

Full Length Research Paper

Histopathological and biochemical disrupting effects of Escravos crude oil on the liver and heart in Chinchilla rabbits

Ngokere Anthony Ajuluchukwu¹, Okoye Jude Ogechukwu^{1*}, Ogenyi Samuel Ifedioramma¹, Bukar Simon Mshelbwala³ and Ude Tochukwu²

¹Histopathology Unit, Department of Medical Laboratory Science, Faculty of Health Science and Technology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.

²Chemical Pathology Unit, Department of Medical Laboratory Science, Faculty of Health Science and Technology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.

³In-vitro Diagnostics Control Laboratory, Medical Laboratory Science Council of Nigeria, 8 Harvey Road, Yaba, Lagos State, Nigeria.

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The benefit of exploration and exploitation of crude oil to the Nigerian economy is not without its negative consequences. Apart from the indirect exposure to crude oil due to spillage, the consumption of this crude oil by the rural populace living in oil rich regions as traditional medicine for illnesses have evoked local and international concerns. The aim of this study was to investigate the histological and biochemical disrupting effects of Escravos crude on the liver and heart in Chinchilla rabbits. A total of thirty Chinchilla rabbits aged twelve to fourteen weeks and weighing 1.2 to 1.45 kg were used. Crude oil was orally given at the doses 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 (control) received distilled water. The result show a dose dependent significant increase in the serum concentrations of total cholesterol, creatine kinase, C-reactive protein, alanine transaminase and aspartate transaminase ($p < 0.05$). The histological findings include: lymphocytic infiltration, cirrhosis, fibrosis, hemosiderin, oedema, mild tissue scaring and tissue necrosis. Thus, this result suggests that Escravos crude oil is a potential biochemical disruptor and can also affect the micro-architecture of liver and heart.

Key words: Escravos crude oil, liver, heart, biochemical parameters, histology, Chinchilla rabbits.

INTRODUCTION

The over dependence on the monetary benefit of crude oil exploration and exploitation and neglect of its environmental consequences has made the problem of crude oil pollution a recurrent issue. The impact of crude oil spill-

age 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

*Corresponding author. Email: judeokoye42@yahoo.com. Tel: +234 703-111-9994.

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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 liberally used by some of the indigenes who believe that it can repel witches when applied either topically or given orally to 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.

Generally, various studies on crude oil have revealed that it has serious deleterious effects on soils (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). However, humans and other animals are also adversely affected. The constituents of crude oil can irritate the skin and mucous membrane on contact. Irritant effects can range from slight reddening to burning, swelling (oedema), pain and permanent skin damage. 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 structural and biochemical disrupting effects of Escravos crude oil on the liver and heart in 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 x 25 x 5 cm) for 24 h at the site of the study 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 30 Chinchilla rabbits aged 12 to 14 weeks and weighing 1.2 to 1.45 kg were obtained from the Faculty of Agriculture, Ebonyi State University Abakaliki (EBSU). The animals were examined, treated for ectoparasites using Ivermectin (Hebei New Century Pharmaceutical CO. Ltd) 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 animals were randomly divided into five groups, containing 6 rabbits each (3 males and 3 females).

The research plan consisted of five groups designated Groups 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 the 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

The experiment was conducted in accordance with the Guidelines of the U.S. National Institute of Health (NIH) on the care and use of laboratory animals. The animals were kept under standard and good laboratory conditions (12 h light and 12 h darkness, temperature $(30 \pm 4.5^\circ\text{C})$, humidity and ventilation). 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 grower pellets (from Vital feed Ltd, Jos, Plateau State, Nigeria) and water *ad libitum* for 28 days.

Sample collection, organ harvest and microscopy

On the 29th day (morning), the animals were anaesthetized using cotton wool damped in chloroform with due consideration of their body weights. The blood samples, obtained by marginal ear vein puncture, were drawn into tubes using 22 gauge sterile needles. For biochemical analyses, blood samples collected into plain test tubes were centrifuged (Rotofix 32@-Hettich) at 3000 g for 10 min; the serum was collected and kept at -20°C until analysis. Animals were sacrificed; the heart and liver excised, blotted dry to remove traces of blood and weighed using an electronic weighing balance (using 210/0.1 mg digital balance ESJ-210-4). The excised heart and liver were fixed in 10% formal saline, processed through paraffin wax, sectioned and slices of 3 μm thickness were stained using Haematoxylin and Eosin (H&E), Van Gieson and the Gordon and Sweet's staining Techniques (Avwioro, 2002). Photomicrograph of the stained tissue sections were taken for documentation. The processing of the heart and liver were made at Histopathology Unit in the Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria.

Biochemical analysis

The serum liver enzyme concentrations were estimated using the colourimetric method (Sood, 2009). The modified- IFCC (UV) method was adopted for the estimation of serum creatine kinase (CK-MB) as described by Stein (1985). Enzyme linked immunosorbent quantitative method as described by Powell et al. (1979) was used to determine the concentration of C-reactive protein while enzymatic end point method was used to estimate the serum total cholesterol concentration (Sood, 2009). Kits from Randox Laboratories, United Kingdom and Diagnostic Automation Inc., Calabasas were used. The concentrations of the biochemical parameters were measured using ELISA machine (MR 96 USA) and spectrophotometer. The biochemical analyses were carried out using the facilities of Reene Laboratories Onitsha.

Statistical analysis

Mean values ($\pm\text{SD}$) of the biochemical parameter and organ weights were taken for analysis. The data was tested for homogeneity of variance and significantly different results were established by one-way ANOVA using the SPSS software application (version 16). Pair-wise comparisons were made using the Post hoc test. The accepted level of significance was set at $p < 0.05$. The Pearson's correlation was made to compare the blood levels of (i) Aspartate transaminase and C-reactive protein, (ii)

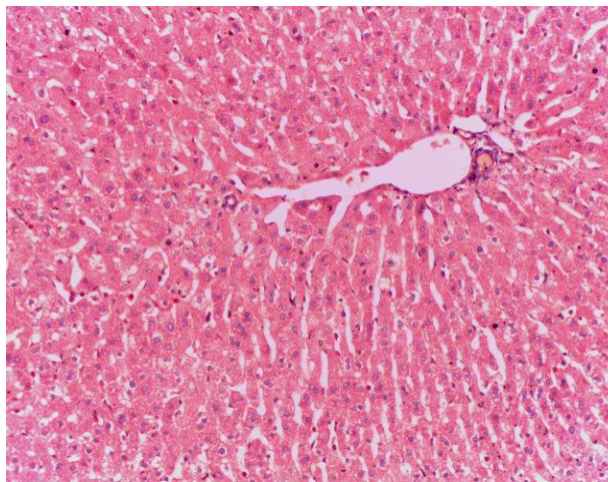


Figure 1. Group A: Photomicrograph of the liver tissue with normal micro-architecture. Stained by Haematoxylin and Eosin stain (H&E), 200x.

Table 1. Mean \pm SD and pair-wise comparison of the biochemical parameters between the control group and the treated groups.

Parameter	Group					P-value
	A (control)	B (15 mg/kg)	C (20 mg/kg)	D (25 mg/kg)	E (30 mg/kg)	
Aspartate transaminase	17.00 \pm 3.16	19.40 \pm 2.07 (0.854)	45.60 \pm 29.06 (0.037)	65.20 \pm 31.00 (0.001)	41.40 \pm 10.52 (0.072)	0.008
Alanine transaminase	27.83 \pm 2.71	35.60 \pm 4.93 (0.061)	40.20 \pm 8.93 (0.005)	45.00 \pm 9.75 (0.000)	40.00 \pm 3.54 (0.005)	0.003
Creatine kinase	9.42 \pm 4.36	13.69 \pm 4.57 (0.412)	16.23 \pm 3.27 (0.196)	30.08 \pm 17.38 (0.001)	20.15 \pm 3.98 (0.048)	0.008
C-reactive protein	0.24 \pm 0.11	0.38 \pm 0.12 (0.147)	0.51 \pm 0.25 (0.008)	0.57 \pm 0.19 (0.002)	0.46 \pm 0.07 (0.032)	0.019
Cholesterol	1.44 \pm 0.18	1.91 \pm 0.33 (0.007)	2.20 \pm 0.19 (0.002)	2.29 \pm 0.24 (0.000)	2.92 \pm 0.23 (0.000)	0.000

P-value is significant at $p < 0.05$; significant increases in the serum concentration of the biochemical parameters when the control group was compared with the treated groups ($p < 0.05$). One-way ANOVA and Post Hoc test.

aspartate transaminase and creatine kinase, and (iii) creatine kinase and C-reactive protein, with the accepted level of significance set at 0.01.

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. Soon after the tenth day, they regained their appetite.

DISCUSSION

Crude oil 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 organ toxicity and disrupt normal metabolic processes in relation to serum concentration of C-

reactive protein (CRP), aspartate transaminase (AST), alanine transaminase (ALT), creatine kinase (CK-MB), cholesterol, weights of the liver and heart.

Significant increases in the weight of the liver and heart were observed when the control group were compared with the treated group (Table 2 Figure 1), which could be attributed to the different histopathological findings observed in the tissues. The significant decrease in the mean change in body weight per week (Table 2) could be linked to the reduction in appetite observed in the treated groups. Cholesterol is an unsaturated steroid alcohol. It is used by the liver, testes and adrenal gland as a major metabolic precursor for the biosynthesis of bile acids, and steroid hormones which include male and female sex steroids (androgens and oestrogens) and adrenal steroid hormones (Morrissey, 2006). In this study, a significant increase in serum cholesterol concentration was observed in the treated group of both sexes (Table 1). The result is in conformity with the findings of Afonne et al. (2013). The observed cholesterol increase may be an indication of tissue fibrosis which culminated to cirrhosis (Figures 4, 5 6 and 7) and or renal retention disease resulting in diminished removal of lipoprotein from the

Table 2. Mean \pm SD change in body weight of animals (kilogram) and weight of liver and heart (kilograms) in the test and control groups.

Weight	Group					P-value
	A (control)	B (15 mg/kg)	C (20 mg/kg)	D (25 mg/kg)	E (30 mg/kg)	
Mean change in weight (kg)	0.012 \pm 0.004	0.09 \pm 0.003	0.02 \pm 0.001	0.08 \pm 0.001	0.08 \pm 0.004	0.019
Weight of Liver (kg)	0.068 \pm 0.05	0.077 \pm 0.08	0.080 \pm 0.05	0.091 \pm 0.06	0.087 \pm 0.08	0.000
Weight of heart (kg)	0.041 \pm 0.005	0.045 \pm 0.005	0.047 \pm 0.003	0.053 \pm 0.005	0.052 \pm 0.003	0.006

P-value is significant at $p < 0.05$; significant decrease in the mean change in body weight per week and significant increases in the weight of the liver and heart ($P < 0.05$). One-way ANOVA.

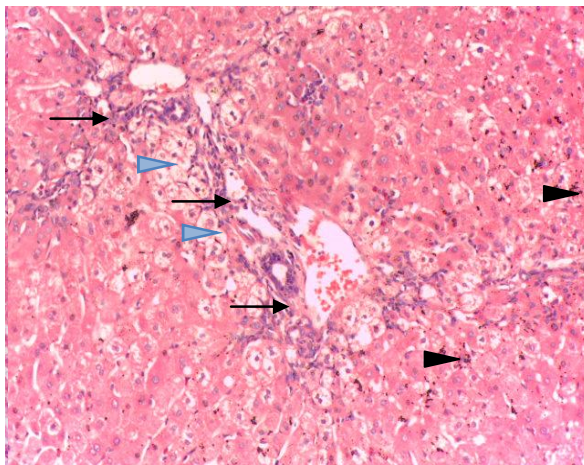


Figure 2. Group B: Photomicrograph section of the liver with evidence of stromal proliferation, hydropic hepatocytes (marked by blue arrow heads), lymphocytic infiltration (marked by arrows) and hemosiderin (coarse, dark brown and refractile granules-marked by black arrow heads). Stained by H&E technique, 200x.

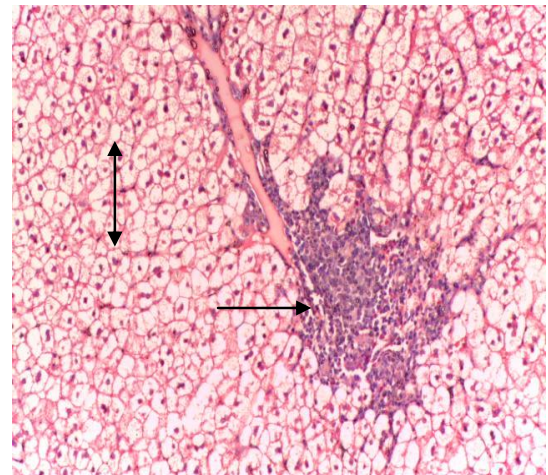


Figure 4. Group E: Photomicrograph of the liver tissue with fat deposits clogging the blood supply to the hepatocytes, marked hydropic cells (ballooning degeneration-marked by the double headed arrow), karyorhexis, cirrhosis and fibrosis (marked by arrow). Stained by H&E technique, 200x.

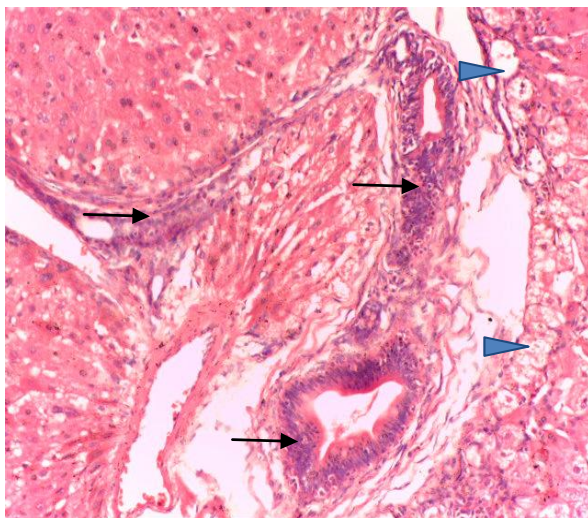


Figure 3. Group C: Photomicrograph of the liver tissue with dilatation of the portal triad, dense stroma, hydropic cells (marked by blue arrows) and lymphocytic infiltration (marked by blue arrow heads) within and around the portal triad. Stained by H&E technique, 200x.

plasma, thus causing the concentration of cholesterol to increase markedly.

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 et al., 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 a significant increase in CRP concentration (Table 1) in the treated animals. The Inflammatory process marked by lymphocytic infiltration (Figures 2 and 3) proves a vivid connection between the assayed CRP and the structural changes evident in the section of the liver. Additionally, measurement of CRP by high-sensitivity CRP assays

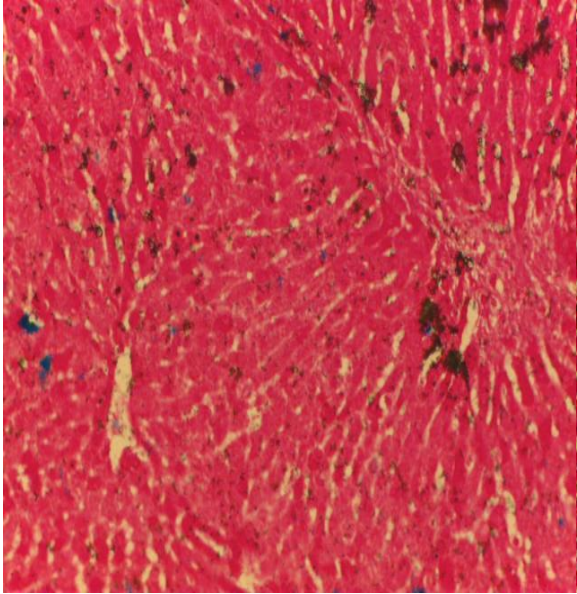


Figure 5. Group A (control): Photomicrographed section of a normal liver. Stained by Gordon and Sweet's technique. X200.

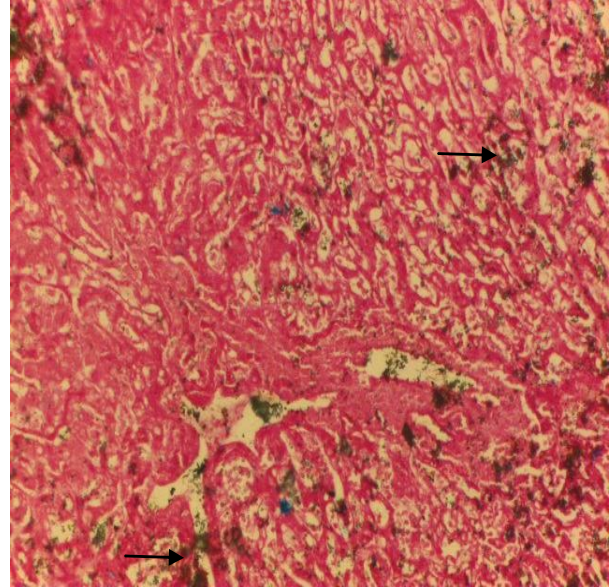


Figure 7. Group E: photomicrograph of the liver tissue with hydropic cells and cirrhosis (demonstrated by poorly marked darkly stained reticulin fibres). Stained by Gordon and Sweet's technique, 200x.

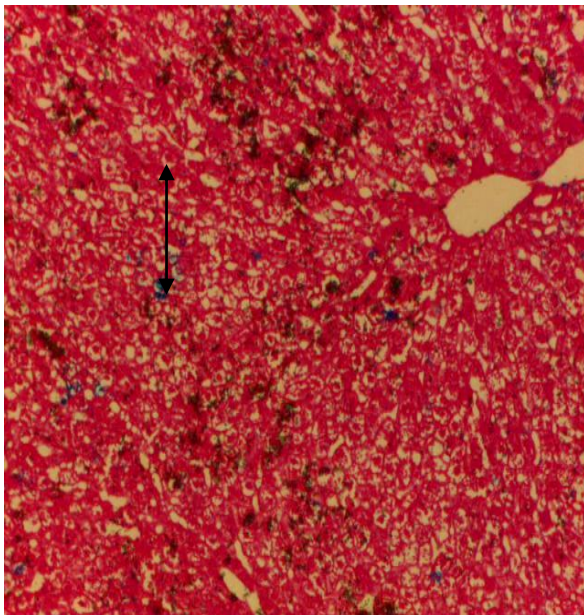


Figure 6. Group D: Photomicrograph of the liver tissue with mild onset of cirrhosis (marked by areas of distorted liver stroma). Stained by Gordon and Sweet's technique, 200x.

may add to the predictive value of other cardiac markers (myoglobin, creatine-kinase-MB, Troponin I and T), which are used to assess the risk of cardiovascular and peripheral vascular disease (Ridker et al., 1997, 1998).

Creatine kinase (CK-MB) is primarily found in striated muscle, brain and heart tissues. Ck-MB activity is useful

in diagnosis of myocardial infarction, re-infarction and the sizing of infarction (Braunwald et al., 2000; Apple and Murakami, 2005). In this study, a significant increase in CK-MB was observed (Table 1). The result from the Pearson's correlation shows a strong correlation between the AST, CK-MB and CRP levels in the serum of the treated animals (Table 3). Tissue necrosis and mild muscle scarring were evident in the histological sections of the heart tissue from group E (treated animals) used in this study (Figure 9 and 11). When compared with the control group (Figure 8). The micro-architectural changes found in the heart could be attributed to the increased cholesterol observed in the treated groups which may have caused arteriosclerosis, ischemia and finally necrosis in the heart tissue. The latter, is in consonance with the reports of Milinkovitch et al. (2013), who found similar effects on juvenile golden grey mullet (*Liza aurata*).

AST is an enzyme found mostly in the heart muscle, liver cells, skeletal muscles and kidneys. Injury to these tissue result in the release of the enzyme in the blood stream. Elevated levels are found in myocardial infarction, cirrhosis and hepatitis (Sood, 2009). The result from this study showed significant increases in the levels of AST and ALT (Table 1). This result is in accordance with the reports of Sese et al. (2013) who reported similar increase after administering Bonny light crude oil to male Chinchilla rabbits. Cirrhosis is a result of advanced liver disease, characterized by replacement of liver tissue by fibrosis (scar tissue), regenerative nodules; lumps that occur due to attempted repair of damaged tissue, elevated

Table 3. Correlation between aspartate transaminase, C-reactive protein (CRP) and creatine kinase (CK).

Variables	R-value	P-value	Remark
Aspartate transaminase correlated with CRP	0.518**	0.007	Positive correlation
Aspartate transaminase correlated with CK	0.622**	0.001	Positive correlation
Creatine kinase correlated with CRP	0.574**	0.002	Positive correlation

Correlation is significant at 0.01 level (2-tailed); significant strong positive correlations between the serum concentration of the biochemical parameters at 0.05 and 0.01 () confidence interval. Pearson's correlation.

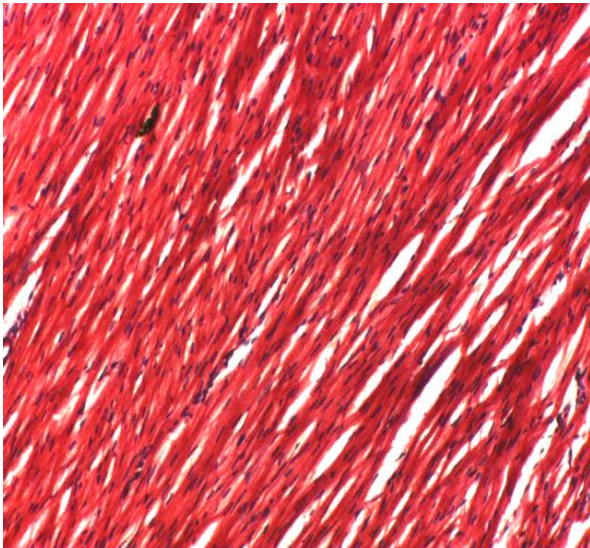


Figure 8. Group A (control): Photomicrograph of the heart tissue with normal architecture. Stained by Van Gieson technique, 200x.

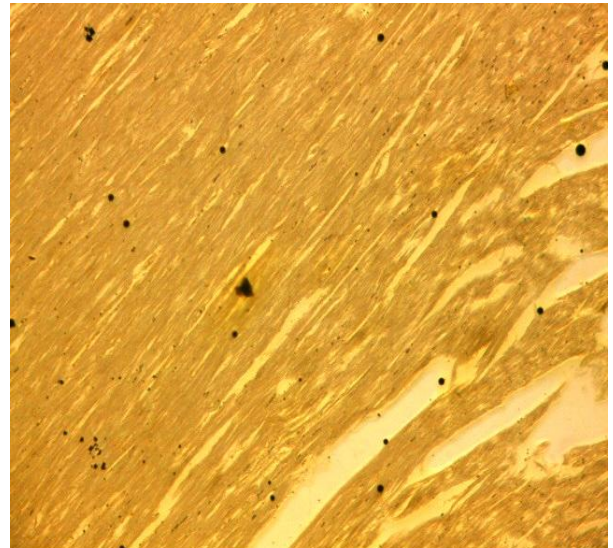


Figure 10. Group A (control): Photomicrograph of the heart tissue with normal architecture. Stained by Van Gieson technique, 200x.

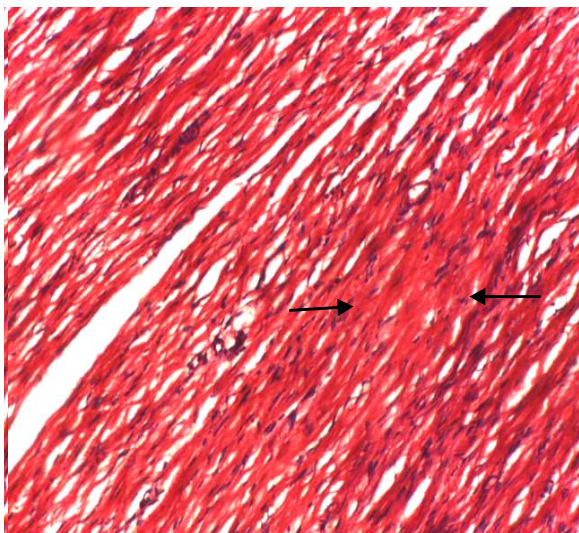


Figure 9. Group E: Photomicrograph of a heart section with mild distortion (and discontinuation) of the muscle alignment. There is also an area of mild tissue necrosis (marked by arrows). Stained by H&E technique, 200x.

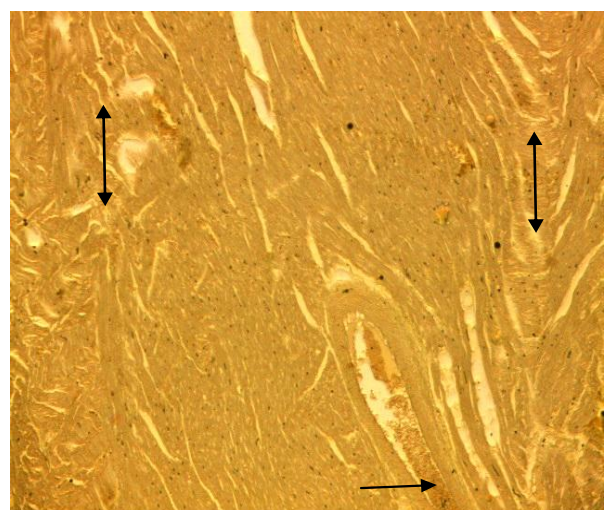


Figure 11. Group E: Photomicrograph of a section of the heart with evidence of increased vascularization, oedema (marked by arrow) and mild muscle scarring (degeneration of collagen fibres-marked by double headed arrows). Stained by Van Gieson's technique, 200x.

cholesterol and hemochromatosis among others. According to the reports by Udemé and Etim (2012), the Nigerian crude oil blends have been observed to contain some trace metals such as Pb, Cd, Cr, Mn, Zn, Cu, and Co at a low concentration but with high values of Ni, V and Fe. The Fe in the crude oil may have caused the iron deposits (hemosiderin) found on the liver tissues of the treated groups (Figures 2 and 4) when compared with the control group (Figure 1).

The biochemical and the histological findings evident in the photomicrographs elucidated the deleterious effect of crude oil on the liver and heart, whether by indirect contact due to oil spillage or by ingestion as a traditional medicine. This result suggests that Escravos crude oil has the potential to cause biochemical toxicity and can affect the micro-architecture of the liver and heart.

Conflict of interests

The author(s) have not declared any conflict of interests.

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