucose	Total Protein	Albumin
Treatment (T)	4	67.30***	2.56*	1992.88**	4.12**	156.13*	33.58*
Sex (S)	1	18605***	1.15ns	0.80ns	0.03ns	57.80ns	5.00ns
T x S	4	37.30**	6.86***	650.18ns	4.07ns	77.43ns	18.38ns
Error	10	4.05	0.58	287.30	0.50	22.50	7.10
Total	19						
*	=	P < 0.05					
**	=	P < 0.01					
***	=	P < 0.001					
Ns	=	not significant					

TABLE II: Biochemical responses of rabbits exposed to subchronic levels of crude oil

Contamination Level/treatment	Alkaline Phosphatase (lmL) Mean±SEM	Urea (mmol/L) Mean±SEM	Creatinine (umol/L) Mean±SEM	Glucose (umol/L) Mean±SEM	Total Protein (g/L) Mean±SEM	Albumin (g/L) Mean±SEM
I:0.00%	.70^{b}	5.40±1.13^{b}	135.50±11.67^{c}	5.23±0.27^{b}	38.50±5.19^{c}	23.00±1.08^{b}
II:0.05%	18.25±1.38^{bc}	36^{ab}	152.25±5.11b^{c}	5.35±0.12^{b}	40.50±1.19^{bc}	23.25±2.32^{b}
III:0.10%	19.25±0.48^{b}	6.45±0.19^{ab}	158.50±6.56b^{c}	6.70±0.57^{a}	42.50±1.44^{bc}	24.00±0.82^{b}
IV:0.15%	23.75±1.18^{a}	7.43±0.52^{a}	178.25±12.58^{b}	7.44±0.40^{a}	46.25±1.44^{b}	24.25±1.44^{b}
V:0.20%	25.75±3.75^{a}	7.23±1.08^{a}	191.50±9.61^{a}	7.08±1.11^{a}	54.25±3.43^{a}	30.00±1.78^{a}

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

Serial determination of biochemical indicators of kidney damage showed that there was a progressive increase of metabolites with increasing concentration of crude oil. The data is presented in Table II. The mean concentration of alkaline phosphatase in the control animals was 15.75±1.38 *IU/L* and increased from

18.25±1.38 *IU/L* to 25.75±3.75 *IU/L* in the treated animals. Mean urea level was 5.40mmo/*L* in the control and increased from 6.45mmo/*L* to 7.43mmo/*L* in treated animals. Creatinine level was 135.50mmo/*L* and increased from 152.25mmo/*L* to 191.50μmol/*L*. The mean values of glucose, total protein and albumin also increased with increasing dietary concentrations of crude oil.

The susceptibility to nephrotoxicity of mammals is attributed to the unique physiological and anatomic features of the kidney. Filtration processed by the kidneys involve concentrating potential toxicant in the tubular fluid. The increased concentration of biochemical indicators of kidney damage observed was confirmed by histological examination. A histological study of the kidney tissues revealed the presence of eosinophilic cast in the lumen of distal convoluted tubule (Fig 1a). Bowman's capsule was observed to be dilated and also contain some loose eosinophilic cast (Fig 1b). There was eosinophilia scattered in many other parts of the kidney (Fig 1 c,d).

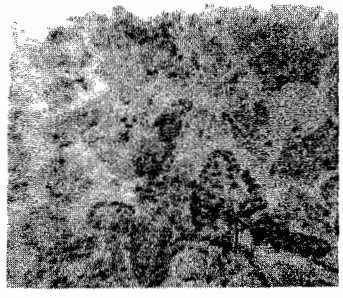


Plate 1a Eosinophilic (proteinaceous) cast in the lumen of distal convoluted tubules x 40.

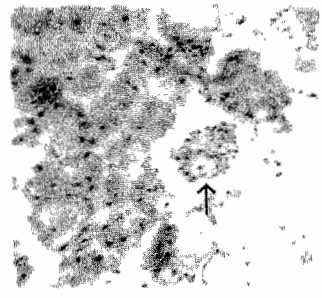
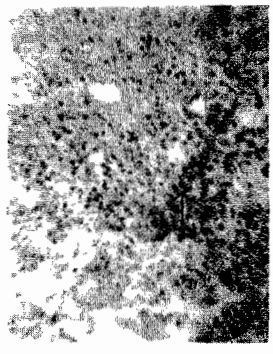


Plate 1b. Dilated Bowman's capsule containing loose eosinophilic cast.



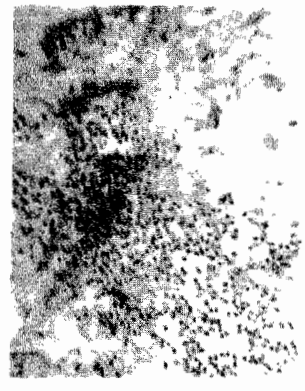


Plate 1c, d
Eosinophilia scattered in many parts of the kidney x 40.

DISCUSSION

The functional integrity of the mammalian kidneys is vital to the total body homeostasis, as they play a principal role in the excretion of metabolic wastes and the regulation of extracellular fluid volume, electrolyte composition and acid-base

balance. Evaluation of kidney function can be accomplished using a variety of methods (Foulkes, 1993; Davis and Berndt, 1994). From the method used the activities of ALP was seen to increase significantly ($P<0.05$) with increasing concentration of crude oil. This enzyme is appropriated in large quantity in the brush borders. The presence of localized enzymes in the serum may reflect brush border damage.

Another important indicator of nephrotoxicity is blood urea and creatinine concentrations. These components were observed to increase with increasing concentration (Table 2). They are normally filtered by the glomerulus; therefore, increased serum concentrations suggest decreases in glomerular filtration rate (Counts *et al.*, 1995). This finding is in consonance with the observations of Bach and Gregg (1985) and Barret (1994) where chemically induced nephrotoxicity resulted in reduced glomerular filtration rate culminating in increased concentrations of urea and creatinine.

Furthermore glucose, total protein and albumin levels were observed to increase. High glucose concentration as well as high molecular weight proteins such as albumin is suggestive of glomerular damage (Christensen and Nielson, 1991). However, specificity is often lacking in some of the non invasive assessment of overall renal functional integrity. Histopathologic evaluation of the kidney following treatment with chemical inducers is crucial in identifying site, nature and severity of the neprotoxic lesion.

The findings of the present study indicate that graded doses of crude oil in the diet caused nephrotoxicity in rabbits. Simple histological study of the kidney show the presence of eosinophilic cast in the lumen of distal convoluted tubules (Fig 1a). Furthermore, the Bowman's capsule is dilated and also contains some loose eosinophilic cast (Fig 1b). Eosinophilia was also observed in many other sections of the kidney (Fig 1 c and 1d). Various constituents of crude oil may initiate cellular injury by initiating toxicity due to its intrinsic reactivity with cellular macromolecules. Since crude oil is made up of hydrocarbons, metal and non-metal constituents, each reacts in its unique way with various components of the kidney. Monks *et al.*, (1994) and Lau and Monks (1993) observed similar nephrotoxicity in mice dosed with bromobenzene, a hydrocarbon. They argued that like the halo-alkenes, the biotransformation of this compound is critical for the expression of nephrotoxicity. The biotransformation follows a series of conjugation which becomes a thousand folds more potent than the bromobenzene itself in producing nephrotoxicity. Again, heavy metals components in the crude oil may also cause toxicity through their ability to bind to sulphydryl group. For instance, mercury is known to produce nephrotoxicity by binding sulphydryl groups on cellular proteins. This effect has been associated with the greater degree of lipophilicity of organic mercury components (Conner and Fowler 1993, Zalups and Lash, 1994).

CONCLUSİON

Crude oil seems to be a nephrotoxicant. The presence of eosimophilic cast in the lumen of distal convoluted tubules as well as the observed dilation of the bowman's capsule may lead to complete collapse of the kidney. These are indications of cellular injury which may eventually lead to cellular death.

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