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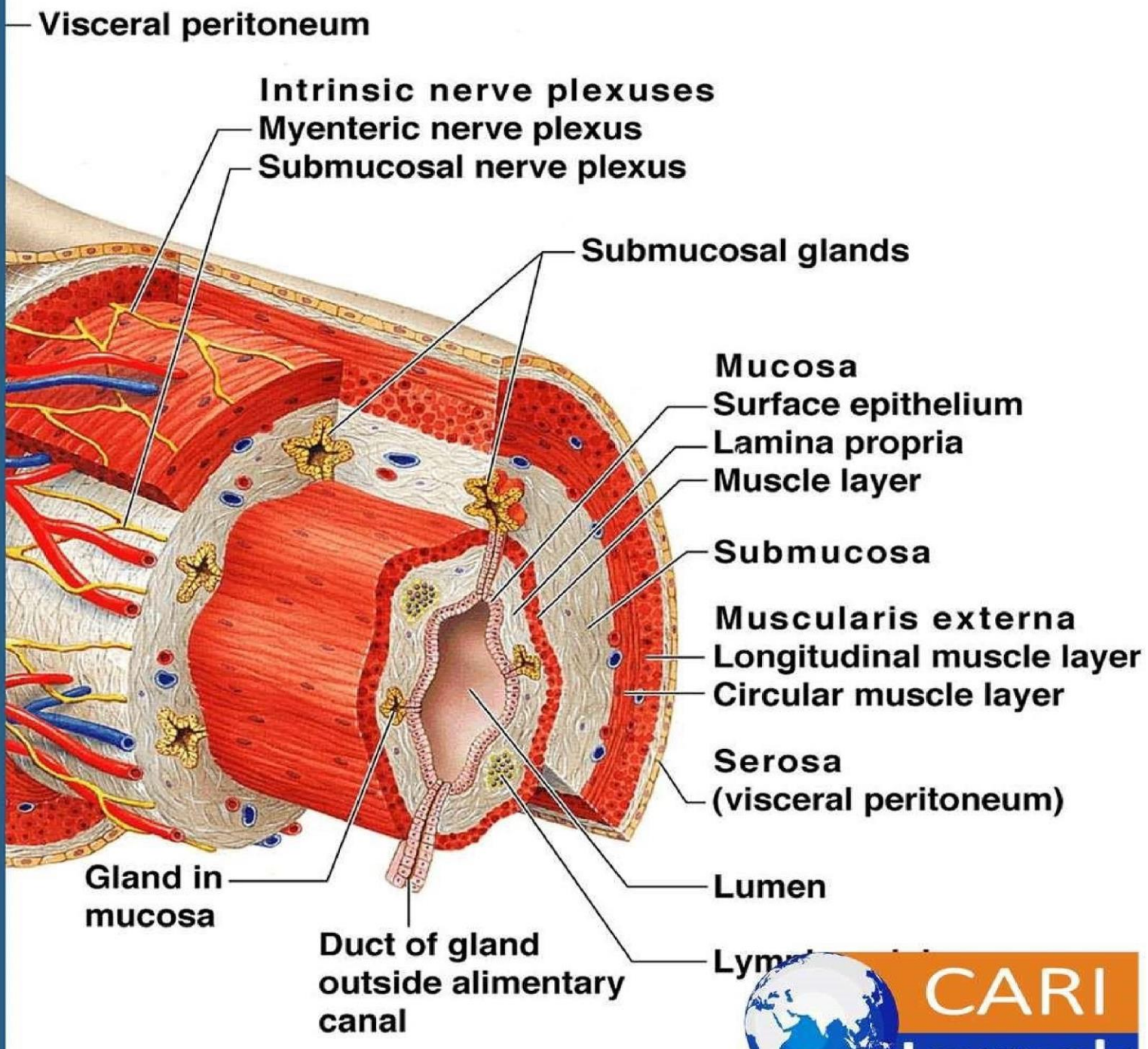

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Biochemical investigations of Albino rats orally exposed to Bonny light crude oil and leaf extract of *Cnidoscolus aconitifolius*

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Abstract:

Purpose: Bonny light crude oil has been used in folkloric medicine in treating different diseases in combination with Medicinal plants. *Cnidoscolus aconitifolius* has been reported to possess medicinal properties. This study is aimed at determining experimentally if the acclaimed benefits are true.

Methodology: This study investigated the biochemical effect of Bonny light crude oil and *Cnidoscolus aconitifolius* leaf extract orally administered to albino rats at different concentrations. In this study, albino rats were orally exposed to different concentrations of Bonny light oil (BLCO) and *Cnidoscolus aconitifolius* leaf extract individually and in combination.

Findings: The results after 21days of exposure revealed that BLCO and *Cnidoscolus aconitifolius* induction at concentrations of 250mg/kgb.wt and 500mg/kgb.wt could induce physiological damage within 21days. This is revealed by the results obtained from the histological analysis of the testes and the detectable heavy metal concentration in the blood samples.

Unique Contribution to Theory, Policy and Practice: As a result, the induction of this leaf extract and BLCO at concentrations of 250mgf/kgb.wt and 500mg/kgb.wt consecutively for 21days may be injurious to health as revealed by the results from the histological analysis. Therefore, it is recommended that the use of this plant leaf extract and BLCO in folkloric medicine at concentrations similar to the ones used in this research should be avoided.

Keywords: *Folkloric, Crude Oil, Leaf Extract, Testis.*

1.0.Introduction

Oil spills have the potential to cause immediate and widespread toxicity to the health and environment depending on the level of exposure [1]. It could directly or indirectly introduce crude oil onto the surface of water bodies, thereby creating serious impact on marine life. In folklore medicine, crude oil is customarily used by native people to manage gastrointestinal problems and male reproductive capacity [2]. It is also used in combination with olive oil to treat burns, ulcers, and witchcraft attacks and poisoning. In Nigeria, it is commonly used as anti-convulsant [3].

Crude oil has been reported to be responsible for oxidative stress [4] which may lead to various diseases like cancer, cardiac dysfunction, blood disorders, hepatic abnormalities, nephrotoxic effects [5]. The toxicity depends on various factors, including the oil composition and characteristics (physical and chemical), condition (i.e. weathered or not), exposure routes and regimen, and the bioavailability of the oil. If the levels of the additive toxic effect of hydrocarbons exceed the threshold concentration, mortality could ensue [6], since the metabolites of polycyclic aromatic hydrocarbons (PAHs) and aliphatic hydrocarbons are highly toxic and carcinogenic [7]. Particularly, PAHs are the principal contributors to toxicity, with different metabolic pathways producing metabolites that possess the ability to attack and bind to DNA and proteins [6].

Some studies have been able to document the use of plants and its products in managing petroleum induced toxicity. These studies include *Monodora myristica* [8], *Gongronema latifolium* [9] and *Chromolaena odorata* [10]. *Cnidoscolus aconitifolius* commonly called chaya is a leafy perennial shrub that is believed to have originated in Yucantan peninsula of Mexico [11]. This plant is commonly called “Hospital too far” in the Niger Delta region in Nigeria and “Efo Jerusalem” in the south-western region of Nigeria. In the folk medicine *C. aconitifolius* leaves has been used for the treatment of malaria, Jaundice, intestinal worm etc. Other studies have reported the role of this plant in maintaining blood sugar level, anti-inflammtory, anti-anemic and antioxidant effect of this plant [11],[12],[13]. Even though studies on the adverse effects on crude oil is on-going, only a few studies have been carried out on the ability of *C. aconitifolius* to mitigate the adverse effects of crude oil exposed animals. Thus, this study helps in determining the possible effects of *C. aconitifolius* on crude oil induced toxicity in experimental albino rats.

2.0 Materials and Methods

Twenty-eight Wistar albino rats were obtained from the animal house of the Department of Pharmacology, University of Port Harcourt, Rivers State, Nigeria. Animals were acclimated for one week to laboratory conditions at Biochemistry Department animal house, and given food (growers mash) and water *ad libitum.*

2.1 Chemicals and Reagents

All chemicals and reagents used throughout the study were of analytical grade.

2.2 Crude oil Sample

The test sample of the Bonny light crude oil was obtained from the Port Harcourt Refining Company (PHRC) Limited, Alesa Eleme, Port Harcourt Rivers State, Nigeria.

2.3 Plant Collection and Extraction

Leaves of *Cnidoscolus aconitifolius* were collected from a garden at Aboh, Amaokpara in Nkwerre Local Governement Area of Imo State, Nigeria. The leaves were thoroughly washed with clean running water to remove unwanted materials and dirt. *C. aconitifolius* leaves were authenticated by the Department of Plant Science and Biotechnology, University of Port Harcourt, and assigned the herbarium number of UPH/P/264. Preparation of the *C. aconitifolius* extracts was done using water and ethanol as solvent extractors using the method described by [10]. The plant leaves were air dried in open air at room temperature for 2 days and further dried in an oven at 45^0C for 48 hours to obtain a constant weight. The dried leaves were pulverized to fine powder using electric blender [10]. After blending, the ethanol extraction was carried out using the method described by [10] with slight modification. In using this method, 131 g of the powdered *A. paniculata* was soaked in 1182ml of 80 % (v/v) ethanol and allowed to stand for 24 hours. The extraction mixture was filtered with cheese cloth and the filtrate was concentrated using a rotary evaporator at 45^0C. Further dryness was achieved using a water bath. From the dried sample extract (crude extract), 1g and 2 g of the extract were separately dissolved in 4ml of distilled water to bring the concentrations to 250 mg/ml and 500 mg/mL, respectively.

2.4 Experimental Design

Animals weighing between 90 and 150g were divided into seven groups of four male rats each. Group 1, the negative control, received neither the Bonny light crude oil (BCLO) nor the leaf extract. Groups 2 and 3 received 250 mg/kg bw and 500 mg/kg bw of BLCO, respectively, while Groups 4 and 5 received 250 mg/kg bw and 500 mg/kg bw of *C. aconitifolius* leaf extracts, respectively. Groups 6 received 250 mg/kg bw of BLCO + 250 mg/kg bw of *C. aconitifolius* leaf extract, while Group 7 received 500 mg/kg bw of BLCO + 500 mg/kg bw of *C. aconitifolius* leaf extract. These doses were based on that used by the local population in folklore medicine [10] and administered daily. All animals were given food and water *ad libitum*. The body weight of the rats was recorded daily.

At the end of the 21 days exposure period, the animals were weighed and sacrificed under chloroform anesthesia. The testes were excised, weighed, and fixed in Bouin's fluid for at least 48 h. They were processed in an automatic processor, and embedded in paraffin wax. Sections 5µm thick were examined and photographed using Lietz light microscope [10].

Blood samples obtained from the jugular vein and placed in EDTA container were used for heavy metals analyses. The levels of the heavy metals (Pb, Cd, As and Hg) in the filtrate from each digested sample was determined with the aid of atomic absorption spectrophotometer Sens AA [14].

Blood samples drawn from the jugular vein, placed in a plane container was used for the measurement of biochemical parameters. Antioxidant capacity of GSH was estimated according to the method of [15]. SOD was, determined by the method of [16] while the [17] method was used for the determination of CAT. The lipid (Cholesterol, HDL, TG and LDL) profiles were determined using the Spectrum kit manufactured by the Egyptian Company for Biotechnology, Cairo, Egypt.

Statistical Analysis

Values were reported as Mean ± SEM. The least significant difference was used to test for differences between individual treatments groups, and the difference in the body weight of the rats over the treatment period using Statistical Package for Social Sciences (SPSS) version 22.0.

3.0 Results:

The oxidative stress enzymes Glutathione (GSH), catalase (CAT), superoxide dismutase (SOD)and Malonaldehyde (MDA) of Wistar rats orally exposed to BLCO and CA leaf extract are presented in Table 1. The SOD enzyme of the control group (0.24 ± 0.05 %) was significantly ($p<0.05$) lower when compared to 250mg/kgb.wt each of CA (0.55 ± 0.01 %), and 500mg/kgb.wt CA (0.52 ± 0.06 %). Similarly, 250mg/kgb.wt CA was significantly ($p<0.05$) higher when compared to 250mg/kgb.wt BLCO + 250mg/kgb.wt CA (0.25 ± 0.03). There was no significant ($p<0.05$) difference between 250mg/kgb.wt CA and 500mg/kgb.wt CA. The MDA of the control (0.62 ±0.02 %) was significantly ($p<0.05$) higher when compared to 250mg/kgb.wt each of CA (0.32 ± 0.01 %) , 500mg/kgb.wt CA (0.35 ± 0.08 %), 250mg/kgb.wt BLCO + 250mg/kgb.wt CA (0.43 ± 0.10 %) , 500mg/kgb.wt BLCO + 500mg/kgb.wt CA (0.56 ± 0.03 %). However, no significant ($p<0.05$) exists between 250mg/kgb.wt BLCO (0.53 ± 0.05 %), 250mg/kgb.wt CA and 250mg/kgb.wt BLCO +250mg/kgb.wt CA and 500mg/kgb.wt BLCO (0.53 ± 0.05 %), 500mg/kgb.wt CA and 500mg/kgb.wt BLCO + 500mg/kgb.wt CA. Also, no significant ($p<0.05$) difference exists between 250mg/kgb.wt CA and 500mg/kgb.wt CA.

Heavy metals: The blood heavy metal concentration of rats orally exposed to BLCO and CA are presented in Table 2.The parameters analyzed includes Lead (Pb), Cadmium (Cd), Arsenic (AS) and Mercury (Hg).The lead (Pb) concentration of the control group (0.75 ± 0.08 %) was significantly ($p< 0.05$) lower when compared to 250mg/kgb.wt BLCO (1.32 ±0.04 %)and 500mg/kgb.wt BLCO (1.87 ± 0.14 %) groups. Also, the 250mg/kgb.wt BLCO and 500mg/kgb.wt BLCO were significantly ($p<0.05$) higher when compared to 250mg/kgb.wt BLCO + 250mg/kgb.wt CA (0.55 ± 0.02) and 500mg/kgb.wt BLCO + 500mg/kgb.wt CA (0.56 ± 0.05). There was no significant ($p<0.05$) between 250mg/kgb.wt CA (0.53 ± 0.07 %), 500mg/kgb.wt CA (0.56 ±0.05 %)and 250mg/kgb.wt BLCO + 250mg/kgb.wt CA, 500mg/kgb.wt BLCO + 500mg/kgb.wt CA. However, the Cadmium (Cd) concentration in the control group (0.33 ± 0.02 %) was significantly ($p<0.05$) higher when compared to 250mg/kgb.wt BLCO + 250mg/kgb.wt CA (0.15 ± 0.02 %), 500mg/kgb.wt BLCO + 500mg/kgb.wt CA (0.02 ± 0.02 %). Also, the control

group was significantly ($p<0.05$) lower when compared to 250mg/kgb.wt BLCO (0.97 ± 0.10 %), 500mg/kgb.wt BLCO (1.16 ±0.10 %) and 500mg/kgb.wt CA (0.63 ±0.11 %). However, the concentration of 250mg/kgb.wt BLCO and 500mg/kgb.wt BLCO were significantly ($p<0.05$) higher when compared to 250mg/kgb.wt BLCO + 250mg/kgb.wt CA (0.15 ± 0.02) and 500mg/kgb.wt BLCO + 500mg/kgb.wt CA. The 250mg/kgb.wt CA (0.41 ± 0.10) was significantly ($p<0.05$) lower when compared to 500mg/kgb.wt CA. However, the As of the control group (0.17 ± 0.01 %) was significantly ($p<0.05$) lower when compared to 250mg/kgb.wt BLCO (0.64 ± 0.01 %), 500mg/kgb.wt BLCO (0.88 ±0.02 %), 250mg/kgb.wt CA (0.55 ± 0.03 %) and 500mg/kgb.wt CA (0.54 ± 0.01 %), 250mg/kgb.wt BLCO + 250mg/kgb.wt CA (0.69 ± 0.07 %), 500mg/kgb.wt BLCO + 500mg/kgb.wt CA (0.70 ± 0.04 %). Also, the 500mg/kgb.wt BLCO had a significantly ($p<0.05$) higher value when compared to 500mg/kgb.wt BLCO+ 500mg/kgb.wt CA. Also, the Hg of the control group (0.02 ± 0.01 %) had a significantly ($p<0.05$) lower value when compared with 500mg/kgb.wt BLCO (0.06 ± 0.01 %).

Lipid Profile: The lipid profile parameters Total cholesterol (TC), High density lipoprotein (HDL), Triglyceride (TG) and low density lipoprotein (LDL) of Wistar rats orally exposed to BLCO and CA leaf extracts are presented in Table 3. The HDL concentration of 250mg/kgb.wt BLCO (62.51 ±1.93 %) was significantly ($p<0.05$) higher when compared to 250mg/kgb.wt BLCO + 250mg/kgb.wt CA (55.67 ±1.16 %). Also 500mg/kgb.wt BLCO (62.32 ±1.93 %) was significantly ($p<0.05$) higher when compared to 500mg/kgb.wt BLCO + 500mg/kgb.wt CA (55.48 ±0.50 %).

Table 1: Oxidative stress enzymes of Wistar rats orally exposed to Bonny light crude oil and *C. aconitifolius* leaf extract

Groups	GSH	CAT	SOD	MDA
Control	1.43 ± 0.35^{a}	4.54 ± 0.94^{a}	0.24 ± 0.05^{a}	0.62 ± 0.02^{a}
BLCO (250 mg/kg b.wt.)	0.97 ± 0.28^{a}	4.18 ± 0.56^{a}	0.22 ± 0.11^{a}	0.61 ± 0.04^{a}
BLCO (500 mg/kg b.wt.)	1.29 ± 0.35^{a}	5.13 ± 0.88^{a}	$0.33\pm0.09^{a,b}$	$0.53\pm0.05^{a,b}$
CA (250 mg/kg b.wt.)	1.38 ± 0.37^{a}	4.92 ± 0.84^{a}	0.55 ± 0.01^{c}	0.32 ± 0.01^{b}
CA (500 mg/kg b.wt.)	1.20 ± 0.44^{a}	5.53 ± 0.79^{a}	$0.52\pm0.06^{b,c}$	$0.35\pm0.08^{b,c}$

BLCO (250 mg/kg b.wt.) + (CA 250 mg/kg b.wt.)	1.07±0.20[a]	6.18±0.46[a]	0.25±0.03[a]	0.43±0.10[a,b]
BLCO (500 mg/kg b.wt.) + CA (500 mg/kg b.wt.)	1.19±0.15[a]	5.50±0.89[a]	0.23±0.04[a]	0.56±0.03[a,c]

Values are reported as mean ± SEM of triplicate determination. Values with different superscript alphabets are significantly different at $p<0.05$. The Least Significant Difference (LSD) was used to test for the difference between individual treatment groups using Statistical Package for Social Sciences (SPSS), version 22.0. BLCO = Bonny light crude oil; CA = *Cnidoscolus aconitifolius*; mg/kg b.wt. = milligram per kilogram body weight.

Table 2: Heavy metal concentration of Wistar rats administered with Bonny light crude oil and *C. aconitifolius* leaf extract

GROUP	Pb	Cd	As	Hg
Control	0.75±0.08[a]	0.33±0.02[a]	0.17±0.01[a]	0.02±0.01[a]
BLCO (250 mg/kg b.wt.)	1.32±0.04[b]	0.97±0.10[b]	0.64±0.01[b,e]	0.05±0.01[a,b]
BLCO (500 mg/kg b.wt.)	1.87±0.14[c]	1.16±0.10[c]	0.88±0.02[c]	0.06±0.01[b]
CA (250 mg/kg b.wt.)	0.53±0.07[d,e]	0.41±0.10[a]	0.55±0.03[b,d]	0.03±0.01[a,b]
CA (500 mg/kg b.wt.)	0.56±0.05[a,e]	0.63±0.11[d]	0.54±0.01[d]	0.04±0.01[a,b]
BLCO (250 mg/kg b.wt.) + (CA 250 mg/kg b.wt.)	0.55±0.02[a,e]	0.15±0.02[e]	0.69±0.07[e]	0.05±0.01[a,b]
BLCO (500 mg/kg b.wt.) +	0.56±0.05[a]	0.02±0.02[f]	0.70±0.04[e]	0.04±0.01[a,b]

CA (500 mg/kg b.wt.)	

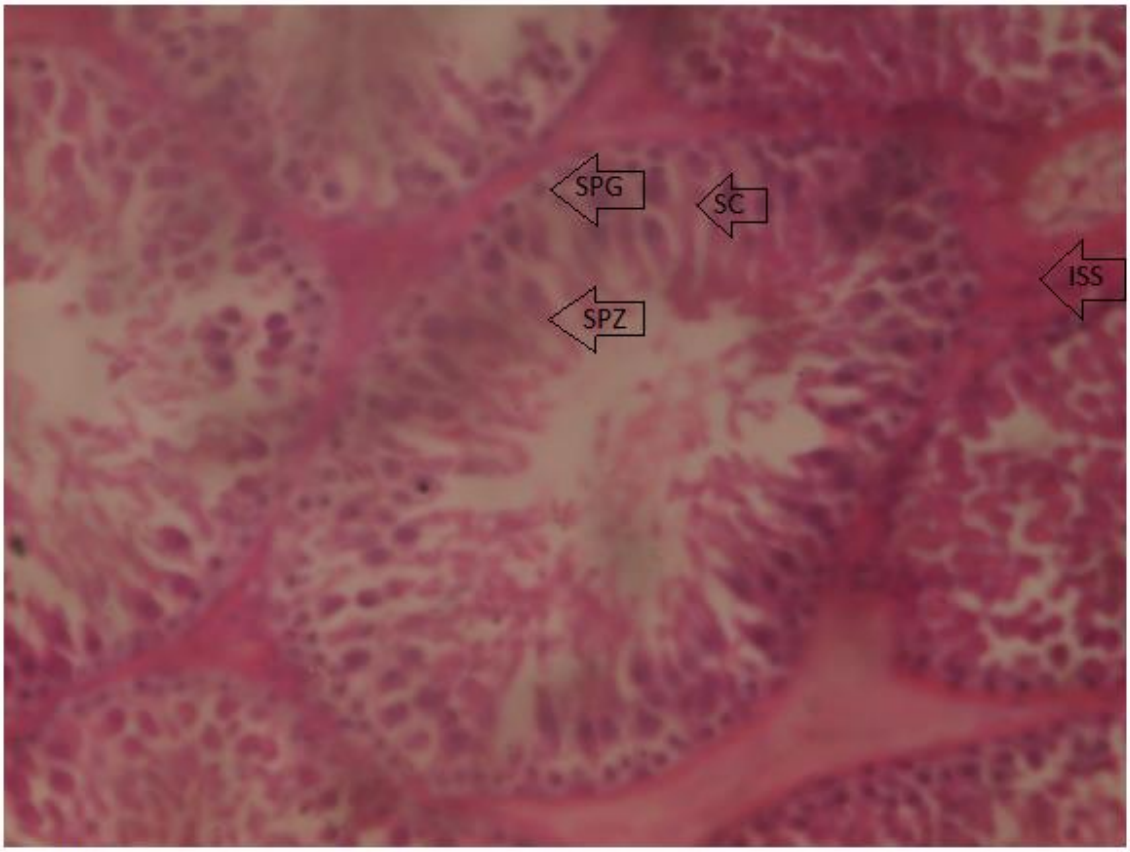
Values are reported as mean ± SEM of triplicate determination. Values with different superscript alphabets are significantly different at $p<0.05$. The Least Significant Difference (LSD) was used to test for the difference between individual treatment groups using Statistical Package for Social Sciences (SPSS), version 22.0. BCLO = Bonny light crude oil; CA = *Cnidoscolus aconitifolius*; mg/kg b.wt. = milligram per kilogram body weight.

Table 3: Lipid profile of Wistar rats administered with Bonny Light Crude Oil and *C. aconitifolius* leaf extract

TREATMENT GROUPS	**TC(**$mgdL^{-1}$**)**	**HDL(**$mgdL^{-1}$**)**	**TG(**$mgdL^{-1}$**)**	**LDL(**$mgdL^{-1}$**)**
Control	$197.97\pm0.71^{a,b}$	$59.09\pm1.33^{a,b}$	66.67 ± 24.80^{a}	125.55 ± 6.22^{a}
BLCO (250 mg/kg b.wt.)	$196.82\pm1.29^{a,c}$	$62.51\pm1.93^{a,c}$	53.76 ± 19.11^{a}	123.55 ± 4.89^{a}
BLCO (500 mg/kg b.wt.)	$195.53\pm1.06^{a,c}$	$62.32\pm1.93^{a,c}$	30.11 ± 13.08^{a}	127.19 ± 5.24^{a}
CA (250 mg/kg b.wt.)	$197.63\pm1.82^{a,b}$	$60.42\pm0.66^{a,b}$	62.37 ± 5.69^{a}	124.74 ± 1.08^{a}
CA (500 mg/kg b.wt.)	$199.60\pm2.15^{b,c}$	63.65 ± 4.11^{a}	81.72 ± 35.21^{a}	119.60 ± 5.13^{a}
BLCO (250 mg/kg b.wt.) + (CA 250 mg/kg b.wt.)	$199.80\pm1.75^{b,c}$	55.67 ± 1.16^{b}	88.17 ± 2.15^{a}	126.49 ± 2.98^{a}
BLCO (500 mg/kg b.wt.) + CA (500 mg/kg b.wt.)	$198.98\pm1.94^{a,c}$	55.48 ± 0.50^{b}	58.06 ± 19.71^{a}	131.89 ± 5.81^{a}

Values are reported as mean ± SEM of triplicate determination. Values with different superscript alphabets are significantly different at $p<0.05$. The Least Significant Difference (LSD) was used

to test for the difference between individual treatment groups using Statistical Package for Social Sciences (SPSS), version 22.0. BLCO = Bonny Light Crude Oil; CA = *Cnidoscolus aconitifolius*; mg/kg b.wt. = milligram per kilogram body weight.

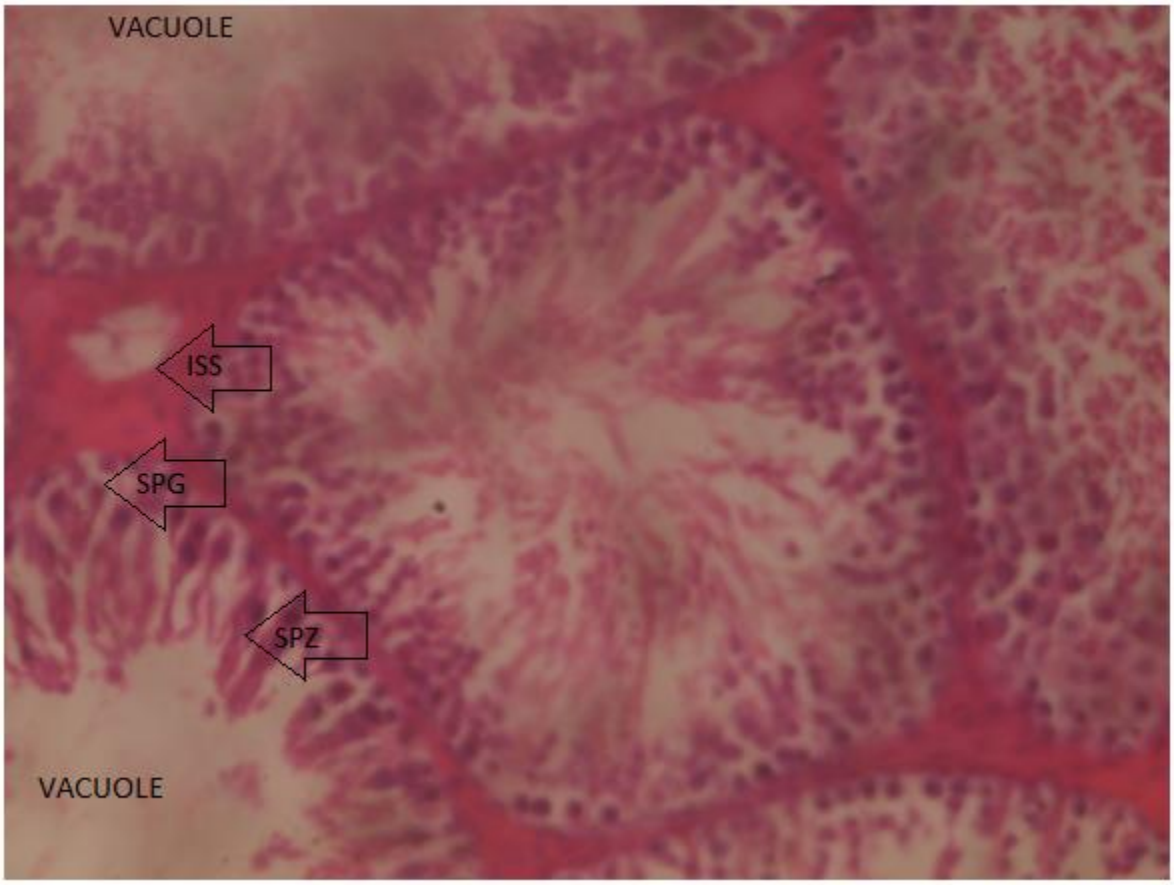


Testis 1

Magnification= X400 H&E

Fig. 1: Photomicrograph of testis from the control group

Histologically normal testis, seminiferous tubule containing sertoli cells (SC), spermatogenic cells (SPG) and spermatozoa (SPZ)

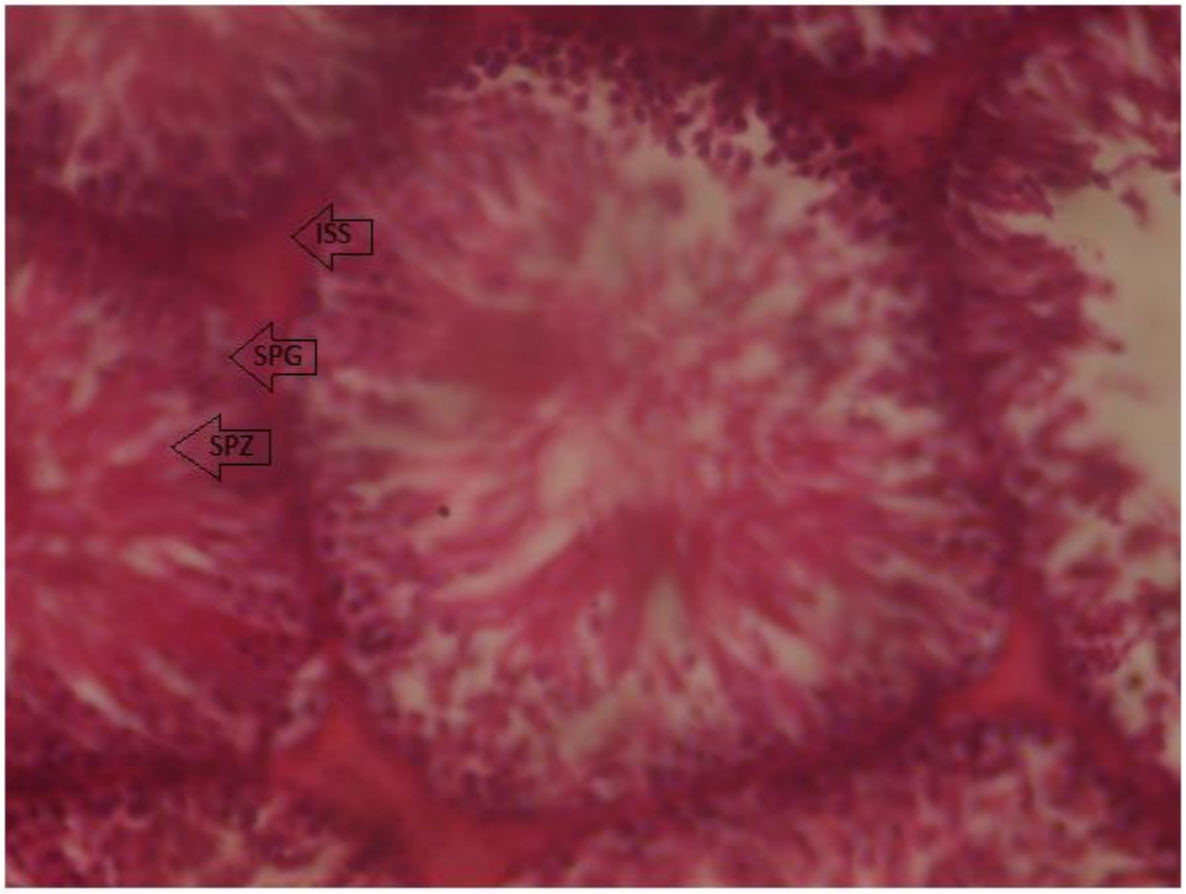


Testis 6

Magnification= X400 H&E

Fig. 2: Photomicrograph showing testis from the group administered 250mg/kgb.wt BLCO

Histologically distorted testis with vacuolated seminiferous tubules containing spermatogenic cells (SPG) and deformed spermatozoa (SPZ), interstitial spaces (ISS) contain Leydig cells

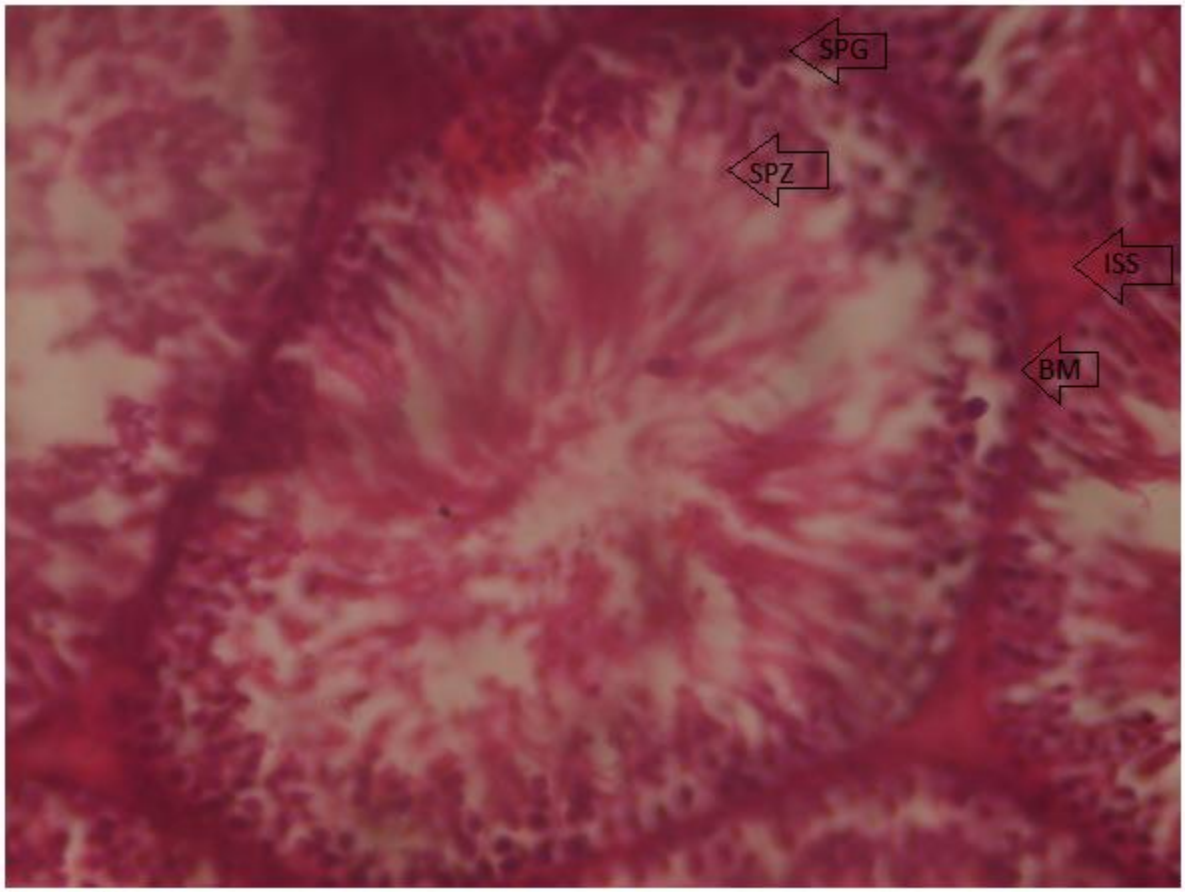


Testis 7

Magnification=X400 H&E

Fig. 3: Photomicrograph of testis from group administered 500mg/kgb.wt BLCO

Histologically normal testis with intact seminiferous tubules containing SPZ and SPG, interstitial spaces containing Leydig cells (ISS)

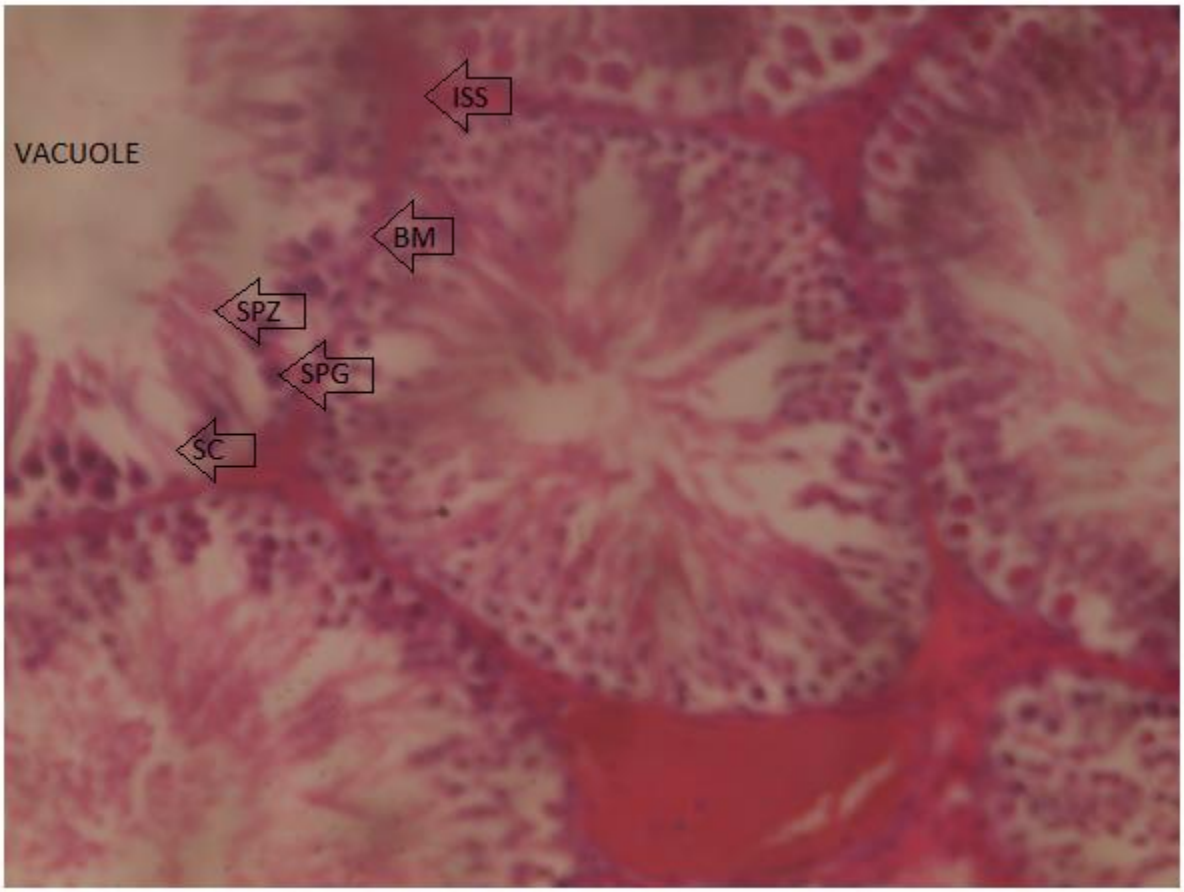


Testis 8

Magnification= X400 H&E

Fig. 4: Photomicrograph of testis from the group administered 250mg/kgb.wt CA

Histologically normal testis with intact seminiferous tubules lined with basement membrane (BM) containing SPZ and SPG, interstitial spaces (ISS) containing Leydig cells

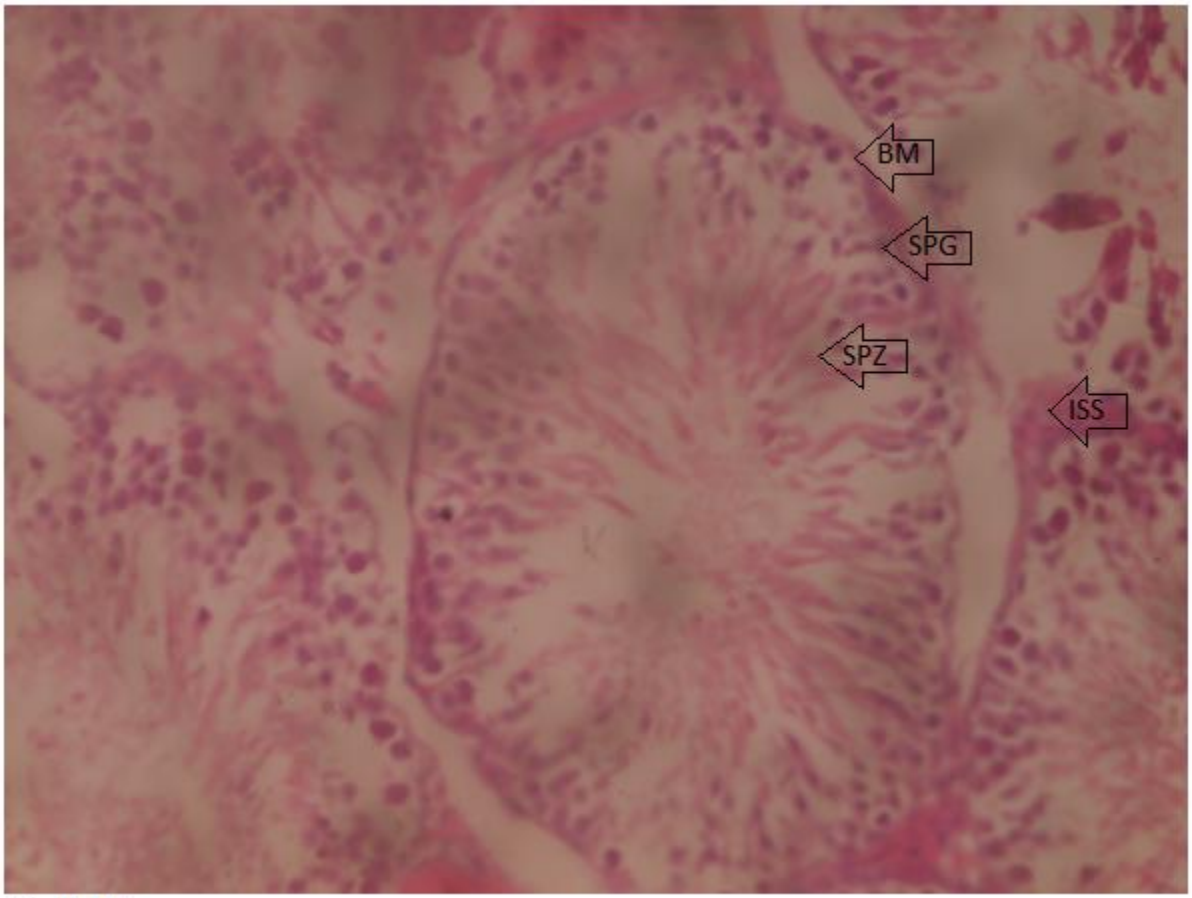


Testis 9

Magnification= X400 H&E

Fig. 5: Photomicrograph of testis from group administered 500mg/kgb.wt CA

Histologically mildly distorted testis with partial vacuolation of seminiferous tubules , seminiferous tubules contains Sertoli cells (SC), deformed spermatozoa (SPZ) and SPG, ISS contain Leydig cells



Testis 10

Magnification= X400 H&E

Fig. 6: Photomicrograph of testis from group administered 250mg/kgb.wt BLCO + 250mgkg/b.wt CA

Histologically normal testis with intact seminiferous tubules containing SPZ, SPG, lined by basement membrane (BM) and ISS containing Leydig cells

4.0 Discussion

The results obtained from this study shows the biochemical implications of Wistar rats exposed to Bonny light crude oil and leaf extract of *C. aconitifolius.* The determination of the oxidative stress status aids in assessing the biological redox status, disease state and progression, and the healthy enhancing effects of antioxidants. A cell can regain its original functional state after overcoming small perturbations but more severe oxidative stress can cause necrosis and cell death. However, the damaging effects of reactive oxygen species (ROS) can be overcome in aerobic cells through the provision of an extensive antioxidant defense mechanism [18]. Endogenous antioxidant enzymes like SOD, CAT and GST and non-enzymatic antioxidants like GSH can limit the effects of ROS but quickly become overwhelmed by large quantities of ROS [19]. The present study

shows that administration of BLCO for 21days consecutively resulted in no dose-dependent difference in GSH and CAT. Nevertheless, an increase in SOD activity which was observed may signify enzyme induction. An increase in SOD activity has been reported to indicate an increased free radical generation [20]. Lipid peroxidation is a degenerative disorder which is mediated through free radicals produced in the cells [21]. This reaction leads to the formation of MDA which is cytotoxic and mutagenic. A large fraction of crude oil components are lipophilic and biological membranes are generally the target sites where the adverse effect occurs. Thus, an increased MDA level shows a state of stress in the liver which may have been induced by BLCO or its metabolites. Nevertheless, the findings of this study revealed that no stress occurred in the animals as a result of the ingested crude oil. Nevertheless, the photomicrograph of the testis from the group administered 250mg/kgb.wt BLCO (fig.2) showed histologically distorted testis with vacuolated seminiferous tubules with deformed spermatozoa. This implies that a concentration of 250mg/kgb.wt BLCO, had a physiologically damaging effect on the testis. This is in line with a similar observation was made by [22],[23]. This was ameliorated on administration of 250mg/kgb.wt BLCO + 250mg/kgb.wt CA were the histological findings showed normal testis with intact seminiferous tubules. This shows the detoxification characteristics of the plant leaf extract at this concentration. Also, at a concentration of 500mg/kgb.wt CA, the histological findings showed mildly distorted testis with partial vacuolation of seminiferous tubules and deformed spermatozoa. This implies that at this concentration the plant extract may have a deleterious physiological effect on the tissues.

Heavy metals are absorbed into the body through inhalation, absorption through the skin and ingestion. A gradual build up of these heavy metals occur when they are accumulated in the tissues faster than the rate at which the are eliminated. The concentrations of the heavy metals (Pb, Cd, AS and Hg) revealed a greater accumulation in the groups that were treated with 250mg/kgb.wt and 500mg/kgb.wt when compared to other groups. Nevertheless, there was a higher accumulation in 500mg/kgb.wt BLCO group when compared with the 250mg/kgb.wt BLCO group. This observation is in line with a similar observation made by [24] which revealed varying heavy metal concentration in the blood, testes and liver of rats. A reduction in the concentration of heavy metals seen in the groups treated with similar concentration of CA and BLCO shows the detoxification ability of the leaf extract when compared with other groups.

There was no significant change in the lipid profile of the animals on administration of BLCO and the plant extract.

4.1 Conclusion:

This study revealed that the induction of BLCO at concentrations of 250mg/kgb.wt, 500mg/kgb.wt and 500mg/kgb.wt of *Cnidoscolus aconitifolius* for 21days consecutively may be injurious to the health. This is evident from the results of the histological analysis carried out on the tissue extracts. As a result, it is recommended that the use of BLCO and *C. aconitifolius* in folkloric medicine at concentrations similar to the ones used in this study should be avoided.

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