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HISTOPATHOLOGICAL CHANGES IN THE LIVER AND KIDNEY OF ADULT BUCK RABBITS EXPOSED TO CRUDE OIL CONTAMINATED FEED

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ABSTRACT
The histological effects of the liver and kidney of adult buck rabbits, age 7 months were allotted to five treatments (control, no contamination), 5 ml, 10 ml, 15 ml and 20 ml in treatment B, C, D and E respectively in completely randomized design (CRD) with six (6) replicates per treatment, one animal per replicates. The rabbits were fed with crude oil contaminated feed for 8 weeks (56 days). At the end of the feeding trial, three rabbits were sacrificed by stunning and decapitation and carefully eviscerated to collect the livers and kidneys .The livers and kidneys collected from each animal were weighed and the tissues were processed for histology. There was a significantly ($p<0.05$) difference in the liver and kidney weight. The histological examinations showed vary degree of cellular inflammation, infiltration and necrosis. This study has shown that adult rabbits exposure to 10 ml/kg or more for a two-month period is a potential health risk to the liver and kidney of the animal.

KEY WORDS: Histopathological changes, liver, kidney rabbits and contaminated feed

INTRODUCTION

The devastating consequences of oil spill with its eventual hazards on both aerial and terrestrial environment manifest as an irreversible chain effect on both the biodiversity and human safety. As this occurs, the oil threatens surface water and a wide range of subsurface marine organisms which are linked in a complex food chain [(13)]. Oil spillage has caused destruction of food resources (23). 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. Aquatic environments are made up of complex inter-relationship between plant and animal species and any adverse alteration of their physical environment will often lead to the death of one or more species in a food chain, which may also 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 the organism (5).A number of investigations have been conducted on the direct and indirect effects of crude oil on poultry (20), goat (18) and rabbits (17, 1, 21). It was reported that there was a decline in feed intake and also severe depression in growth as the level of crude oil contamination increased in the feed (17,1, 21). Research results have indicated the adverse effects of crude oil on feed intake and growth performance (10, 17, 1 and 19). These reports documented that the consequences of crude oil contamination in mammals were lethality, depressed growth and reduction in organ weights. It is therefore the aim of this study to determine the histopathological changes in the liver and kidney in mammals (rabbits) within the Niger Delta environment. The choice of rabbits for this study is predicated on the fact that they are known mammals that feed on forage and could therefore be prone to accidental ingestion of crude oil contaminated feed resources when spillage occurs.

MATERIALS AND METHODS

The study was conducted at the Teaching and Research Farm, Rivers State University of Science and Technology, Port Harcourt, Niger Delta region of Nigeria and located within the typical tropical rain forest belt. The environment is hot and humid with mean daily maximum and minimum temperatures of 21-33 °C and 20-23 °C respectively. Although a relatively photostable climate with 12 hours of diurnal light all year round. Precipitation is quite high (1,700- 4,500 mm annually) with correspondingly high relative humidity of 50-90%, depending on the season of the year. There are two seasons (rainy and dry), and in some years the rains fall every month of the year. Thirty (30) adult rabbits aged 7 months were randomly assigned into five groups each of six rabbits: A (0 ml crude oil/ kg feed) served as the control, B (5 ml crude oil/kg of feed), C (10 ml crude oil/kg of feed), D (15 ml crude oil/kg of feed) and E (20 ml crude oil/kg of feed). They were all subjected to standard husbandry routine. The crude oil was obtained from the Bonny Terminal, Rivers State, Nigeria. All groups were fed *ad-libitum* for twelve weeks (56 days). At the end of the feeding trial, three rabbits were sacrificed by stunning and decapitation and carefully eviscerated to collect the livers and kidneys .The livers and kidneys collected from each animal were weighed and the tissues were embedded in paraffin blocks, then sliced into 5_m in thickness and placed onto glass slides. After hematoxylin–eosin (HE) staining, the slides were observed and the

photos were taken using optical microscope (Nikon U-III Multi-point Sensor System, USA), and the identity and analysis of the pathology slides were blind to the pathologist at the Anatomical Pathology laboratory of University of Port Harcourt Teaching Hospital (UPTH). All the data collected on organ weights were subjected to Analysis of Variance (ANOVA) according to Steel (25).

RESULTS

Weights of Liver and kidney

The weights of rabbits exposed to graded levels of crude oil contaminated diets are presented in Fig. 3. The weights of the kidney in rabbits exposed to crude oil contaminated feed were significantly ($P<0.05$) different from those of the untreated rabbits, the mean weight of the kidney significantly ($P<0.05$) decreased with increasing concentration of crude oil. There were indications of gross morphological alterations in the liver of crude oil treated rabbits. The mean liver weight in the rabbits significantly ($P<0.05$) increased with increasing concentration of crude oil.

Histopathological evaluation

The result of histological photomicrographs of the kidneys and liver sections of treated and untreated rabbits are shown in Figs. 1–2. Only the photographs of treatment A, C and E are shown. The liver of the untreated rabbits showed no visible lesion, there was normal hexagonal architecture of the hepatocytes, hepatic vein and sinusoid (figs 1a). In the 10ml crude oil/kg feed treated rabbits, there was a mild periportal lymphocytic and histiocytic cellular infiltration in the liver (fig 1b) and in the 20 ml crude oil/ kg feed rabbits liver showed widen of sinusoids, lymphocytic infiltrates with interface destruction of hepatocytes, cytoplasmic vacuolation (fatty change), (Fig 1c). The kidney in the untreated rabbits showed normal kidney, having normal glomerulus, Bowman'scapsule and tubules (fig 2a). In the 10 ml/kg feed treated rabbits there are multiple foci of haemorrhages into the intertitium. There were few loci of tubular necrosis and presence of hyaline casts with interstitial cellular infiltration by macrophages (fig 2b), while the 20 ml/kg crude oil/kg feed treated rabbits shows eosinophilic (proteinaceous) cast in the lumen of distal convoluted tubules (fig 2c)

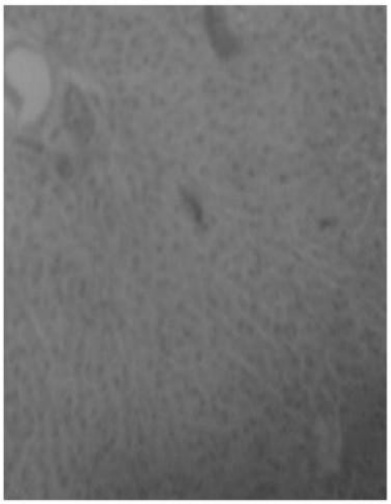


Fig 1a:H & EX 100 Normal Liver
Treatment A

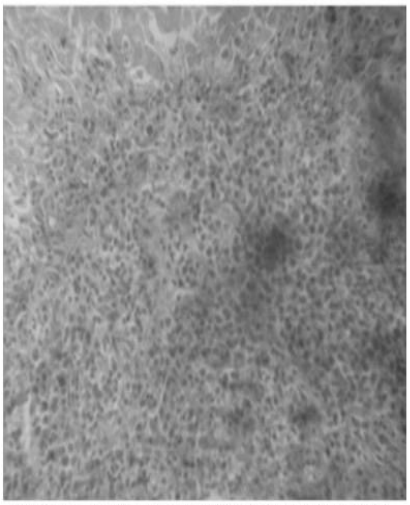


Fig 1b: H & E 400 Liver. Lymphotic infltration with interface destruction of hepatocytes
Treatment C

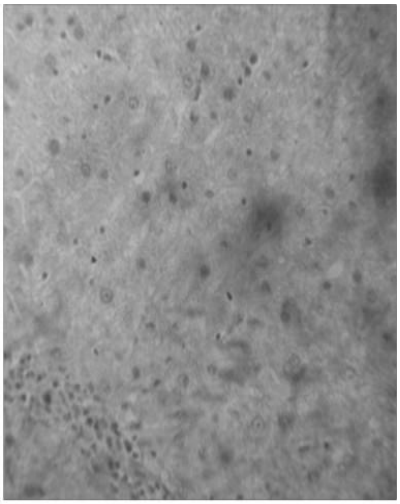


Fig 1b: H & E 400 Liver. Ballon degeneratiom of hepatocytes
Treatment E

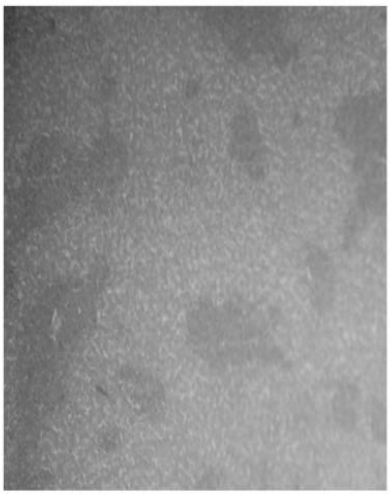


Fig 2a: H & E 400 Normal Kidney,
Treatment A

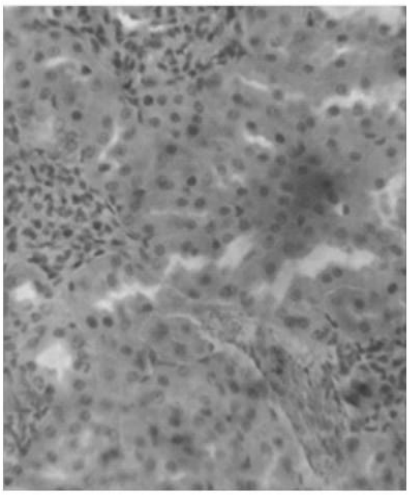


Fig 2b: H & E 400. KIDNEY. glomeruli are cellular due to infiltration by lymphocytes,
Treatment C

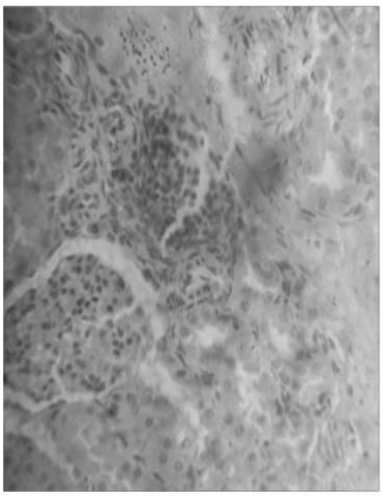
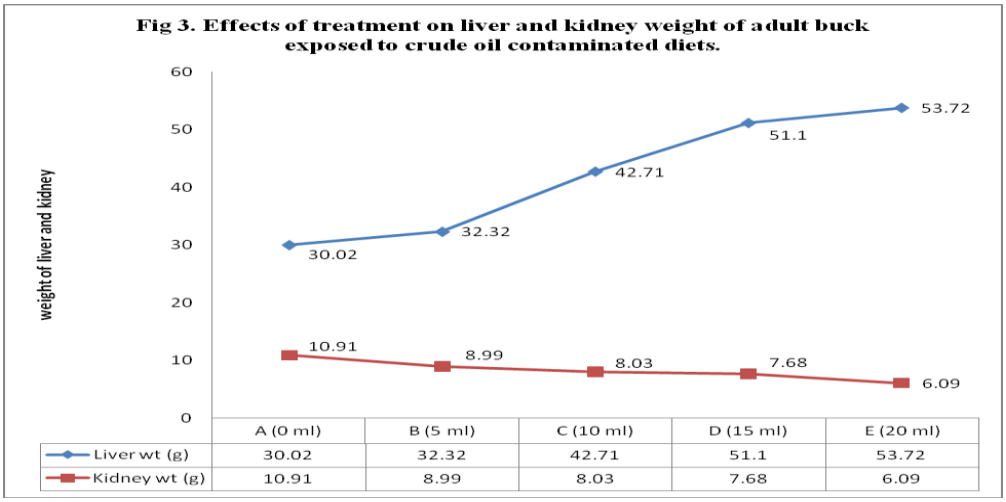


Fig 2c: H & E 400. KIDNEY. glomeruli are cellular due to infiltration by lymphocytes andshowing lobulations
Treatment E



DISCUSSION

Liver is a targeted organ and primary site of detoxification and is generally the major site of intense metabolism and is therefore prone to various disorders as a consequence of exposure to the toxins of extrinsic as well as intrinsic forms (6,7). Liver plays important role in metabolism to maintain energy level and structural stability of the body (9). It is also site of biotransformation by which a toxic compound has been transformed in less harmful form to reduce toxicity (10). The present study has shown that rabbits exposed to crude oil contamination have enlarged liver as shown by increased weights of liver. The increase in liver weight may be due to a reflection of greater specicic metabolism rate, as a result of the toxic effect of the crude oil, since liver is the site of biotransformation (9).This evidence seems to corroborate with the report of (12), in which chronic fuel oil toxicity on American mink (Mustela vision): system and hematological effects of ingestion of a low-concentration of bunker C fuel oil was investigated. They reported that, as the concentration of the fuel oil increase, the livers also increase significantly. The result of histological photomicrographs of the liver manifested an inflammation and necrosis, which supports the weight evaluation of the liver in this study. It is supports the report of Ovuru (22) and Voss (8) , lymphocytic infiltration, which is in agreements with the findings of (15, 27), and interface destruction of hepatocytes (24).This suggests an excessive accumulation of triglycerides within the hepatocytes resulting in a defect in hepatic lipid metabolism which result in severe injury, (4) The kidney, which is also an important organ in the elimination of waste or toxic matters from the body (2), was also seen to be adversely affected in the this study. As the level of crude oil is increasing, the kidneys of the treated rabbits were decreasing. The reduction in size of the kidneys may be an indication of inadequate blood and nutrient supply from the heart, which may negatively affect the kidneys resulting in kidney dysfunction and invariably leading to accumulation of toxins in the body (14). This observation confirm the reports of (21), who fed rabbits with crude oil contaminated forage and observed a reduction in the size of the kidneys. The result of histological photomicrographs of the kidneys were observed to have cellular glomeruli due to infiltration by lymphocytes, thus supporting the findings of (16), who observed that mice treated with bromobenzene, a hydrocarbon. Presence of heavy metals in crude oil components may also cause toxicity through ability to bind to Sulphydryl group especially mercury in animal (2,28).

CONCLUSION

Based on the findings in this study, a diet containing about 10 ml crude oil /kg feed and above may alter liver and kidney weight and has characteristics severe histo-pathological response.

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