Global Science Research Journals

ISSN: 2449-1888 Vol. 6 (4), pp. 296-301, November, 2018

http://www.globalscienceresearchjournals.org/

Global Journal of Medicine and Medical Sciences

Full Length Research Paper

Biochemical, haematological and histological effects following Escravos crude oil ingestion by Chinchilla rabbits

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Accepted 28 January, 2016

The negative consequences of crude oil exploration and exploitation on the health status of exposed individuals cannot be over emphasized, regardless of its financial benefits. Besides, consumption of this crude oil by the rural populace living in oil rich regions as traditional medicine for illnesses have raised local and international questions as to its safety. The aim of this study was to investigate the pathological effects related to Escravos crude oil ingestion by Chinchilla rabbits. A total of thirty Chinchilla rabbits of age twelve to fourteen weeks and weighing 1.2 to 1.45 kg was used. Crude oil was orally given at the dose of 15, 20, 25 and 30 mg/kg body weight, corresponding to groups B, C, D and E, respectively for 28 days, while group A was the Control. The result showed a significant increase in the total white blood cell, monocyte, granulolytic leucocyte, platelet counts, C-reactive protein and serum creatinine ($p < 0.05$). Microscopy of the stained tissue sections showed marked deposition of collagen fibers, glomerulonephritis and atrophic glomeruli among others. There is an agreement between the biochemical, haematological and histological findings. Thus, Escravos crude oil is suggested to have a potential to cause haematoxicity and alter the architecture of the kidney.

Keywords: Rabbits, creatinine, C-reactive protein, collagen fibers, Escravos crude oil, granulolytic leucocyte, kidney, microscopy, monocytes, platelets.

INTRODUCTION

Crude oil exploration is the mains stay of the Nigerian economy and constitute about 90% foreign exchange earning of the nation (Eyong et al., 2004). The over dependence on the monetary benefit of exploration and exploitation and neglect of its environmental conesquences has made the problem of crude oil pollution insurmountable. The impact of crude oil spillage and discharge on the ecosystem as a result of oil exploration activities is an obvious problem of environmental concern (Otitoju and Onwurah, 2007; Ovuru and Ekweozor, 2004). The largest contributor to the oil spill in total, besides corrosion of pipes and tanks, is the rupturing or leaking of production infrastructures that are described as, “very old and lack regular inspection and maintenance” (Nwilo and Badejo, 2001).

According to Dede et al. (2002), cases of misuse of this substance by individuals have been reported, as it is known to be used liberally by some of the indigenes who believe that it can repel witches when applied either topicaly, or by oral administration on afflicted individuals,

while other countries such as Kenya, Tanzania, Zimbabwe, Ghana and Tunisia depend on crude oil for unorthodox treatment of ailments such as stomach ache, diarrhoea, respiratory distress and convulsion. The hydrocarbons in crude oil are mostly alkanes, cycloalkanes and various aromatic hydrocarbons while the other organic compounds contain nitrogen, oxygen, sulphur and trace amount of metals such as iron, nickel, copper and vanadium (Speight, 1999).

Generally, various studies on crude oil have revealed that it has serious deleterious effects on soils (Erdogan and Karaca, 2011; Jeroh et al., 2011; Mary and Dolor, 2007), plants (Baek et al., 2004; Agbogidi et al., 2007), aquatic life (Ndimele et al., 2010; Daka and Ekweozor, 2004) and even organisms such as the macrobenthic invertebrates (Arimoro and Adamu, 2008). Commonly reported effects of acute exposure to crude oil through inhalation or ingestion include: difficulty in breathing, headaches, nausea, confusion and other central nervous system effects (Akpofure et al., 2000). The aim of this study was to investigate the haematological effects and renal problem ascribed to Escravos crude oil ingestion by Chinchilla rabbits.

MATERIALS AND METHODS

Test sample

The Escravos blend crude oil (with reference number 863) used in this study was provided by Warri Refining and Petrochemical Company Effurun, Delta State. The crude oil was exposed to sunlight in shallow pans (25 cm × 25 cm × 5 cm) for 24 h at the site of the project to allow the extremely light and volatile fractions to evaporate leaving behind the stable components. This product simulates the naturally occurring condition following spillage (Neff et al., 2000).

Animals/experimental design

A total of 30 Chinchilla rabbits aged 12 to 14 weeks weighing 1.2 to 1.45 kg were obtained from the Faculty of Agriculture, Ebonyi State University Abakaliki. The animals were examined, treated for ectoparasites (using Lymectin; Hebei New Century Pharmaceutical C0. Ltd) and Bacterial infections (using Spectropan; Pharma swede-Egypt) by a veterinarian and allowed to acclimatize for two weeks at the Animal House of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus. The rabbits were sex-matched and divided into five groups, containing 6 rabbits each (3 males and 3 females). The research plan used consist of four groups designated Group A (control), B, C, D and E. Group B to E were orally given a sub-lethal dose of 15, 20, 25 and 30 mg/kg body weight of Escravos crude oil, respectively with due consideration of their body weight (those with greater body weights have their dose divided into two; one in the morning one at night). The different doses of the liquid Escravos crude oil were measured in weight on an electronic weighing balance and given orally (oral gavage) for 28 days.

Animal treatment/sample collection

The protocol for animal handling and collection of samples in this study was approved by the Ethics Committee of the Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, Nigeria. Overnight, prior to exposure, the animals (rabbits) were starved of solid food and their body weights were taken weekly and for the duration of the study to check for weight loss or gain which is associated with toxicity. The rabbits were fed vital grower pallets and water *ad libitum* for 28 days.

Organ harvest and tissue processing for light microscopy

The animals were anaesthetized using cotton wool damped in chloroform with due consideration of their body weights and sacrificed (on the 29th day morning). 5 ml of blood samples, obtained by marginal ear vein puncture, were drawn into tubes using 22 gauge sterile needles. For biochemical analyses, 3 ml of blood samples collected into plain test tubes were centrifuged (ROTOFIX 32®-HETTICH) at 3000 rpm for 10 min; the serum was collected and kept at -20°C until analysis. For the haematological investigations, 2 ml of whole blood samples were dispensed into ethylene diamino-tetra-acetic acid (EDTA) containers. The excised kidneys were blotted dry to remove traces of blood and then weighed electronically (using 210/0.1 mg digital balance ESJ-210-4) and fixed in 10% formal saline. Tissues of 3 µm thickness were stained using Haematoxylin & Eosin (H&E) and Gomori's trichrome staining techniques, and photomicrograph of the stained tissue sections was taken for documentation (Awvioro, 2002). The kidneys were processed at the University of Nigeria Teaching Hospital (UNTH), Enugu State.

Biochemical analysis and haematological investigation

Serum creatinine and urea were estimated using the Jaffe slot alkaline picrate and Urease-bertholot methods, respectively (Sood, 2009). The enzyme linked immunosorbent quantitative method was used to determine the concentration of C-reactive protein in the serum. Kits from Randox Laboratories, United Kingdom and Diagnostic Automation Inc., Calabasas were used. These biochemical analysis were done using ELISA machine (MR 96 USA) and spectrophotometer. The haematological investigations were carried out by means of automation, using the Erma Inc. Hematology Analyzer Model PCE-210. The experiment was carried out using the facilities of Reene Laboratories Onitsha and the Nnamdi Azikiwe University Teaching Hospital (NAUTH).

Statistical analyses

Mean values ± standard deviation (SD) of the sex hormones, cholesterol, body and ovary weights were taken for analysis. The data was tested for homogeneity of variance and significantly different results were established by one-way analysis of variance (ANOVA) using the statistical package for social sciences (SPSS) software application (version 16). The multiple comparisons were made using the Post hoc test. The accepted level of significance was set at $p < 0.05$.

RESULTS

Behavioural effect

After two days of the crude oil administration, the animals in the treated groups D and E became restless. The latter was followed by loss of appetite and decreased locomotion. They regained their appetite after the tenth

Table 1. Mean ± SD of the biochemical parameters change in body weight of animals per week (kilogram) and weight of kidney (kilograms) in the test and control groups.

Weight	Group					F-value	P-value
	A (control)	B (15 mg/kg)	C (20 mg/kg)	D (25 mg/kg)	E (30 mg/kg)		
C-reactive protein (mg/dl)	0.24±0.11	0.38±0.12	0.51±0.25	0.57±0.19	0.46±0.07	3.751	0.019
Creatinine (µmol/l)	0.33±0.08	0.33±0.06	0.36±0.08	0.48±0.09	0.38±0.06	3.946	0.015
Urea (mmol/l)	4.97±1.44	5.66±0.87	6.00±1.16	6.60±1.68	6.00±1.35	1.102	0.385
Mean change in weight (kg)	0.12±0.04	0.09±0.03	0.02±0.01	0.08±0.04	0.05±0.02	4.636	0.019
Weight of kidneys (kg)	0.081±0.009	0.092±0.011	0.103±0.010	0.108±0.011	0.103±0.06	8.109	0.001

P is significant at $p<0.05$

Table 2. Total white cell counts and differential values in rabbits exposed to crude oil (mean ± SD).

Groups/dose	WBC (10^3/µl)	Lymphocytes (10^3/µl)	Monocytes (10^3/µl)	Granulolytic leucocytes (10^3/µl)
	Mean±SD			
A (00 mg/kg)	4.50±0.57	1.50±0.14	0.30±0.01	2.70±0.71
B (15 mg/kg)	5.20±0.10	2.23±0.31	0.87±0.21	2.90±1.42
C (20 mg/kg)	6.17±0.21	1.53±0.71	0.50±0.26	4.10±1.04
D (25 mg/kg)	7.17±0.61	2.50±0.66	0.90±0.30	3.80±0.17
E (30 mg/kg)	9.60±0.53	2.77±0.74	1.33±0.55	5.50±0.87

day, though not completely. Table 1 shows significant increase in the serum concentrations of C-reactive protein, creatinine, mean change in body weight per week and kidney weight ($p < 0.05$), with an insignificant increase in serum urea concentrations. The pair-wise comparison was made between the control group and the treated groups. The post hoc test (LSD) showed significant increase in total white blood cells (WBC), monocyte and granulocytic counts ($p < 0.05$) in a dose dependent manner, with slight increases in other haematological parameters.

DISCUSSION

Crude oil(s) extracted from different wells and locations have different chemical compositions, which may finally determine their toxicity (Neff et al., 2000). The result of this study elucidates the potency of Escravos crude oil to induce haematoxicity and renal pathology in relation to haematological parameters, CRP, creatinine, urea and histology.

According to Jacob and Al-Muzaini (1995), petroleum pollution could range from diffused chronic exposure to considerably large single doses. These sub-lethal concentrations may not necessarily lead to outright mortality but may have significant effects which can lead to physiological stress and dysfunctions in animals (Omoregie, 1998). The high total WBC counts observed in this study (Tables 2 and 4) is in accordance with the report of Eyong et al. (2004) who orally administered the Bonny Light crude oil to rabbits but discordant with the reports of Ovuru and Ekweozor (2004) who administered Nigerian Agip crude oil to rats in doses of 0.05, 0.10, 0.15 and 0.20%. The increase in WBC counts could be adduced to the normal physiologic response following perception of a foreign attack by the body defence mecha-nisms. The result also showed the granulocytic leucocyte count (neutrophils and eosinophils) significantly increased linearly with increasing concentration of crude oil (Tables 2 and 4) which is in accordance with the reports of Ovuru and Ekweozor (2004). This increase is an indication of stress imposed by crude oil fraction in the diets and supported by Selye (1963), finding that a stress stimulus elicits a defence response. Leu-cocytosis is found in several conditions which include

Table 3. Total red cell counts, platelets and dependable factors in rabbits exposed to crude oil.

Groups/dose	RBC (10^3/µl)	Platelets (10^6/µl)	Haemoglobin (g/dl)	PCV (%)
A (00 mg/kg)	6.11±0.49	178.50±33.23	10.50 ±0.99	32.05±3.46
B (15 mg/kg)	6.03±0.48	288.67±44.97	10.50±0.43	30.97±2.38
C (20 mg/kg)	6.31±0.60	201.00±49.15	10.53±0.84	32.10±2.75
D (25 mg/kg)	6.42±0.30	235.33±30.37	10.97±0.83	32.20±3.74
E (30 mg/kg)	6.24±1.39	318.00±74.54	10.53±2.86	31.33±5.91

Table 4. The pairwise comparison (Post hoc test) of mean ± SD of the haematological parameters.

Parameter	WBC	Lymphoctes	Monocytes	Granulocytes	Rbc	Hgb	PCV	Platelets
A (00 mg/kg)	-	-	-	-	-	-	-	-
B (15 mg/kg)	0.115	0.158	0.097	0.824	0.913	1.000	0.767	0.041
C (20 mg/kg)	0.002	0.952	0.530	0.144	0.782	0.981	0.989	0.637
D (25 mg/kg)	0.000	0.098	0.082	0.240	0.674	0.742	0.967	0.249
E (30 mg/kg)	0.000	0.044	0.008	0.011	0.859	0.981	0.845	0.014
F-value	56.02	2.555	3.694	3.733	0.114	0.052	0.058	3.572
P-value	0.000	0.112	0.048	0.047	0.974	0.994	3.572	0.052

P is significant at $p<0.05$.

include: inflammation, tissue necrosis and polycythaemia vera (Hoffbrand et al., 2001). Also observed is a significant increase in the monocyte count (Table 2), which suggests that the crude oil increased the susceptibility of the rabbits to bacterial infection- resulting in monocytosis. This is in contrast to the works of Ovuru and Ekweozor (2004) who reported a decrease in the monocyte count after administering 0.05, 0.10, 0.15 and 0.20% of Nigerian Agip oil. The main function of platelets is to form mechanical plugs during normal haemostatic response to vascular injury (Hoffbrand et al., 2001), which could be the which could be the reason for the observed high platelet Count (Table 3).

Increase in haemoglobin (Hb), red blood cell (RBC) count, packed cell volume (PCV), and platelet count are found in myeloproliferative disorders such as polycythaemia vera (Hoffbrand et al., 2001), this could be related to high RBC count, lymphocyte count, and Hb values observed in this study (Table 3 and 4). Furthermore, Hb concentrations are reported to be moderately increased in haemolytic anaemia and any condition associated with rapid intrascular haemolysis and haemoglobinuria (Bolarin, 1997).

C-reactive protein (CRP) is an acute phase protein synthesized by the liver and is normally present as a trace constituent of serum or plasma at levels less than 0.3 mg/dl (Kushner and Rzewnicki 1994; Macy et al., 1997). Its physiological roles are numerous and varied, but with several functions similar to those of immunoglobulins, CRP appears to function in host defense (Schultz and Arnold, 1990). As elevated CRP values are always associated with pathological changes, the CRP assay provides useful information for the diagnosis, therapy and monitoring of inflammatory processes and associated disease (Shine et al., 1981; Dixon, 1984; Hind and Pepys, 1984; Kushner, 1991). The result of this study showed high CRP concentration in the treated groups when compared with the control group. The Inflammatory process marked by lymphocytic infiltration and hyperchromatic fibroblast evident in the stained tissue section (Figure 1 to 6) could be related to the high CRP and granulolytic leucocytes.

Renal azotaemia occurs when urea is retained primarily due to impaired glomerular filtration which results in acute or chronic renal disease. The acute state may be due to glomerulonephritis, nephrotoxic drugs, or renal cortical necrosis (Ochei and Kolhatkar, 2000). The high serum concentrations of creatinine and urea observed in the treated groups when compared with the control group are in agreement with the findings of Azeez et al. (2013) who exposed rats to petroleum hydrocarbon.

In diseases such as necrotizing, crescentric glomeronephritis where there is disruption of Bowman capsule, fibroblasts can migrate from the interstitium into glomeruli and produce interstitial collagens (Guillermo and Elba, 2010). The latter was evident in the stained tissue sections of the treated groups in this study (Figures 4 and 6). A replacement of mesangium with eosinophilic amorphous material is an indication of light chain amyloidosis (Guillermo and Elba, 2010). This eosinophilic material was observed in the stained sections of the treated groups (Figure 5).

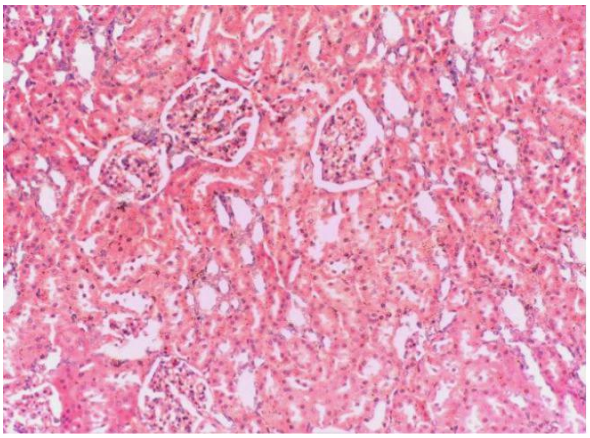

Figure 1. Group A: Photomicrograph of kidney section with no obvious pathology.
Stained by H&E technique. ×200.

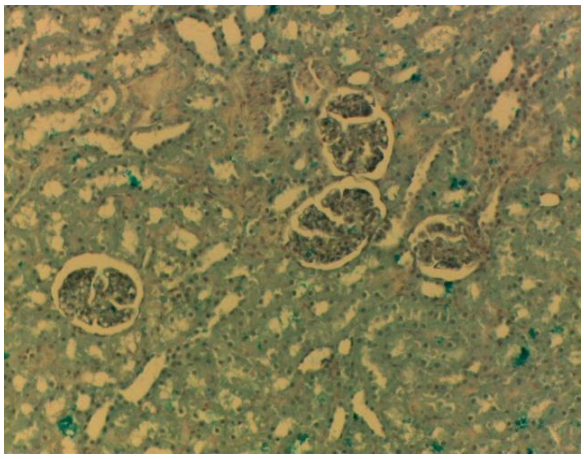

Figure 2. Group A: Photomicrograph of a kidney section with no obvious pathology.
Gomori's Trichrome technique. ×200.

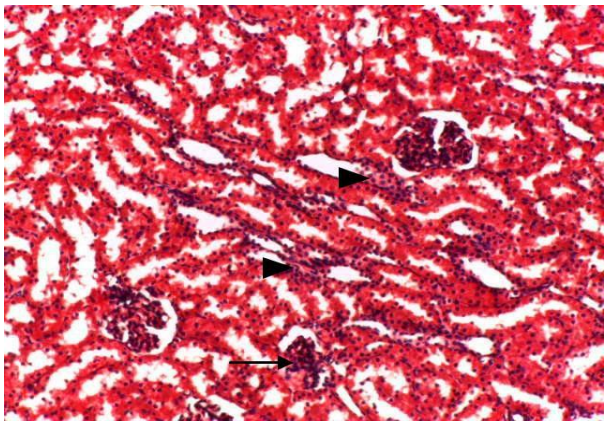

Figure 3. Group B (15 mg/kg): Photomicrograph of the kidney with marked lymphocytic infiltration (glomerulo-nepheritis marked by arrow heads), marked cellularity within the tuft (migrated fibroblasts). The tubules are slightly enlarged and with a shrunken glomerulus (arrow). Stained by H&E technique. ×200.

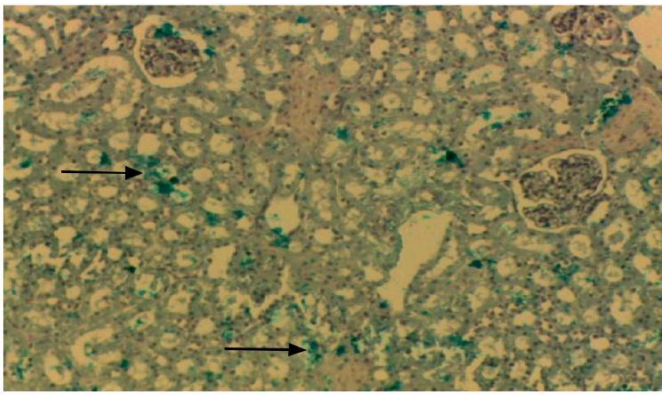

Figure 4. Group C (20 mg/kg): Photomicrograph of a section of the kidney showing enlarged renal tubules, marked cellularity and mild deposition of collagen fibres (green marked by arrows).
Stained by Gomori's trichrome technique. ×200.

Figure 5. Group D (25 mg/kg): Photomicrograph of the kidney with some congested tubules with hyperchromatic fibroblasts and fibrosis (arrow heads). There is an enlarged cystic space with eosinophilic material (a possible indication of light chain associated amyloidosis). Some of the tubules are enlarged with single layer of epithelium.
Stained by H&E technique. ×200.

Figure 6. Group D (25 mg/kg): Photomicrograph of a section of the kidney with enlarged Bowman's capsule, cystic space, shrunken glomeruli, lymphocytic and fibroblastic infiltration mar-ked deposition of collagen fibers (green marked by arrow) and stromal erosion. Stained by Gomori's trichrome technique. ×200.

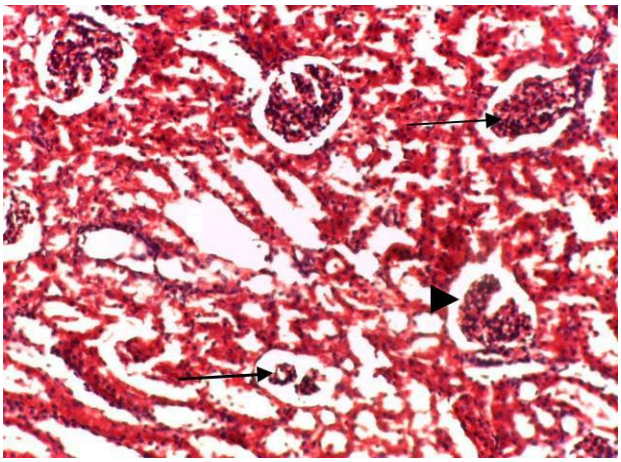

Figure 7. Group E (30 mg/kg): Photomicrograph of the kidney with multicystic stromal tissue with few identifiable tubules, Slightly shrunken (arrow) and atrophic glomeruli (arrow), and enlarged Bowman's capsule (arrow head). Stained by H&E technique. ×200.

Conclusion

The findings of this study suggest that Escravos crude oil, even in low concentration, has the potential to affect haematological, biochemical parameters and can cause renal diseases following exposure.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the technical support of Francis Ifediata (Haematology Unit, NAUTH), Lawrence Ogbodo (Department of Morbid Anatomy, UNTH) and Dr. Kingsley Achinowa (Histopathology unit, Federal Medical Center, Owerri). We also wish to thank the Managing Director, Warri Refinery and Petrochemical Company Ltd, Efuru for providing the test samples.

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