The Journal of Anatomical Sciences
Email: journalofanatomicalsciences@gmail.com

J. Anat Sci 15(1)

Gestational Exposure to Bonny Light Crude Oil alters Renal Function Indices, Cardiac Muscle Integrity, and Oxidative Stress Indices in Offspring of Wistar Rats

[1]*Jeje S.O., [1]Omayone T.P., [1]Adenawoola M.I., [2]Ola-Mudathir F.K., [3,4]Ijomone O.M.

[1]Department of Physiology, School of Basic Medical Sciences, Federal University of Technology, Akure, Ondo State
[2]Department of Physical and Chemical Sciences, Elizade University, Ilara-Mokin, Ondo State
[3]Department of Anatomy, Faculty of Basic Medical Sciences, University of Medical Sciences, Ondo, Nigeria.
[4]Laboratory for Experimental and Translational Neurobiology, University of Medical Sciences, Ondo, Ondo State, Nigeria.

Corresponding author: Jeje S.O

E-mail: sojeje@futa.edu.ng

ABSTRACT

In this study, the cardio-renal health of offspring of mother exposed to bonny light crude oil (BLCO) during gestation was assessed in Wistar rats. Ten pregnant rats were divided into 2 groups (n=5). Distilled water (0.75 mL/Kg/days) was administered to group 1 while BLCO (0.75 mL BLCO/Kg/day) was administered to group 2. The administration was done orally from gestation day 1-21. Cardiac and renal MDA, Superoxide dismutase, and catalase activities were assessed as measures of oxidative stress. Serum and urine creatinine and creatinine clearance were assessed as indices of renal function. Serum creatinine kinase was used as an index of cardiac integrity. Histopathology and histomorphometry of the heart and kidney were also assessed. Data were subjected to Two-way ANOVA to assess the interaction between sex and treatment. The result showed a significant reduction ($P < 0.05$) in body weight at birth, postnatal days 21 (weaning) and 3 months of postnatal life in the male and female offspring of BLCO group. Cardiac and renal Catalase and SOD activities, urine creatinine, and creatinine clearance were significantly reduced ($P < 0.05$) in the female offspring of BLCO group. The cardiac MDA, renal MDA and serum creatinine kinase activities were significantly raised ($P < 0.05$) in both male and female offspring of BLCO treated dams. Histopathology results also suggest pathological changes in the heart (including fibres fragmentation and disintegration, and loss of striation) and kidney (including atrophic glomeruli, some glomeruli appeared inflamed with occlusion of the Bowman's space, and occasional hemorrhagic reaction.) of the male and female offspring of BLCO treated rats. In conclusion, the result suggests an alteration in renal and cardiac function and increased oxidative stress in these tissues.

Key Word: bonny light crude oil, cardiac, renal, oxidative stress, offspring, environmental pollution

INTRODUCTION

Environmental pollution remains a major public health issue especially in areas where oil exploration has become a daily activity. Study by Ovuru and Ekweozor [1] reported that environmental contaminants like crude oil can affect both terrestrial and aquatic creatures. The fundamental sources of hazard and pollution in the oil producing communities of Nigeria include; oil spillage during production, exploration and discharge from storage facilities and refineries and bursting of pipelines[2].

Crude oil is a complex mixture of various substances, with varying hydrocarbon contents depending on its source of exploration. Bonny light crude oil (BLCO), named after the Bonny Island area on the southern edge of Rivers State in the Niger Delta region of Nigeria is characterized with low sulphur content and low corrosive property[2]. Studies conducted in the past have indicated that animals are susceptible to crude oil and its constituents, particularly Polycyclic Aromatic Hydrocarbons (PAH)[3].

Bonny light Crude Oil is known to contain PAHs and heavy metals that contribute to varying degree of toxicity including carcinogenicity, mutagenicity,

endocrine imbalance and organ dysfunction[4,5]. Another showed that the serum of Wistar rats exposed to BLCO had various level of cadmium, Lead, Chromium, Nickel and Vanadium[6]. There are report that cadmium, Lead, Nickel and Chromium induce various level of toxicity in tissue.

Reports have suggested that crude oil and its constituent hydrocarbon are considered to be a developmental toxicant and as such have the ability to cause adverse effects including pregnancy terminations and malformations in the offspring[7,8]. Nigerians living in Bonny Highland and its environs are unavoidably exposed to BLCO because of their nearness to refineries around and prevalent occurrence of oil pipe leakages in this area. Women around this area also drink and use this oil polluted water domestically during gestation which may result to different complications such as pregnancy terminations and malformations[7].

Recent understanding of fetal origin of adult diseases indicated that abnormal environment during gestation can alter physiological function in the offspring without resulting in obvious structural malformation at birth[9,10]. Epidemiological studies in human and experimental findings from laboratory animals showed that extreme *in utero* environment altered adult phenotype and predispose the individuals to lifelong development of cardio-renal disease and other health problems. Although much study has been conducted on crude oil, very few studies have been aimed to investigate the gestational effects of chronic crude oil exposure in offspring.

The sensitivity of heart as an organ have been emphasized in different research showing its exposure to crude oil leads various heart abnormalities, including diminished cardiac looping and pericardial edema[11]. Additionally, in fish embryos, crude oil has been linked to cardiac dysfunctions such as decreased ventricular contractility, an increased risk of arrhythmias, and variable heart rates[12]. Moreover, there are reports that bonny light crude oil could also induced pathological changes in the kidney and affect indices of renal function[13].

In humans, research have shown that oxidative stress played significant role in the development of cardiovascular diseases such as atherosclerosis, heart failure, myocardial infarction and chronic kidney diseases[14,15]. According to Ola-Mudathir et al.[16], oxidative stress results from an imbalance between the generation of reactive oxygen species and the antioxidant defense system. Base damage and DNA strand breakage are caused by oxidative stress from oxidative metabolism. Reactive oxygen species (ROS) such as superoxide radical (O^{2-}), hydroxyl radical (OH), and hydrogen peroxide (H_2O_2) are the main source of indirect base damage. Damage to DNA is the primary source of most long-term effects of oxidative stress[17].

Thus, the purpose of this study was to see how exposure to BLCO during gestation affected measures of heart and kidney function, as well as oxidative stress in Wistar rats' offspring.

METHODOLOGY

Animals

We used adult male and female Wistar rats (10 weeks old) from the Department of Physiology Animal House, Federal University of Technology, Akure, Nigeria. These rats were not from the same litter. Throughout the study, these animals were housed in cages in the Department of Physiology Animal House and given full access to food and water provided by Ladokun Feeds Limited, Ibadan, Nigeria. The males used for mating were established male breeders, while the females were nulliparous. The whole investigation was conducted with a 12-hour dark–light phase. At the start of mating, the male and female rats weighed 180-200 and 150-170 g, respectively. Animals were given two weeks to acclimate to the laboratory surroundings. The research was carried out in compliance with the 2010 revision of NIH publication/80-23, which contains the International Ethical Norms on Animal Care and Use

Bonny Light Crude Oil (BLCO)

The Nigerian National Petroleum Corporation (NNPC) in Warri, Nigeria, provided BLCO. The treatment group received 0.75ml of BLCO/Kg/day orally every day. The dose is within the previously published range of subacute toxicity levels (0.25-3.5 mL/Kg) in the literature[2,3].

Experimental protocol

We used ten Wistar female rats (10 weeks; 150–170 g) with a regular estrous cycle in total. Every day, the rats' estrous cycle was observed using the methodology outlined by Marcondes *et al.*[18]. Proestrous rats were mated overnight at a 1:1 ratio with a verified male breeder; the next morning, sperm in the vaginal or copulatory plug was used to identify GD 1. Following confirmation of pregnancy, the animals were divided into two groups at random, each consisting of five individuals, and their gestation period was observed appropriately. During gestation days 1 through 21, administration was done orally between the hours of 8 and 10 a.m. Distilled water (0.75 mL/Kg/days) was administered to group 1 while BLCO (0.75 mL BLCO/Kg/day) was administered to

group 2. Six puppies in each litter was the maximum number used. Following delivery, the following parameters were measured: birth weight, PND 90 (at adulthood), and PND 21 (end of weaning). Histopathology and histomorphometry of the heart and kidney, malondialdehyde levels, catalase and superoxide dismutase activities in the homogenate of the heart and kidney were also assessed at PND 90. Serum creatinine kinase level were also assessed as a measure of integrity of the heart muscle. Serum and urine creatinine and urea levels, in addition to creatinine clearance were assessed as measure of kidney functions.

Collection of Urine

Twenty-four hours urine collection was done in the rat's offspring at PND 88 using a specialized metabolic cage. An aliquot was obtained and stored for further examination after the volume of voided urine was recorded.

$$\text{Creatinine Clearance (ml/min)} = \frac{\text{Urine creatinine concentration (mg/dl) X 24 hours Urine Volume (ml)}}{\text{Serum Creatinine concentration (mg/dl) X 1440}}$$

Serum and Tissue collection

Blood samples were taken from the orbital sinus of both male and female offspring while under sodium thiopentone anesthesia (50 mg/kg, i.p.) (PND 90 days). The samples were then put into polythene tubes and allowed to clot for an hour. After that, the blood samples were centrifuged for 10 minutes at 3000 rpm. After being aspirated, serum was kept at 4°C. The integrity of the heart muscle was evaluated by measuring serum creatinine kinase. As a measure of kidney function, creatinine clearance and levels in the serum and urine were measured.

Following the procedure for drawing blood samples, the rats—male and female—were meticulously slain by cervical dislocation. During dissection, the kidney and heart were carefully removed and then cleaned in an ice-cold 1.15% KCl solution. The dry weights of the tissues were recorded. After that, they were minced in 0.1 M potassium phosphate buffer (pH 6.5) with a homogenizer. The homogenate was spun for ten minutes at 10,000 rpm in a cold centrifuge. The supernatant was removed and stored in a refrigerator at around 4°C to assess oxidative stress. The biochemical assay was completed within 48 hours after the sample was collected.

Biochemical Assays

Determination of serum creatinine kinase

Creatinine kinase activities was assessed using commercially available kit (Fortress diagnostic CK kits, United Kingdom) according to the manufacturer's manual. The rate of NADH formation was measured photometrically and was proportional to catalytic concentration of creatinine kinase present in the samples.

Determination of creatinine concentration in serum and urine

Serum and urine creatinine concentration were determined using commercially available kits from Fortress Diagnostics Limited, United Kingdom.

The technique is based on creatinine's capacity to create a deep yellow complex in an alkaline media by reaction with picric acid. The amount of creatinine in the sample directly correlates with the amount of complex that forms.

Creatinine Clearance was calculated according to manufacturer's manual as thus:

Determination of Oxidative Stress (Lipid Peroxidation Assessment)

The thiobarbituric acid reactive substances (TBARS) generated during lipid peroxidation were measured in order to assess lipid peroxidation. This was done using the procedures outlined by Buege and Aust[19].

Determination of Catalase Activity

The Claiborne (1985)[20] approach was used to measure the amount of catalase present. The technique is based on the absorbance loss that occurs when catalase breaks hydrogen peroxide, which is seen at 240 nm. Although hydrogen peroxide doesn't have a maximum absorbance at this wavelength, its absorbance and concentration can be correlated sufficiently for use in a quantitative experiment. The employed extinction coefficient was 0.0436 mM-1cm-1.

Determination of Superoxide Dismutase (SOD) Activity.

The SOD activity was assessed using the Misra and Fridovich (1972) techniques[21]. The SOD assay is based on the process by which, at pH 10.2, superoxide dismutase prevents adrenaline from autooxidizing. It is known that the superoxide anion (O2) generated by the xanthine oxidase enzyme oxidizes adrenaline to adrenochrome. Both the pH and the quantity of adrenaline increased the output of adrenochrome generated per superoxide anion. These gave rise to the theory that SOD could prevent the autooxidation of adrenaline by one of the two possible routes, which involves superoxide radicals and free radical chain reactions.

Determination of tissue protein level

Protein estimation was performed using the Lowry et al. (1951) method[22]. Lowry employed the Folin-Ciocalteau reagent in the measurement of proteins. The reagent identifies tyrosine residues due to their phenolic nature in its most basic state. The Folin reagent reacts with a protein in solution in two stages: reactivity with Cu++ in alkaline medium and reduction of the phosphomolybdic-phosphotungstic reagent by the Cu++ protein complex. The reduced compound produces a blue solution with absorption in the visible red band (600-800 nm).

Histopathology of heart and kidney

Normal paraffin wax embedding procedures were performed on tissues preserved in 10% neutral buffered formalin. Hematoxylin and Eosin (H&E) staining was performed routinely to obtain sections that were 5 µm thick and assessed for histological alterations. The slices were examined using a digital bright field microscope, and a photomicrograph was captured.

Histomorphometry

Heart

Photomicrographs were imported onto Image J software (NIH, USA), for analysis of myocytes nuclei count and wall thickness. For myocyte nuclei count, non-overlapping photomicrographs obtained at x400 magnification were imported unto the Image J software, and count was done using the Image J cell counter tool as previously described[23]. To measure wall thickness, photomicrographs were obtained at x40 magnification for each stained section, and imported unto Image J software. The wall thickness was measured as the length from the inner to the outer layer of the heart wall at three non-overlapping locations using the straight-line tool.

Kidney

Photomicrographs were obtained at x100 magnification and imported unto Image J software. Glomeruli present per field were identified and counted using the Image J cell counter tool as previously described[24].

Statistical analysis

The expression of the data was means ± S.E.M. Two-way ANOVA was utilized for conducting statistical comparisons. Turkey's Post-hoc test was conducted after the ANOVA. When only significant treatment effects were shown by ANOVA, an independent student t-test was utilized. P-values for the differences between the treatment groups showed statistical significance when they were less than 0.05. Version 8 of GraphPad Prism (GraphPad® Inc., CA, USA) was used to analyze the data.

RESULTS

Effects of Bonny light crude oil administered during gestation on Body Weight and Relative Organ Weight of the offspring of Wistar rats.

Statistical analysis of birth weight of the offspring following maternal exposure to BLCO showed no significant effects due to sex and interaction, whereas the treatment effects on birth weight was significant (Table 1). Body weight at weaning showed that sex and interaction effects on body weight were not significant but the treatment effects was also statistically significant. At 1 month of postnatal life, the result showed that sex and interaction effects on body weight were not significant but the treatment effects were found to be significant when compared to the control. Moreover, at 3 month of postnatal life, association between sex and interaction effects on body weight were not significant, as it was seen with the treatment effects that was significant. Additionally, post-hoc analysis showed a significant reduction ($p < 0.0001$) in body weight of the male and female offspring at birth, weaning, 1 month and three months of postnatal life (Table 1).

As shown in Figure 2, statistical analysis of the relative heart weight of the offspring at 3 months postnatal following maternal exposure to BLCO during gestation showed a significant effect across all compared group and parameters: sex, interaction and treatments. In addition, analysis of the relative kidney weight of the offspring at PND 3 months showed a significant effect of sex, interaction and treatments (Figure 2).

The post hoc analysis showed that at 3 months postnatal, the male offspring treated with BLCO had significantly larger relative heart and kidney weight ($p < 0.05$) as compared to the control group. When compared to control female offspring, the female offspring's relative heart and kidney weight showed a substantial decrease ($p < 0.05$) (Figure 1a&b).

Effects of bonny light crude oil administered during gestation on heart and kidney oxidative stress indices of the offspring of Wistar rats

Statistical analysis of the heart MDA level revealed a significant effect in treatment, sex and Interaction (Figure 2a). Moreover, renal MDA level was statistically affected by sex, treatment and interaction (Figure 2b). The Post hoc test showed that there was a

significant increase ($p < 0.05$) in the heart and kidney malondialdehyde level in both male and female offspring of treatment group when compared with the control (Figure 2a&b).

As shown in figure 3, the analysis revealed a non-significant effect of Catalase activities on the heart of the treatment group. However, a statistically significant effects was seen between sex and Interaction. Moreover, renal catalase activities were statistically affected by sex, treatment and interaction as shown in Figure 3b.

Additionally, post hoc analysis revealed that, in comparison to the control group, the male offspring's heart and kidney had significantly higher catalase activity. Nonetheless, the t-test analysis (figures 3a&b) revealed a substantial decrease ($p < 0.05$) in the catalase activity in the kidney of the female offspring of the treatment group when compared to that of the control. Analysis of SOD activities on the heart showed that interaction and treatment does not significantly affect the SOD activities. However, effects due to sex was statistically significant (Figure 4a). In addition, renal SOD activities were statistically affected by sex, treatment and interaction (Figure 4b).

Post hoc analysis demonstrated that, in comparison to the control group, the increase in SOD activity in the kidney of male offspring in the treatment group was statistically significant ($p < 0.05$). Nevertheless, when compared to the control, the female offspring of treated rats had significantly lower renal SOD activity ($p < 0.05$), according to an independent t-test analysis (Figure 4a&b).

In addition, the result showed that there was no significant difference in the protein level of the kidney of male and female offspring. Analysis of the heart protein revealed that, the total tissue protein was significantly affected by sex, treatment and interaction. However, post hoc analysis showed that only the protein level in the heart of the female offspring of the treated group was significantly reduced when compared with the control group (Table 2).

Effects of bonny light crude oil administered during gestation on creatinine kinase activities of the offspring of Wistar rats

Analysis of Creatinine kinase activities showed that interaction and sex does not significantly affect the creatinine kinase activities of the tissue. However, creatinine kinase activity was significantly affected by treatment (Figure 5). Post hoc analysis revealed that, the serum creatinine kinase activity was significantly ($p < 0.05$) higher in the male and female offspring when compared with the control (Figure 5).

Effects of bonny light crude oil administered during gestation on serum and urine creatinine concentration and creatinine clearance in the offspring of Wistar rats

Serum Creatinine concentration was significantly affected by interaction, sex and treatment. Post doc analysis, however showed that, the serum creatinine level was significantly raised in the treatment group compared to that of control group in the male offspring. There was no statistically significant difference in the serum creatinine levels between the control and treatment groups in the female offspring (Table 3).

In addition, Urine Creatinine concentration was significantly affected by interaction, sex and treatment. Moreover, Post hoc analysis further showed that Urine creatinine concentration was significantly higher in treatment group compared when compared to that of control group in both male and female offspring (Table 3).

Creatinine clearance level was significantly affected by interaction, sex and treatment. When comparing the male offspring of the treatment group to the control group, post hoc analysis revealed that there was no statistically significant difference in the mean creatinine clearance level. In contrast to the control group, the treatment group's female offspring's creatinine clearance level was significantly ($p < 0.05$) lower (Table 4).

Effects of bonny light crude oil administered during gestation on histopathology and histomorphometry of the heart and kidney of the offspring of Wistar rats.

The normal histology of myocytes (cardiac muscle cells) was observed in control rats. Myocytes are seen to contain one or two nuclei and widespread eosinophilic cytoplasm. Numerous endothelia nuclei are seen in the capillary walls between myocytes. In longitudinal section, myocytes are well arranged in parallel orientation, with elongated appearance of the nuclei. In the transverse section, the mesh-like appeared of network of capillaries between myocytes was obvious (Figure 6). Treatment with BLCO induced marked degenerative changes in myocytes, including fibre fragmentation and disintegration, and loss of striation. Complete necrosis of myocytes was also observed accompanied with inflammatory infiltration in tissue interstitial (Figure 6).

In addition, control rats showed mostly normal histology of the renal cortex; glomeruli appeared

normal with clearly delineated Bowman's space, and mostly intact tubules (Figure 7). BLCO-treated rats showed histopathological alterations including atrophic glomeruli. Also, some glomeruli appeared inflamed with occlusion of the Bowman's space, and occasional hemorrhagic reaction. Furthermore, occasional appearance of densely eosin-stained ('colloid') cast in renal tubules were observed (Figure 7).

Moreover, histomorphometry of the heart suggests a significant effect of interaction and sex. But treatment group have no effects on the cardio-myocyte length. In addition, there was also no significant effects in the treatment and sex on the glomeruli cell count. Meanwhile, the result suggests a significant effect due to interaction (Table 3). In addition, post hoc analysis of histomorphometry of the kidney showed a significant ($P < 0.05$) increase in the glomeruli counts in the male and female offspring of the treated group when compared with control (Table 3).

Table 1: Effect of Bonny light crude oil administration during gestation on Body Weight of the Male and Female Offspring

TREATMENTS	BIRTH WEIGHT (g)	WEIGHT AT WEANING (g)	WEIGHT AT 1 MONTH PND (g)	WEIGHT AT 3 MONTH PND (g)
CONTROL				
Male	7.194±0.170	30.319±0.337	41.405±1.204	183.409±2.135
Female	7.182±0.416	32.129±1.436	43.214±1.360	183.409±1.32
BLCO GROUP				
Male	5.364±0.113*	20.575±0.716*	34.90±0.900*	165.80±2.071*
Female	5.263±0.332*	21.423±1.651*	36.210±0.351*	167.919±1.322*

N=5, *P < 0.05 = significantly different from control

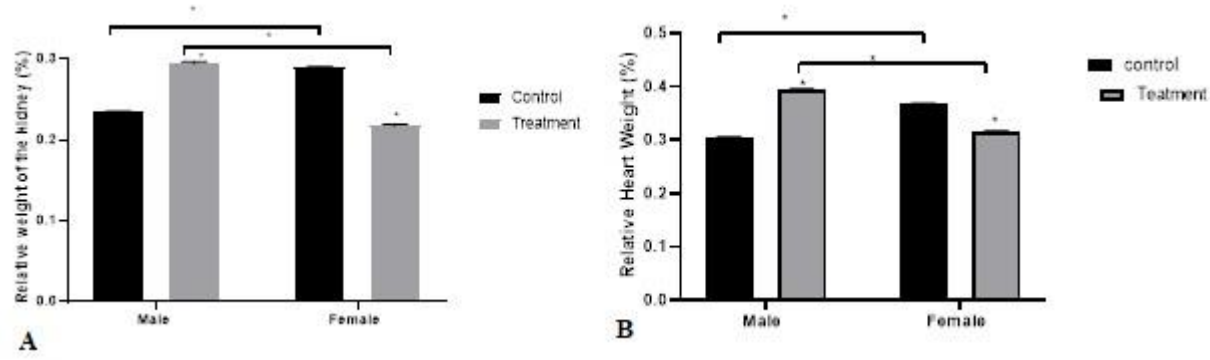

Figure 1: Effect of Bonny light crude oil administration during gestation on (A) relative kidney weight and (B) relative heart weight of the male and female offspring; *p < 0.05 = significantly different from control

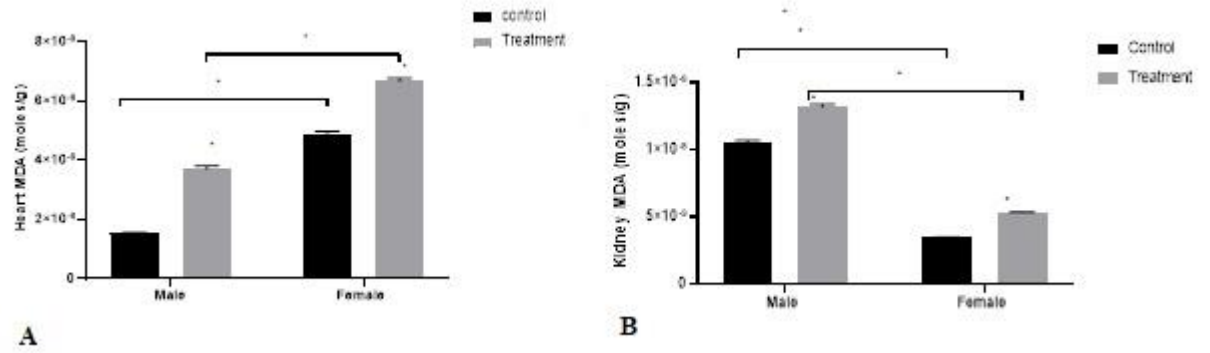

Figure 2: Effect of Bonny light crude oil administration during gestation malondialdehyde (MDA) level in (A) heart and (B) kidney of the male and female offspring; *p < 0.05 = significantly different from control.

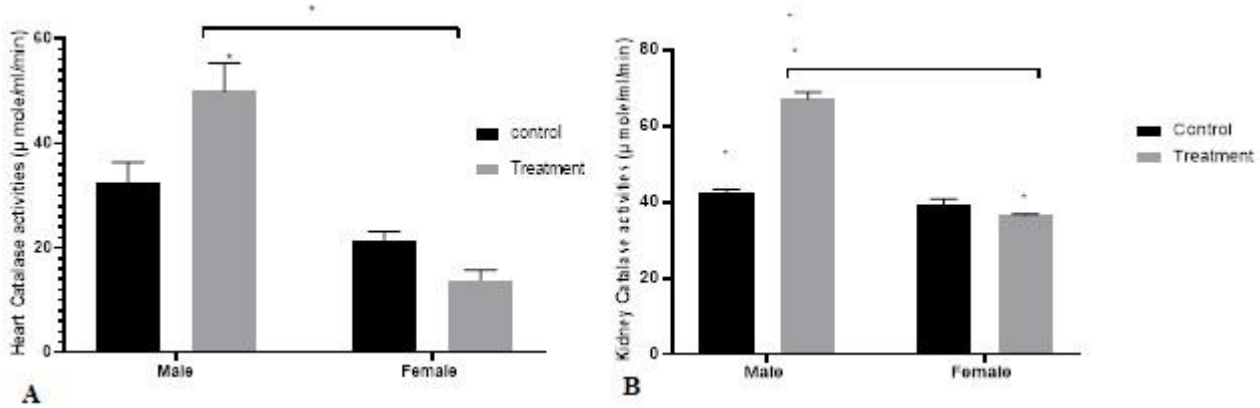

Figure 3: Effect of Bonny light crude oil administration during gestation on catalase activities in (A) heart and (B) kidney of the male and female offspring; *p < 0.05 = significantly different from control**.**

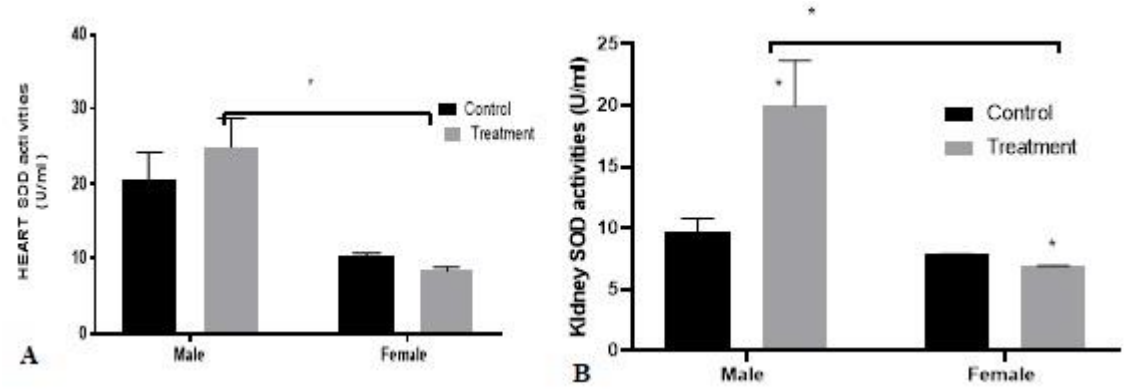

Figure 4: Effect of Bonny light crude oil administration during gestation on superoxide dismutase (SOD) activities in (A) heart and (B) kidney of male and female offspring; *p < 0.05 = significantly different from control

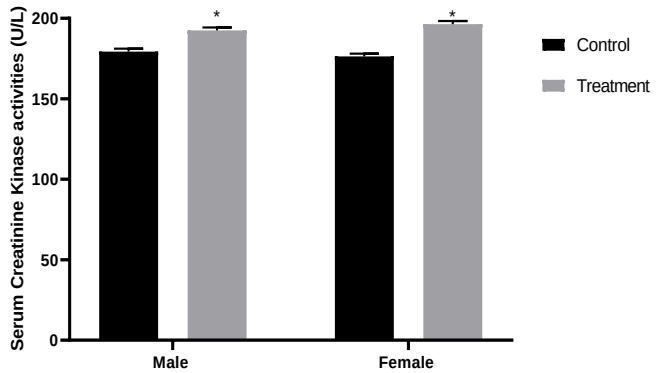

Figure 5: Effect of Bonny light crude oil administration during gestation on serum creatinine kinase activities of the male and female offspring; *p < 0.05 = significantly different from control

Table 2: Effect of Bonny light crude oil administration during gestation on heart and kidney protein of the male and female offspring

TREATMENTS	MALE OFFSPRING		FEMALE OFFSPRING	
	Protein KIDNEY (mg/dl)	Protein Heart (mg/dl)	Protein KIDNEY (mg/dl)	Protein Heart (mg/dl)
CONTROL	21.567±2.187	10.939±1.912	25.295±1.187	61.408±7.572
BLCO GROUP	26.426±2.326	8.745±1.215	28.773±1.356	37.968±3.043*

N=5, *P < 0.05 = significantly different from control

Table 3: Effect of Bonny light crude oil administration during gestation on histomorphometry of the heart and kidney of the male and female offspring

TREATMENTS	MALE OFFSPRING			FEMALE OFFSPRING		
	Glomeruli Count	Cardio-Myocyte Count	Cardio-Myocyte Walls Thickness	Glomeruli Count	Cardio-Myocyte Count	Cardio-Myocyte Walls Thickness
CONTROL	10.50±0.644	26.38±3.285	961.95±56.531	13.33±0.773	25.25±1.201	2010±143.760
BLCO GROUP	14.0±0.649*	24.00±2355	1614±70.782*	10.313±0.717*	28.83±2.266	1630±68.768*

N=5, *P < 0.05 = significantly different from control

Table 4: Effect of Bonny light crude oil administration during gestation on renal function indices in the male and female Offspring

TREATMENTS	MALE OFFSPRING			FEMALE OFFSPRING		
	Serum Creatinine (mg/dl)	Urine Creatinine (mg/dl)	Creatinine Clearance (mg/dl)	Serum Creatinine (mg/dl)	Urine Creatinine (mg/dl)	Creatinine Clearance (mg/dl)
CONTROL	1.382±0.243	73.353±1.573	0.099±0.005	0.468±0.051	49.650±1.325	0.290±0.0002
BLCO GROUP	2.103±0.101*	82.138±1.513*	0.127±0.026	0.482±0.030	10.786±1.112*	0.010±0.0001*

n=5, *p < 0.01 = significantly different from control

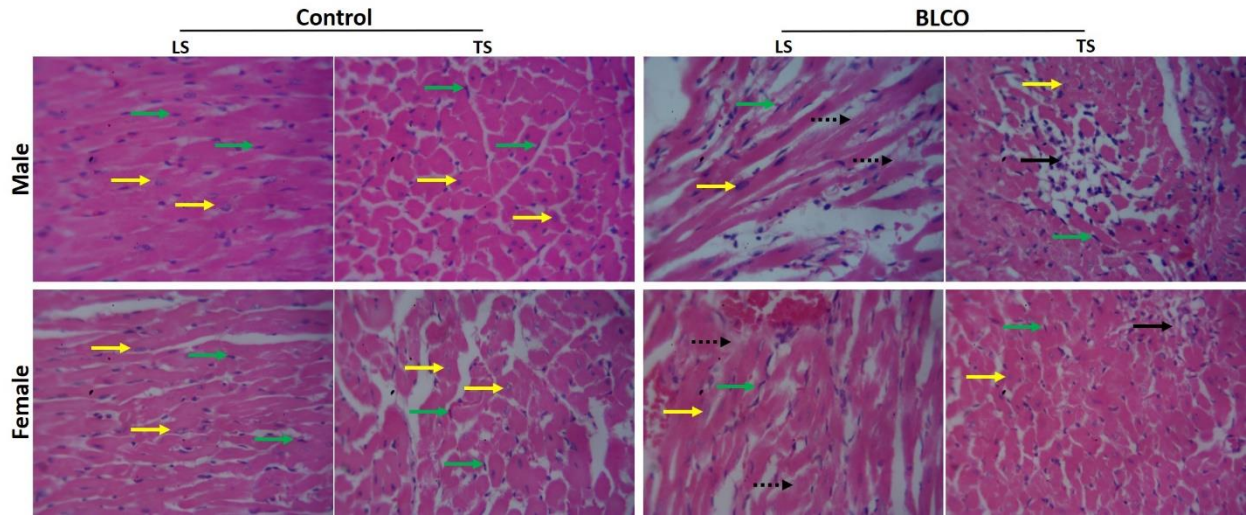

Figure 6: Histology of cardiac muscle (myocardium) of control and BLCO-treated rats. H&E x 400. LS – longitudinal section; TS – transverse section; Myocyte nuclei – yellow arrows; endothelial cell nuclei – green arrows; myocyte degenerative features – black dashed arrows; inflammatory infiltration – black arrows.

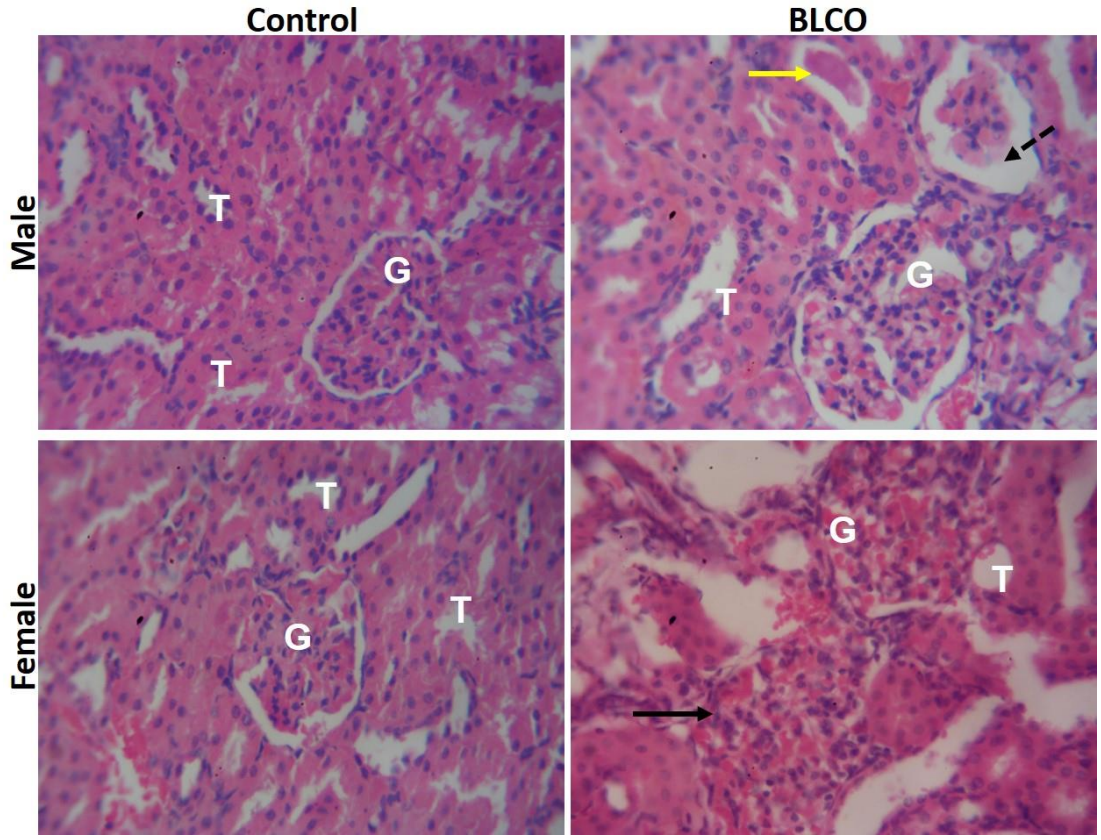

Figure 7: Renal cortex of control and BLCO-treated rats. H&E x 400. G – glomerulus; T – tubules. In stressed group several glomeruli appear atrophic (dashed arrows), and also show inflammation (arrow). Also, observe occasional appearance of eosin-stained ('colloid') cast in renal tubules (yellow arrow).

Discussion

In this study, the effects of maternal exposure to BLCO during gestation on cardiac and renal function biomarkers and oxidative stress in the offspring was investigated. Studies into the toxicity effects of BLCO have focused on direct exposure[2,3], however little attention has been paid to indirect effects on the offspring following prenatal exposure. This study's findings demonstrated that BLCO exposure to mothers during pregnancy dramatically lowered the birth weight and subsequent growth rate of both male and female offspring. It is well reported in literature that somatic growth depends on several factors including nutritional, metabolic and endocrine factors[25,26,27]. Although, the extent to which these factors have been altered in the offspring is not known. It has been reported in literature, that BLCO at a dose of 3.5ml/Kg body weight could become anti-androgenic[2]. It has been previously reported that, endocrine disruptors with anti-androgenic properties could reduce birth weight in Wistar rats[28]. In addition, hepatic toxicity (a major metabolic organ) effects of BLCO have been reported in literature[3]. If these factors (endocrine disruption and altered hepatic function) are responsible for the reduced somatic growth remained to be identified.

In this study, it was observed that the serum creatinine kinase activities were significantly raised in the male and female offspring. Creatinine kinase is an important enzyme used in the assessment of cardiac damage[29]. Histomorphometry examination revealed an increased in the wall thickness in the male offspring compared to the control. Increase in wall thickness may suggest certain level of hypertrophy which may result in architectural changes in cardiomyocytes.

The results of this study also demonstrates that the female offspring's creatinine clearance was lowered when exposed to BLCO during gestation. The male offspring's creatinine clearance did not differ substantially from the control group. The kidneys' role in the bloodstream includes the removal of several compounds, such as creatinine[30]. Reduced creatinine clearance may be associated with several factors associated with reduced functionality of the nephron due to damage or reduced number in the kidney[30]. Histomorphometry analysis results in this study suggests a reduced number of glomerulus in the female offspring following prenatal treatment with

BLCO. In agreement with this observation, the male offspring that exhibited increased number glomerulus cleared creatinine better. The creatinine clearance in the male offspring was not significantly different from the control despite significant higher level of serum creatinine level.

Histopathology of the kidney and the heart showed some degree of atrophic changes in glomeruli and cardiomyocytes. There are also marked architectural changes in the cardiomyocytes. These alterations may be secondary to increased oxidative stress. According to Shafer *et al*.,[31] and Ola-Mudathir *et al*.[16], oxidative stress results from an imbalance between the production of reactive oxygen species and endogenous antioxidants. It has a critical role to play in the development of so many non-communicable diseases[32] including disorders of the cardio-renal systems.

Rats that were exposed to free radicals developed a number of defense mechanisms, including catalase and superoxide dismutase (SOD), which are examples of enzymatic antioxidant defense. According to Hayyan et al.[33], superoxide dismutase is an enzyme that catalyzes the dismutation (or partitioning) of the superoxide (O^{2-}) radical into hydrogen peroxide (H_2O_2) or regular molecular oxygen (O_2). As a byproduct of oxygen metabolism, superoxide can damage cells in a variety of ways if it is not controlled[33]. Additionally, hydrogen peroxide is also harmful and is broken down by other enzymes like catalase[34]. Therefore, SOD is an essential antioxidant defense that is found in nearly all living cells that come into contact with oxygen. Catalase converts hydrogen peroxide into oxygen and water. It is an essential enzyme that protects the cell from oxidative damage brought on by reactive oxygen species (ROS). Catalase is an enzyme that has one of the highest turnover rates of any other enzyme; in a single second, it may convert millions of hydrogen peroxide molecules into water and oxygen[35]. Free radicals cause lipid peroxidation, which is crucial in pathogenic processes. Malondialdehyde, which plays a vital role in showing oxidative stress, can be used to quantify the damage caused by free radicals. It has been identified as the primary product for evaluating lipid peroxidation. Overproduction of MDA is caused by an increase in free radicals[36].

Results from this study show that male offspring of treatment rats had significant activities of Catalase, SOD and MDA levels in the heart and kidney homogenates. Increase MDA level suggests possible presence of increased lipid peroxidation in this tissue. However, there is an increase level of antioxidant enzymes SOD and catalase that could savage the situation by reducing the extent of the lipid peroxidation. Conversely, in the female offspring, the increase heart and kidney MDA levels was associated with reduced antioxidant enzymes SOD and Catalase. These confirm possible increase in oxidative stress in these tissues. The reason for sex dependent response of the offspring to maternal BLCO exposure during gestation is unknown, however, this kind of situation is not uncommon in developmental programming. It was clear from this study, that despite the difference in the level of antioxidant enzymes between male and female offspring, the extent of lipid peroxidation in the heart and kidney of both sexes were higher than their respective control. It can therefore be suggested that BLCO increased oxidative stress in the male and female offspring, but the male offspring may exhibit high level of ability to protect its tissues against reactive oxygen species that induced oxidative stress.

The presence of PAHS and heavy metals (such as Cadmium, Lead, Nickel, Vanidium, and Chromium) in BLCO has been documented in the literature[6]. These substances have been separately linked to the production of reactive oxygen species and the induction of oxidative stress. Nickel-induced neurotoxicity in Wistar rats, according to Ijomone *et al*.,[23] is connected with increased oxidative stress in brain tissue. Cadmium has also been linked to oxidative stress in the literature[16].

It has been shown that the cytochrome P-450-linked polysubstrate monooxygenase system converts the hydrocarbon molecules in crude oil through metabolic processes to highly reactive and carcinogenic chemicals[37,38].

In addition, Dichloromethane which is one of the constituents of BLCO[39] has been reported by Owumi and Najophe[40] to be able to inhibit to an extent the defensive activities of antioxidants in protecting animal's tissues against cellular oxidative damage which may explain increased level of MDA as a result of lipid peroxidation.

In conclusion, the results from this study suggest an alteration in renal and cardiac function and increased oxidative stress in the offspring following maternal exposure to BLCO during gestation. The extent of the damage due to oxidative stress may be more pronounced. The oxidative stress may be associated with heavy metal and PAH contained in the BLCO.

Acknowledgement: The authors acknowledge the technical assistance of Mr. Seyi Olabanji Aleruwa and Mr. Ismail Akanni Oguntade, and Ms Comfort Okoh, of the Department of Physiology and Neuro- Lab, respectively.

STATEMENT AND DECLARATIONS

Data Availability:

The datasets generated during and analyzed during the current study are available from the corresponding author

Animal Research (Ethics):

The study was conducted in accordance with the International Ethical Norms on Animal Care and Use as contained in NIH publication/80-23, revised in 2010 and Approved by Federal University of Technology, Akure, Ethical Committee.

Author Contribution:

Author (Sikirullai Olatunde Jeje and Michael Adenawoola) contributed to the study conception and design. Material preparation, data collection and analysis were performed by (Sikirullai Olatunde Jeje), (Michael Adenawoola) and (Fausat Kikelomo Ola-Mudathir) and (Omamuyovwi M. Ijomone) The first draft of the manuscript was written by (Sikirullai Olatunde Jeje) and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest: The authors does not have any conflict of interest to declare

Funding: The authors declared that no funding was received during the preparation of this manuscript

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