Original Research Article

Bonny Light Crude Oil Toxicity: Histopathological and Biochemical Disrupting Effects on Cardiac and Hepatocellular Tissues

ABSTRACT

Aim: Evaluate the acute toxicity effect of bonny light crude oil on histopathological and biochemical disrupting effects on cardiac and hepatocellular tissues.
Study Design:A total of 50 albino rats were randomly divided into three (3) groups; Control group, Low dose group, and High dose group. The Control group consisted of 10 albino rats while the low dose and high dose groups consisted of 20 albino rats each. The control group was fed with normal (uncontaminated) feeds and water only (that is, a dosage of 0.00mL/g of rat feed), and the low dose (0.005mL/g) group was fed with 300g of rat feeds mixed with 1.5mL of BLCO while high dose (0.01mL/g) group was fed with 300g of feeds mixed with 3.0mL of BLCO. The treated feeds were administered once every day for 35 days.
Methodology: After day 35, the rats were allowed to fast overnight and anesthetized with chloroform (CHCl3). Blood specimens (5 ml) were collected by slitting the neck of the rats into an anticoagulant labeled bottle. The livers and hearts of the experimental rats were harvested and preserved in 10% formalin in different labeled plastic containers prior to tissue processing and histological examinations. Blood specimens were centrifuged at 4500 rpm for 10 minutes to obtain plasma. Plasma levels of ALT, AST, ALP,cTnT, cTnI, MDA, and SOD were estimated. All weights were measured in grams.
Results: The result indicated that low and high-dose treated groups showed a significant decrease in body weight. The SOD and MDA indicated significantly lower and higher values respectively in the low and high-dose treated groups when compared to the control rats. However, no significant difference in SOD values were seen between the low and high dose treated groups. The ALT, AST, and ALP values indicated significantly higher values in the low and high-dose treated groups compared to the control group. More so, dose-dependent increases were also observed in AST and ALT. However, ALP indicted no significant difference between the control and the low-dose treated rats. In addition, cTnT and cTnI values indicated significantly higher values in the low and high-dose treated groups compared to the control group. However, cTnT and cTnI indicted no significant difference between the high-dose and the low-dose treated rats. The *P* value was set *P*=.05
Conclusion: Bonny light crude in feed at small doses over a period of 35 days induced myocardiaciand hepatic injuries indicated by increased levels of AST, ALT, ALP, cTn-T and cTn-I. More so, histopathological changes further showed the disrupting changes in hepatic and cardiac tissues.

Keywords: Crude Oil; Cardiac Markers; Troponins; Hepatocellular Markers; Oxidative Markers

INTRODUCTION

Crude oil is a diverse blend comprising hydrocarbons, sulfur, nitrogen, oxygen compounds, trace elements, and water[1]. The primary constituents are hydrocarbons, encompassing paraffins, naphthenes, aromatics, and olefins. The existence of sulfur compounds contributes to its corrosive attributes, while nitrogen and oxygen compounds may lead to the creation of nitrogen oxides (NOx) when combusted[2]. Additionally, crude oil can contain trace elements and metals. The most common metals are iron, nickel, copper, and vanadium [2]. Petroleum hydrocarbons are organic substances derived from crude oil, a blend of hydrocarbons obtained from geological formations. These compounds, consisting mainly of carbon and hydrogen atoms, exhibit a wide array of physical and chemical properties [3]. Petroleum hydrocarbon concentrations in marine, coastal, and estuarine ecosystems have increased as a

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result of rising petrochemical demand [4]. Nigeria is the largest oil producers and most populous country in Africa[5].

One of the most important contaminants for aquatic ecotoxicology is crude oil pollution[6]. Crude oil exploration is the key support of the Nigerian economy and constitutes about 90 percent of the foreign exchange earnings of the nation[7]. Exploration of crude oil results in pollution of the environment and exposure to crude oil pose risk to both aquatic and terrestrial life[6, 8]. Oil spills (stemming from factors like pipeline ruptures, equipment malfunctions, sabotage, and illicit oil bunkering), gas flaring, natural seeps, industrial discharge, and the destruction of ecosystems have led to ecological devastation, health problems, and socioeconomic challenges for communities in the Niger Delta; one of the world's largest oil-producing regions [6]. The pollution of waterways and farmlands has impacted agricultural activities (diminishing food availability), and the livelihoods of local communities, inflicting devastating consequences on both the local ecosystem and communities[9]. Crude oil spillage has been a persistent and severe environmental concern in the Niger Delta for many years [6]. The spilled oil covers water surfaces, diminishes oxygen levels, and poses significant health risks to aquatic life, birds, loss of biodiversity, and the well-being of the human population that heavily relies on these resources for their sustenance[6].Long-term health consequences are a major concern associated with oil spills in the Niger Delta[10,11]. The toxic elements found in crude oil, particularly polycyclic aromatic hydrocarbons PAHs, have been connected to an increased susceptibility to lung, liver, and skin cancers[11, 12]. Evidence from studies indicates elevated PAH levels in the blood and tissues of Niger Delta residents, indicating prolonged exposure to these cancer-causing substances[13].

The mechanism of crude oil toxicity is centered on the production of reactive metabolites such as Reactive Oxygen species (ROS) and Reactive Nitrogen Species (RNS) and metabolic conjugates of these reactive species after undergoing systemic metabolism. For instance, Wiesman et al.,14], stated that cytochrome P450 isoenzymes metabolize polycyclic aromatic hydrocarbons (PAHs) to create reactive epoxides, which then target essential macromolecules such as proteins, RNA, and DNA.Malini & Maithily, [15], also stated that the metabolism of petroleum hydrocarbons produce free radicals and cause oxidative stress in experimental animals. An imbalance between the production ROS an RNS and the body's inability to detoxify them can lead to oxidative stress [16, 17]. Oxidative stress can damage various cellular components, including lipids, proteins, and DNA, and has been linked to the development and progression of various diseases [18].

The consequences of exposure to crude oil on human health have been thoroughly researched. However, there is paucity of data on the effect of crude oil on cardiac muscles and cardiac troponins.Acute or chronic cardiac muscle damage has long been a risk for cardiac failure. Several markers such as creatine kinase MB, myoglobin, ischemia-modified albumin, natriuretic peptides, C-reactive protein (CRP) and soluble CD4 homocysteine 0-ligand (sCD40L) have been investigated in heart and heart-related issues[19]. However, cardiac troponins have surpassed CK-MB and myoglobin in terms of clinical significance to emerge as the preferred Cardiac diagnostic due to its highly sensitive and accuracyfor myocardial insults [20]. Cardiac troponins are regulatory proteins that regulate the calcium-mediated interaction of myosin and actin, which causes striated muscle to contract and relax [21]. These troponins are specific biomarker for Cardiac muscle damage, is important in determining the severity of cellular damage [21]. Following Cardiac injury or damage, the regulatory protein troponin T, which is present in Cardiac muscle fibres, is released into the bloodstream.

More so, cardiac failure and liver disease has been observed to frequently coexist [22]. Hepatocellular enzymes are essential for evaluating the health and function of the Liver. The functionality of the Liver can be accessed by measuring the levels of these enzymes[23]. Hepatic damages resulting in inflammation and degeneration are most times induced by its exposure to chemicals during metabolism. Numerous studies have shown that oxidative stress and inflammation are the most important pathogenic processes in Liver illnesses, regardless of the etiology [24].

Therefore, this study investigated the oral ingestion of Bonny Light Crude Oil in diet on cardiac troponin T (cTn-T) and cardiac troponin I (cTn-I), hepatocellular enzymes as well as oxidative stress markers in albino rats. In order to observe any morphological changes, histological evaluation of the liver and cardiac tissue will also be done.

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2. MATERIALS AND METHODS

2.1 Materials

The materials used include Scout-pro electronic weighing balance, Bonny light crude oil (BLCO),H&E stains, Memmert Incubator, Stat-Fax 4200 microplate reader, automatic tissue processor (MTPN-Series), rotary microtome, and light microscope. Reagent used include, Aspartate aminotransferase (AST) and Alanine Aminotransferase (ALT) kits procured from Randox Diagnostics whileAlkaline Phosphatase (ALP) kit was procured from Teco Diagnostics. Rat-specific ELISA kits for cardiac Troponin T (cTn-T) and cardiac Troponin I (cTn-I) were procured from Bioassay Technology, while malondialdehyde (MDA) and superoxide dismutase (SOD) were gotten from Elabscience.

2.2 Experimental Animals

Fifty (50) albino rats weighing approximately 135grams were purchased from Olive Green Laboratory Animals Company, Abia State, Nigeria. The conventional housing cages were used to house the rats and they were allowed to acclimatize for 2 weeks before the commencement of the experiment. The rats were provided with clean water for drinking and were fed with rat premix pellet feeds given *ad libitum* for the entire period of the experiment. The body weights of the rats were measured daily in the treatment process. This experiment was carried out with regard to the Helsinki [12] declaration on the guiding principles of care and use of experimental animals.

2.3 Preparation of Treated Feeds

2.3.1 Control

Feed 300g of rat pellets only (that is, a dosage of 0.00mL/g of rat feed).

2.3.2 Low dose

Feed 300g of rat pellets were mixed thoroughly with 1.5mL of bonny light crude oil making it a dosage of 0.005mL/g of rat feeds.

2.3.3 High dose

Feed 300g of rat pellets were mixed thoroughly with 3.0mL of bonny light crude oil making it a dose of 0.01mL/g of rat feeds. The method of treatment was similar to the technique described by Ogara et al. [26]

2.4 Administration of Crude Oil Contaminated Feeds

Bonny Light Crude Oil (BLCO) obtained from the Department of Petrochemical Engineering, Rivers State, Port Harcourt, Nigeria was used. The rats were allowed to feed on the feds contaminated with the crude oil for 35 days. Feds given were measured before and after daily.

2.4.1 Experimental Design

A total of 50 albino rats were randomly divided into three (3) groups; Control group, low dose group, and high dose group. The Control group consisted of 10 albino rats while the low dose and high dose groups consisted of 20 albino rats each. The control group was fed with normal (uncontaminated) feeds and water only (that is, a dosage of 0.00mL/g of rat feed), and the low dose (0.005mL/g) group was fed with 300g of rat feeds mixed with 1.5mL of BLCO while high dose (0.01mL/g) group was fed with 300g of feeds mixed with 3.0mL of BLCO. The treated feeds were administered once every day for 35 days.

2.5 Study Area

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The study was carried out and samples were analyzed in the Department of Clinical Chemistry, Faculty of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

2.6 Specimen Collection, Preparation, and Analysis

After day 35, the rats were allowed to fast overnight and anesthetized with chloroform (CHCl3). Blood specimens (5 ml) were collected by slitting the neck of the rats into an anticoagulant labeled bottle. The livers and hearts of the experimental rats were harvested and preserved in 10% formalin in different labeled plastic containers prior to tissue processing and histological examinations. Blood specimens were centrifuged at 4500 rpm for 10 minutes to obtain plasma. Plasma levels of ALT and AST were estimated as described by Reitman-Frankel [27], ALP was estimated as described by King&Angstrom [28], while cTnT, cTnI, and MDA, were estimated by ELISA procedure as described by Engvall &Perlmann [29] while SOD activity was estimated by the method described by Marklund et al. [30]. All weights were measured in grams.

2.7 Statistical Analysis

The data were statistically analyzed using Graphpad prism version 9.02 (San Diego, California, USA). Results were presented as Mean ± Standard Deviation (SD). The comparison of values in the exposed groups and control group was done using One-way ANOVA. Statistical significance was set at *P*=.05

3. RESULTS

3.1 Results of Feeds and Body Weights in Rats Treated with Crude oil Contaminated Feds

The result indicated that the rats eat significantly higher amount of the feds given on a daily basis compared to the remains measured (table 1). Also, the body weights of the control and crude oil-treated groups, after the experiment, were compared, the low and high-dose treated groups showed a significant decrease in body weight at p< 0.05 (Table 2)

Table 1: Mean Average of Feed Contaminated with Crude Oil Consumed Over a Period of 35 Days

Dosage	Initial Weight of feed given (g)	Final Weight of feed remaining (g)	Weight of feed consumed (g)	P value	F value	Remark
Low dose	300.0 ± 0.00^{a}	51.23 ± 43.11^{b}	248.8 ± 43.11^{c}	<0.0001	487.6	S
High dose	300.0 ± 0.00^{a}	74.03 ± 52.73^{b}	226.5 ± 53.35^{c}	<0.0001	247.9	S

Post-Hoc: Values in the same row with different superscripts differ significantly when initial weights, final weights, and weights of feed consumed were compared. S=Significant at p<0.05.

Table 2: Results of Weight of Rats Exposed to Bonny Light Crude Oil Contaminated Feeds

Category	Weight Before (g)	Weight After (g)	T value	P value	Remark
Control	134.1±5.877	148.7±9.262	4.209	0.0005	S
Low Dose	128.8±7.587	110.0±7.255	7.988	<0.0001	S
High Dose	133.8±6.463	113.5±6.509	9.872	<0.0001	S

S=Significant at p<0.05.

3.2 Results of SOD and MDA in Rats Treated with Crude Oil Contaminated Feds

The plasma value of SOD was significantly reduced in the low and high-dose treated groups when compared to the control rats. Also, there was no significant difference in SOD value between the low and high dose treated groups when compared at p < 0.05. However, in MDA no significant difference were observed between control and low-dose treated groups but significantly higher values were seen in highdose treated rats compared to control and lowdose treated rats over a period of 35 days (Table 3).

Table 3. **Results of Superoxide Dismutase (SOD) and Malondialdehyde (MDA) in Rats Exposed to Feeds Contaminated With Bonny Light Crude Oil**

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Parameters	Control	Low Dose	High Dose	*P* value	F value	Remark
SOD (ng/ml)	6.90±0.94[c]	4.18±1.24[b]	2.72±0.82[a]	<0.0001	54.76	S
MDA (ng/ml)	148.3±34.64[b]	161.6±21.24[b]	178.1±11.27[a]	0.0022	6.968	S

PostHoc (Tukey's): Values in the same row with different superscripts differ significantly at *P*=.05.
Key: SOD = superoxide dimutase
MDA= Malondialdehyde

3.3 Results of ALT, ALP and AST in Rats Treated with Crude Oil Contaminated Feds

The ALT, AST, and ALP values indicated significantly higher values in the low and high-dose treated groups compared to the control group at p< 0.05. Significantly higher values were also observed high-dose treated groups compared to the low dose group at p< 0.05. However, ALP indicted no significant difference between the control and the low-dose treated rats (Table 4). More so, cTnTand cTnIvalues indicated significantly higher values in the low and high-dose treated groups compared to the control group at p< 0.05. However, cTnT and cTnI indicted no significant difference between the high-dose and the low-dose treated rats (Table 4).

Table 4:Results of Liver Enzymes and Cardiac Troponins in Serum of Rats Exposed to Bonny Light Crude Oil Contaminated Feeds

Parameters	Control	Low Dose	High Dose	*P*value	Fvalue	Remark
ALT(U/L)	27.25±11.05[c]	35.76±12.17[b]	49.11±19.97[a]	0.0043	5.962	S
AST(U/L	36.17±13.13[c]	44.88±21.47[b]	64.88±21.47[a]	0.0001	4.840	S
ALP(U/L)	26.83±15.09[b]	31.25±7.95[b]	42.75±17.88[a]	0.0012	7.558	S
cTnI (ng/mL)	29.32±11.37[b]	40.66±14.37[a]	49.55±14.56[a]	<0.0001	12.26	S
cTnT (ng/mL)	9.920±6.551[b]	20.18±10.34[a]	24.04±6.832[a]	17.60	<0.0001	S

PostHoc (Tukey's): Values in the same row with different superscripts differ significantly at *P*=.05.
Key: ALT= Alanine aminotransferase
ALP= Alkaline Phosphatase
AST= Aspartate aminotransferase
cTnI= Cardiac Troponin I
cTnT= Cardiac Troponin T

3.5 Histological Examination

The photomicrograph of the control rat liver tissue showed a normal central vein, distinct hepatocytes arranged within the hepatic plate separated from one another by a well-defined sinusoids originating from the central vein (Fig. A). In the low dose rats, the central vein shows mild filtration of parenchyma materials. Hyperplasias with nuclear aggregation of the hepatocytes were seen. The hepatic plate and sinusoids were mildly affected as well (Fig. B). In the highdose treated rat, mild infiltration of the central vein as well as vacuolation at the periphery of the central vein was observed. The hepatocytes appeared deeply stained (hyperchromatic) with poorly defined hepatic plate. The sinusoids also appeared distorted (Fig. C).

When the cardiac tissue was considered, thephotomicrograph of the control showed normal heart tissues fibres and nucleus with anastomosing cardiac fibres and normal myocytes (Fig D). In the Low dose, the section showed regenerating heart tissue fibres and cardiac myocytes with regenerating fibres (Fig E) while in the high dose, the photomicrograph section of heart tissue showed hypertrophied myocardial fibres and enlarged nuclear lesions with pyknosis (Fig F).

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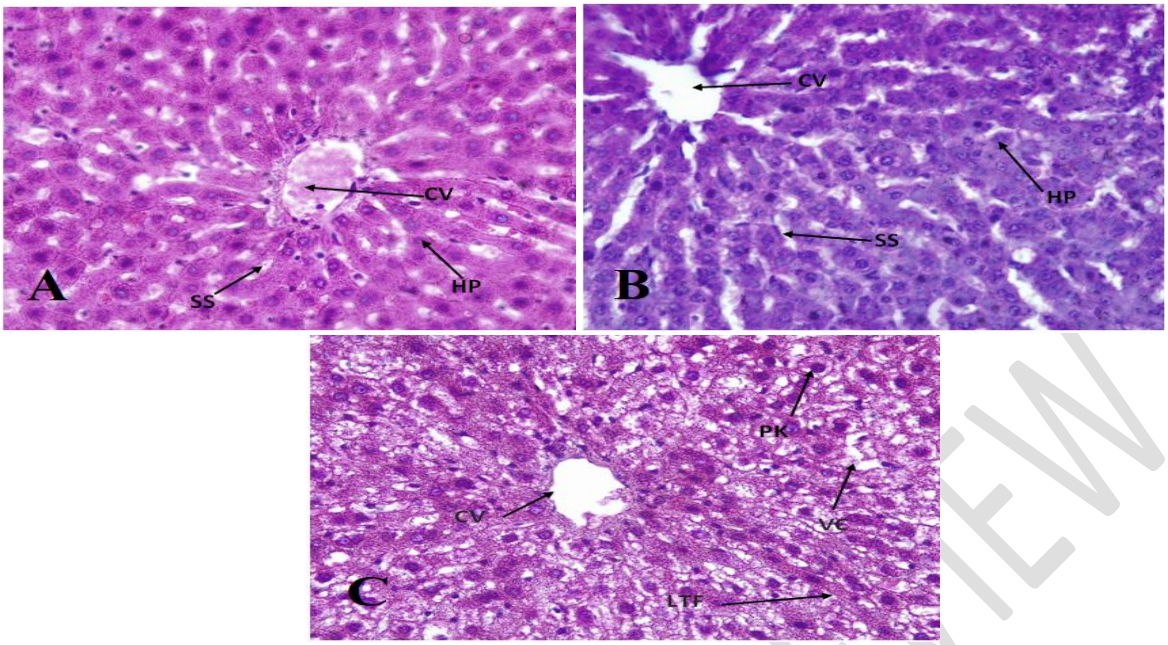

Figure A: Control: Photomicrograph section of normal control liver tissue with central vein (CV), Hepatocytes (H), and sinusoids (S). Mag. x400, Stain: H& E. **Figure B:** LOW DOSE: Photomicrograph section showing distorted central vein (CV), and hypercellurization of Hepatocytes (Hp), as well as dilated sinusoids (SS) x400, Stain: H& E. **Figure C:** HIGH DOSE: Photomicrograph section of liver tissue showing hepatic tissue vacuolation(VC), hypertrophied hepatic cells, nuclear pyknosis (PK) and liver tissue fibrosis (LTF), as well as CV infiltration x400.stain, H&E

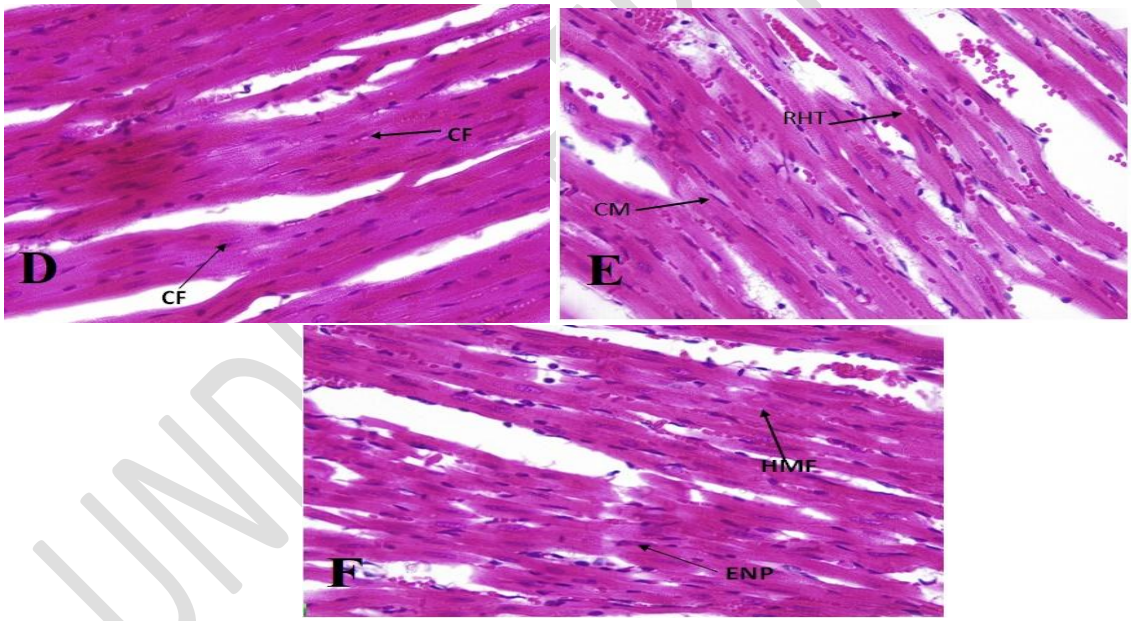

Figure D: Control: Photomicrograph section of heart tissue (cardiac muscle). Section showed normal heart tissues fibres and nucleus with anastomosing cardiacfibres (CF) and normal myocytes (M) x400. **Figure E:**Photomicrograph section of heart tissue (cardiac muscle) (Low dose). Section showed regenerating heart tissue fibres and cardiac myocytes with regenerating fibres. **Figure F:** High Dose: Photomicrograph section of heart tissue showing Hypertrophied myocardial fibres and enlarged nuclear lesions with pyknosis x400.

DISCUSSION

The significant decrease in the weight of the rats fed with low and high dose of crude oil is an indication of toxicity of the crude oil. Our findings are in similar to the reports of Oritseweyinmiet al.,[31], who documented significant loss of body weight after being exposed to an increasing dose of BLCO for 14 days. Drastic weight loss within a short period has been reported as a sign of toxicity when animals are

exposed to chemicals. The weight could be the loss of parenchymal materials of the body's organs system.

The significant higher values of cTn-T and cTnI concentrations in the low and high dose compared to control indicate myocardial injuries induced by crude oil. Our findings are similar to the reports of Anakwue&Otamiri, [32], who documented significant increase in cardiac enzymes including cardiac troponin I (cTn-I) in rats exposed to petroleum products in wistar rats. Anthony et al. [33], also reported dose dependent increase in cardiac markers like CK-MB and C-Reactive Proteins in rabbits treated with Escravos light crude oil for 28 days at doses of 15, 20, 25, and 30 mg/kg. Our findings suggest that these injuries could have been initiated by oxidative stress in the metabolism of crude oil in the liver. The induction of ROS and RNS is supported by the significantly lower values of SOD and significantly higher value in MDA in the low and high dose treated rats. The higher MDA values further indicated increased degree of lipid peroxidation following induced oxidative stress in the rats. The resultsof SOD and MDA obtained are similar to the findings in our previous studies [34] were the effect of crude oil on hepatocellularr enzymes, female reproductive parameters, and oxidative stress markers were studied. The result in this study further suggest that the degree of ROS and RNS produced by crude oil surpasses the rate at which these species were been eradicated from the system. The histology of the cardiac tissue further suggested myocardial disturbances showing hypertrophied myocytes alongside pyknosis (figure F). In another similar study, documented by Iroh et al., [35],cTnT not TnIwas found to be elevated when rats were treated over a period of 60 days with tartrazine dyes at 7.5mg/kg. Similarly, Anakwue&Otamiri, [32], further reported histological changes in wistar rat petroleum products- induced cardiotoxicity in animals.

The significantly higher levels of ALT, AST, and ALP in the low and high dose treated rats are similar to our findings in previous study as documented by Elekima et al [34]. It was observed that crude oil given in same dose over 35 days induced increased ALP, AST, ALT values in the plasma. Similarly, Imo*et* al., [36],documented significant increase AST and ALP of rats exposed to inhalation of petroleum products for5 hours daily for 21 days. The significantly higher values of AST, ALT, and ALP are indication of hepatic toxicity and damage. The mechanism of induced-toxicity could also be related to induce oxidative stress as previously discussed. The increase in ALT and ALT were also observed to be dose-dependent. In other words, as the doses of crude oil were increased, the degree hepatic toxicity also increased proportionately. This finding was also reported by Anthony et al. [33]. They reported dose dependent increase in ALT and AST in rabbits treated with Escravos light crude oil for 28 daysat doses of 15, 20, 25, and 30 mg/kg. The histology of the liver in the low and high rats further revealed the distortion of hepatic sinusoids and hepatic plates, infiltration of central vein by parenchymal materials as well as hyperchromatic and hpyercellularisation of hepatocytes (figure B and C). These results also concur with the finding of Anthony et al. [33]. They reported hepatic infiltration, cirrhosis, and oedema (hyperplasia) in rabbits treated with Escravos light crude oil for 28 days at doses of 15, 20, 25, and 30 mg/kg. Our findings are indicative of hepatic inflammation and injuries that could result in the higher values of AST, ALT, and ALP in the plasma as shown by the biochemical parameters. Oritseweyinmiet al, [31],also reported severe histological changes in the liver after being exposed to an increasing dose of BLCO for 14 days.

Conclusion

Bonny light crude in feed at small doses over a period of 35 days induced myocardiacand hepatic injuries indicated by increased levels of AST, ALT, ALP, cTn-T and cTn-I. More so, histopathological changes further showed the disrupting changes in hepatic and cardiac tissues.

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