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Alterations in Hepatocellular, Reproductive, and Oxidative Stress Parameters in Female Albino Rats Exposed to Crude Oil

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Authors' contributions

This work was carried out in collaboration among all authors. Authors IE and TPO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TPO, OMF and APO managed the analyses of the study. Authors NB and WHA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To study the effect of exposure to crude oil on the liver, ovary, and some oxidative stress parameters in albino rats.
Study Design: A total of 50 female albino rats were used in the experiment. The rats were grouped into three: The control group which consisted of 10 rats, the low dose group which consisted of 20 rats, and the high dose group also consisted of 20 rats. The low dosage group was orally administered 1.5 mL crude oil mixed with 300 grams of rat feeds (0.005 mL/g) and the high dosage

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group was orally administered 3.0 mL crude oil mixed with 300 grams of rat feeds (0.01mL/g), while the control group was fed with normal rat feeds. The treated feeds were given once a day for 35 days.
Place of Study: The study was carried out in the Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.
Methodology: On the 36th day, the rats were sacrificed and then 5mL of blood from each rat was collected by cardiac puncture into labeled lithium heparin bottles for liver enzymes assay, hormonal assay, and oxidative stress parameters assay, while the livers and ovaries were harvested and fixed in 10% formal saline before tissue processing and histological examinations using H&E staining technique. The collected blood specimens were spun; the plasma was extracted and analyzed in the laboratory for Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), follicle Stimulating hormone (FSH), luteinizing hormone (LH), Prolactin, Malondialdyde (MDA), and Superoxide dismutase (SOD). Statistical analysis was performed using Graphpad prism version 8.02.
Results: Significantly higher plasma levels of AST, ALT, and MDA in the treated groups, except for ALP which was only significantly higher in the high-dose group. FSH, LH, Prolactin, and SOD indicated significantly lower levels in the crude oil-treated rats. The histological examinations showed marked distortion in the architecture of the livers and ovaries of the treated groups, also, there was a reduction in ovarian cellularity and massive degenerated tissues.
Conclusion: It is shown that exposure to crude oil contaminants orally could have a significant effect on the plasma level of hepatocellular enzymes, reproductive hormones, and oxidative stress parameters which in turn could lead to hepatocellular dysfunction, infertility, or impaired reproduction in mammals and cellular injuries caused by excess free radicals as signaled by plasma level of oxidative stress parameters.

Keywords: Crude oil; hepatocellular enzymes; reproductive hormones; oxidative stress markers; Niger Delta.

1. INTRODUCTION

Crude oil exploration is the mainstay of the Nigerian economy and constitutes about 90 percent of the foreign exchange earnings of the nation [1]. The Southern part of the country especially the Niger Delta area provides most of the space for the exploration and exploitation of crude oil in Nigeria. It has been reported that an average of 240,000 barrels of crude oil are spilled in the Niger Delta every year, mainly due to unknown causes (31.85%), third-party activity (20.74%), and mechanical failure (17.04%). This region habours numerous rivers and streams through which freshwater empties into the Atlantic Ocean. Most of the time, these rivers provide the only sources of drinking water and marine food for the local communities within the area. The economic benefits of crude oil exploration and exploitation are accompanied by the discharge of harmful substances into the environment [2]. Crude oil is found to contain several poisonous compounds [3]. Over the years, there have been concerns about the effects of acute and chronic exposure to crude oil from direct contact with petroleum products, oil spills, pipeline vandalism, tanker accidents, as well as indirect ingestion of contaminated water and foods [4].

Crude oil composition is very ambiguous, consisting of a complex combination of hydrocarbons, oxygen, sulfur, nitrogen, and trace metals. The hydrocarbons of crude oil consist of paraffin, cycloparaffins, and aromatic substances containing at least one benzene ring. Bonny light crude oil (BLCO) also contains polycyclic aromatic hydrocarbons (PAHs). The majority of these elements (vanadium, nickel, asphaltenes, and polyaromatic hydrocarbons) are considered to be toxic [5].

PAHs have been linked to liver damage in humans and cancers, especially lung cancers [6]. PAHs in agricultural crops add to organisms' exposure to these compounds via the dietary route [7]. Exposure to crude oil may occur directly or indirectly through inhalation, skin contact, and ingestion [8]. Consumption of crude oil by humans may affect the state of well-being however; the actual evidence of pathological and psychological effects on the health of local communities is poorly understood [9].

Symptoms such as anxiety, throat and eye irritation, headache, depression, and sore throat may occur. It may also cause several problems to the heart, lungs, kidney liver, endocrine, DNA, and brain, etc. [8]. Children, given crude oil to treat febrile convulsion, show symptoms of acute renal failure, a sequel of shocks, intestinal obstruction, extensive epidermolysis, mucositis, conjunctivitis, pneumonitis, and oesophagitis. The symptoms of exposure may appear spontaneously (acute) or emerge after some time passes (chronic) [8].

This research is centered on the effects of acute exposure to crude oil on the liver, ovary, and some oxidative stress markers. Globally, liver disease is a leading cause of illness and mortality [10]. Defects or injuries to the ovary may lead to female infertility. Infertility is the inability of a couple to achieve pregnancy after one year of unprotected sexual intercourse. The prevalence of infertility varies, in Nigeria; its prevalence is up to 25% [11]. Malondialdehyde (MDA), is a lipid peroxidation product, while Superoxide dismutase (SOD) acts as a catalyst for mopping excess free radicals (reactive oxygen species). These oxidative markers are vital in determining the extent of cellular damage. The disturbance of the integrity of the liver, ovaries, and oxidative stress markers may be influenced by hereditary, lifestyle, and environmental variables [10]. Therefore, there is a need to attempt to investigate the toxicological influence of crude oil on different organ systems.

2. MATERIALS AND METHODS

2.1 Materials

The materials used in this study include Ohaus scout-pro electronic weighing balance (Ohaus Corporation, New Jersey, USA), polypropylene gavage tubes (Intect Laboratory Incorporated, Plymouth Meeting, USA), Bonny light crude oil (BLCO) and albino rats. Aspartate aminotransferase (AST) and alanine amino transferase (ALT) kits were purchased from Randox Diagnostics, United Kingdom, while alkaline phosphatase (ALP) kit was purchased from Teco Diagnostics, United Kingdom. Rat-specific ELISA kits for follicle stimulating hormone (FSH), luteinizing hormone (LH), and prolactin were purchased from Bioassay Technology, China while that of malondialdehyde (MDA) and superoxide dismutase (SOD) were purchased from Elabscience Houston, Texas, USA. H&E stains, Memmert Incubator, Stat-Fax 4200 microplate reader, automatic tissue processor (MTPN-Series), rotary microtome, and light microscope. Other materials used include; automatic pipettes dissecting board, pins, measuring cylinder, beakers, cotton wool staining trough, slides, cover slips, syringes, and needles.

2.2 Experimental Animals

Fifty (50) female albino rats, eight weeks old weighing 132.1±2.23g were used. The rats were purchased from Olive Green Laboratory Animals Company in Obieche, Aba, Abia State, Nigeria. The housing of the rats was made of a conventional wire mesh metal cage of dimension (36 × 71 inches). The cage was separated into three compartments of equal size. The compartments were properly aerated; the bedding of the cage was made of pine shavings. Each group of rats was placed in separate compartments of the cage and kept under standard rat laboratory conditions (room temperature of 18-25^{0}C, 40-50% humidity, and proper ventilation). The rats were avoided from exposure to rainfall and high sunlight with clean tap water for drinking and were fed with rat pre-mix pellet feeds given *ad libitum* for the entire period of the experiment. The body weights of the rats were assessed daily before to treatment. 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. [13].

2.4 Administration of Crude Oil Contaminated Feeds

The crude oil used in this experiment was the Bonny Light Crude Oil (BLCO) and was gotten from the Department of Petrochemical Engineering, Rivers State, Port Harcourt, Nigeria. 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. Before the crude oil-contaminated feeds were administered, the rats were first made to acclimatize to the environment for two weeks in their cages.

2.4.1 Acute toxicity study

A total of 50 female 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 with an average weight of 134.1±5.877. The low dose consisted of 20 albino rats with an average weight of 128.8±7.587, and the high-dose group consisted of 20 albino rats with a weight of 133.8±5.646 on average.

2.4.1.1 Dose and duration

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 thirty-five (35) days.

2.5 Study Area

The study was carried out and samples were analyzed in the Department of Medical Laboratory Science. Port Harcourt, Nigeria. While the histological examination of the selected organs was carried out in the Anatomical Laboratory, College of Medical Science, University of Port Harcourt, Rivers State, Nigeria.

2.6 Specimen Collection, Preparation, and Analysis

On the 36th day, the experimental rats were anesthetized with chloroform ($CHCl_3$) then the rats were placed on the dissecting board, lying supine with the four limbs pinned to the board, and 5mL of blood was collected by cardiac puncture using a syringe and then transferred into an anticoagulant labeled bottle (lithium heparin bottle) for laboratory investigation. Organs were harvested using a surgical blade to make a longitudinal incision and another incision on the upper left part of the thorax. The livers and ovaries of the experimental rats were harvested and preserved in 10% formalin in different labeled plastic containers prior to tissue processing and histological examinations. The collected blood specimens were spun at 4,500 rpm for 10 minutes to obtain plasma. The plasma was extracted into clean labeled plain bottles and stored in the refrigerator at 4 ºC prior to the laboratory analysis. During the laboratory analysis, Plasma levels of ALT and AST were determined as described by Reitman-Frankel [14], ALP was determined as described by King-Angstrom [15], while FSH, LH, Prolactin, and MDA, were determined by Enzyme-linked immunosorbent assay (ELISA) procedure which were based on technique as described by Engvall & Perlmann [16] while SOD activity was determined by the method described by Marklund et al. [17]. All weights were measured in grams.

2.7 Statistical Analysis

The data were statistically analyzed using Graphpad prism version 8.02 (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 $p < 0.05$.

3. RESULTS

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

The comparison of ALT, AST, and ALP values between the control and the treated groups over a period of 35 days of treatment showed significantly higher values of ALT, ALP, and AST in the low and high-dose treated groups at $p< 0.05$. However, in ALP there was no significant difference between the control and the low-dose treated rats. In addition, significantly higher values were observed in rats treated with high doses of crude oil compared to those treated with low doses (Table 1).

3.2 Results of FSH, LH and Prolactin in Rats Treated with Crude Oil Contaminated Feds

The control and the treated groups on comparison of their FSH, LH, and prolactin levels showed significantly lower values of FSH, LH and prolactin in the low and high-dose treated rats. However, there was also no significant difference in FSH, LH, and prolactin values when compared between the low and high-dose treated rats, at $p < 0.05$ (Table 2).

3.3 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 a 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 high-dose treated rats compared to control and low-dose treated rats over a period of 35 days (Table 3).

3.4 Results of Body Weights in Rats Treated with Crude oil Contaminated Feds

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 4).

Table 1. Results (Mean ±SD) of liver enzymes in rats exposed to feeds contaminated with bonny light crude oil

Parameters	Control	Low dose	High dose	P value	F value	Remark
ALT(U/L)	$27.25±11.05^{a}$	$35.76±12.17^{b}$	$49.11±19.97^{c}$	0.0043	5.962	S
ALP(U/L)	$26.83±15.09^{a}$	$31.25±7.95^{a}$	$42.75±17.88^{b}$	0.0012	7.558	S
AST(U/L)	$36.17±13.13^{a}$	$44.88±21.47^{b}$	$64.88±21.47^{c}$	0.0001	4.840	S

Key: ALT= Alanine aminotransferase, ALP= Alkaline Phosphatase, AST= Aspartate aminotransferase. Tukey's post-Hoc: Within the same row, values with different superscripts differ significantly at p <0.05

Table 2. Results (Mean ±SD) of follicle stimulating hormone (FSH) and luteinizing hormone (lh) and prolactin in rats exposed to feeds contaminated with bonny light crude oil

Parameters	Control	Low dose	High dose	P value	F value	Remark
FSH (ng/ml)	$4.07±1.20^{a}$	$2.03±0.81^{b}$	$1.63±0.62^{b}$	<0.0001	31.63	S
LH (ng/ml)	$2.10±0.1^{a}$	$1.20±0.1^{b}$	$0.98±0.1^{b}$	<0.0001	31.45	S
Prolactin (ng/ml)	$1.09±26^{a}$	$0.85±0.19^{b}$	$0.78±0.13^{b}$	0.0002	10.25	S

Key: FSH= Rat-specific Follicle Stimulating Hormone, LH= Luteinizing Hormone. Tukey's Post-Hoc: Within the same row, values with different superscripts differ significantly at p <0.05

Table 3. Results (Mean±SD) of Superoxide Dismutase (SOD) and Malondialdehyde (MDA) in plasma of rats exposed to feeds contaminated with bonny light crude oil

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

Key: SOD= Superoxide Dismutase, MDA= Malondialdehyde. Tukey's Post-Hoc: Within the same row, values with different superscripts differ significantly at p <0.05

Table 4. Results (Mean±SD) of weight of rats exposed to bonny light crude oil contaminated feeds

Group	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

3.5 Histological Examinations

3.5.1 Histology of the Liver

The photomicrograph of the control rat liver tissue showed a normal central vein as well as distinct hepatocytes that are deeply stained with the primary dye (haematoxylin). The hepatocytes are well arranged within the hepatic plate separated from one another by well-defined sinusoids radiating from the central vein (Fig. 1A). The photomicrograph of the low-dose rat showed the filtration of the central vein by parenchyma materials of the hepatocytes. The hepatocytes showed aggregation of nuclear material, these hepatocytes indicated hyperplasia with nuclear aggregation. The hepatic plate and sinusoids are completely distorted (Fig. 1B). The photomicrograph of high-dose treated rat indicates a distorted central vein as well as vacuolation at the periphery of the central vein. The hepatocytes appeared to be hyperchromatic within and slightly distorted hepatic plate. The sinusoids also appeared distorted (Fig. 1C).

3.5.2 Histology of the ovaries

The photomicrograph of the control rat showed a normal ovarian cortex with primordial follicles, growing follicles, and mature ovum, while the photomicrograph of the low-dose treated rat indicated mildly distorted growing follicles. The high-dose treated rat showed degenerated follicles as well as the absence of primordial follicles.

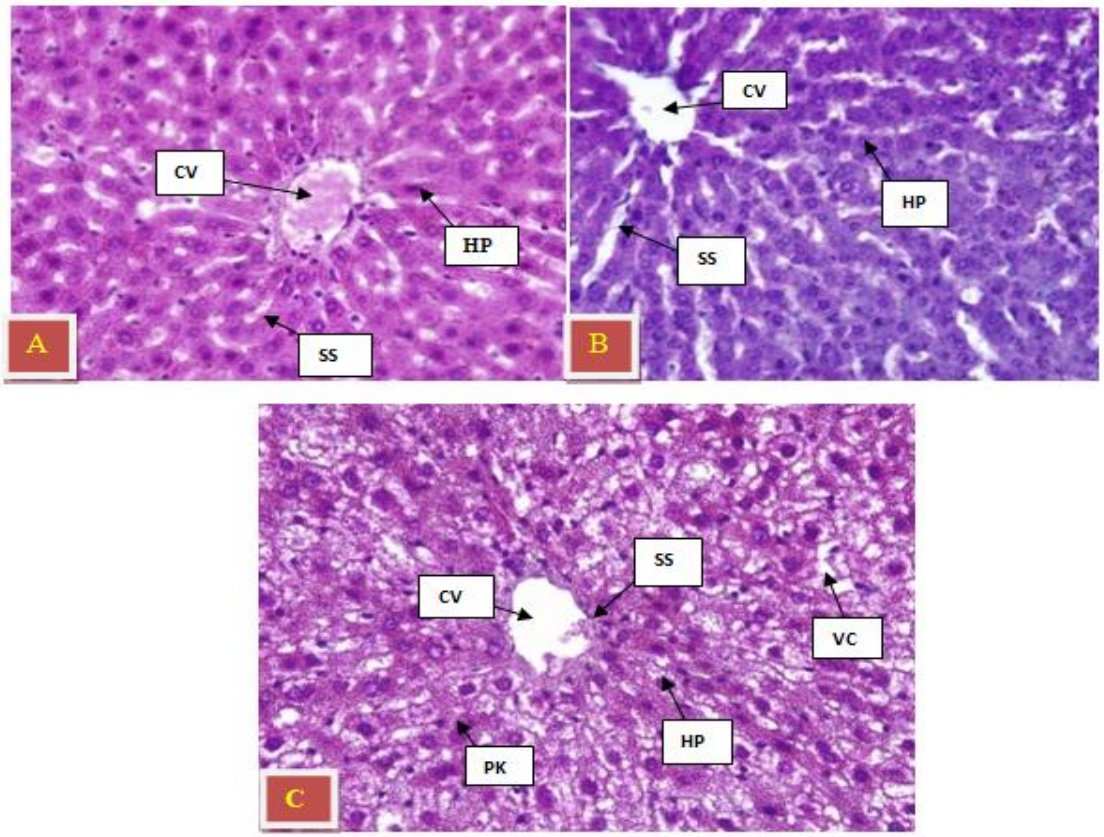

Fig. 1. Histological images of Liver sections of female albino rats. Magnification X400 Stain: H&E. Image A: A photomicrograph liver section of a control rat showing Central Vein (CV), Hepatocytes (HP), and Sinusoids (SS). Image B: A photomicrograph liver section of low dose treated rat showing Distorted Central Vein (CV), and Hypercellurization of Hepatocytes (HP), as well as Dilated Sinusoids (SS). Image C: A photomicrograph liver section of high dose treated rat showing Hepatic Tissue Vacuolation (VC), Hypertrophied Hepatic Cells, Nuclear Pyknosis (PK), and Liver Tissue Fibrosis (LTF), as well as CV Infiltration

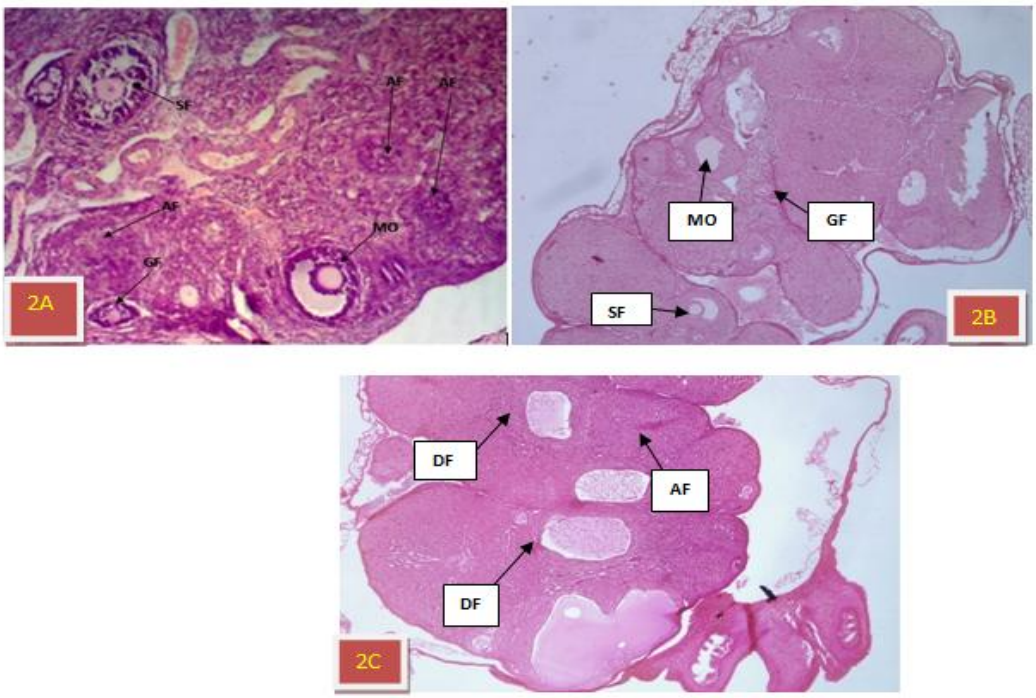

Fig. 2. Histological images of ovaries sections of female albino rats. Magnification X400 Stain: H&E. Image 2A: A photomicrograph liver section. Image 2A: photomicrograph section of normal control tissue showing ovarian cortex with primordial follicles, growing follicles (GF) and mature ovum (MO), atretic follicles (AF), secondary follicles (SF) at different stages of development. Image 2B: Photomicrograph section of ovarian tissues from rats treated with low dose, showing recovered secondary follicles (SF), mature ovum (MO), germinal, and growing follicles, (GF). Image 2C: Photomicrograph section of ovarian tissue from rats treated with high dose of extract showing atretic (AF), and degenerative follicles (DF)

Table 5. Results (Mean±SD) of feed contaminated with crude oil consumed over a period of 35 days

Dosage	Weight of feed given (g)	Weight of feed remaining (g)	Weight of feed consumed	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 (a, b, c) differ significantly when compared to one another at p <0.05. S=Significant

4. DISCUSSION

The study of the rats' plasma level of MDA, SOD, ALT, AST, ALP, FSH, LH, Prolactin, and the histology of the livers and ovaries provided insight into the potential impacts of crude oil using the albino rat model. The liver is a multifunctional organ that is vulnerable to xenobiotic damage due to its major involvement in xenobiotic metabolism [18]. Hepatic toxicity and potential liver damage are indicated by ALT, AST, and ALP [19]. The increased levels of these plasma enzymes seen in the groups exposed to contaminated feeds with crude oil are indicative of cellular leakage and loss of cell membrane integrity as agreed with the works of Ubani and Joshua [20] and Ubani et al. [21] who determined the kerosene has a similar effect in albino rats. Photomicrographs of liver tissue from rats in the control group revealed substantially normal histoarchitecture. The liver tissue of rats in group low-dose group had a skewed arrangement of laminae plate and a dilated hepatic triad (SS) with a spindle-shaped slightly larger central vein (CV). The majority of hepatocytes have hyperchromatic nuclei, with others having hollow

nuclei (HP). The liver necrosis within the dilated hepatic triad was seen in histological sections of rats in the low dose group. In the high dose group, the stroma appears heavily fibrous, with hypochromatic nuclei in some hepatocytes (HP). The central vein (CV) is enlarged and lined by single-layer epithelial cells with multicystic spaces within which are seen as pyknotic hepatocytes (PK). The hepatic triad is slightly dilated (SS). Also seen are numerous ghost cells within the stroma. In a study done by Obidike [22] on the effect of crude on the stomach, his work also revealed degeneration of mucosal villi of the stomach. This study also recorded a significant decrease in body weight of the exposed groups when compared to the control rats, this is in line with the findings of Obidike [22].

FSH, LH, and prolactin are one of the most important endocrine parameters to evaluate ovarian function [23]. Measurement of these hormones can establish the derangements of the female reproductive system. The FSH, LH, and prolactin were significantly reduced in the treated groups when compared to the control group at ($p<0.05$). The photomicrograph of the control rats showed normal primordial follicles and secondary follicles at the stage of development while the photomicrograph of the low-dose treated rats indicated mildly distorted growing follicles. The high-dose treated rat showed degenerating follicles as well as the absence of primordial follicles. In a similar study carried out by Orisakwe et al. [24] in male rats, it was reported that crude oil induced a mild to complete degeneration and necrosis of the seminiferous tubules and reduction in the epididymal sperm count following administration of BLCO dissolved in drinking water to rats. The significant lower values seen in the glycoprotein hormones especially in LH and FSH could be due probable distortion of the theca membrane and cells of the ovaries. These distortions could lead to complete loss of estrogen response resulting in reduced values of LH and FSH owing to poor feedback responses. Therefore, it could also be possible that FSH, LH and PRL values may indicate significant derangements (significantly lower levels) if the duration of treatment was extended.

Malondialdehyde (MDA) is a useful biomarker for lipid peroxidation and oxidative stress. Different researchers have used MDA assay as a parameter for different sample types. Increased levels of oxidative stress have been associated with various disease patterns. MDA and MDA-DNA adduct determination is a valuable tool in finding out the associations between oxidative stress levels and the occurrence of various pathologies. Conclusively, MDA estimations can be used as a reliable tool to assess oxidative stress levels and find their relationships with different disease patterns [25]. SOD has a crucial antioxidant role in defending the cell from superoxide damage [26]. Numerous diseases, such as hepatocellular cancer [27], an accelerated loss of muscle mass with aging, and a shorter life span [28] are all present in mice lacking SOD1, in the midst of extreme oxidative stress. During extreme oxidative stress, SOD2 knockout mice pass away a few days after birth [29]. This study is in concordance with Adedara et al. [30], as they recorded a significant increase in lipid peroxidation and a significant decrease SOD in rats exposed to BLCO for 7 days. In a study done on the exposure of crude oil on plants, Skrypnik et al. [31] reported a significant increase in MDA and SOD activities in the shoots of plants. Plants also showed a similar increase in MDA level in line with this study but showed an increase in SOD level contrary to this study.

5. CONCLUSION

The oral administration and ingestion of two different doses of crude oil mixed with rat feeds; for 35 days, revealed mild derangement of the liver and ovary in the low-dose treated rats, and the derangement was more pronounced in the high-dose treated rats**.** This study further reveals that irrespective of how little the quantity of crude oil contamination, it will induce toxicological and detrimental effects as seen in this study.

6. RECOMMENDATION

Due to the mild derangements observed in the treated groups, it is important to enlighten the local duelers, on the dangers of directly or indirectly ingestion crude oil**.**

ETHICAL APPROVAL

We hereby declare that principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as specific national laws where applicable. All experiments have been examined and approved by the Rivers State University research/ethics committee with file No: RSU/CV/APU/74/VOL.VIII/104.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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