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Gonadal Histo-Morphology and Antifertility Effects of Bonny Light Crude Oil in Male Rats

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Abstract
The study investigated the antifertility effects of ingestion of Bonny Light Crude Oil (BLCO) at a dose of 0.5, 1.5 2.5 and 3.5ml/kg body weight in adult male rats following oral administration for 60days. The animals were sacrificed, the epididymal spermatozoa expressed out and a homogenate made for semen analysis. The testes were removed for histological processing. The sperm count was significantly reduced (21.80±0.57, 19.00±0.85, 16.20±0.00 and 12.90±0.99 million/ml respectively) when compared to their corresponding control groups 26.30±1.27, 25.50±0.71, 26.70±2.69, and 25.60±0.57 million/ml) at $P<0.05$. Degenerative and necrotic changes of cells in the seminiferous tubules and interstitium was observed in the histology of the testes. BLCO has adverse effects on male reproductive system. This may imply possible antifertility for male rats exposed to BLCO ingestion.
Keywords: Bonny light crude oil, testes, antifertility, male rat

1. Introduction
Crude oil is a mixture of hydrogen molecules, which are organic compounds of carbon and hydrogen atoms that may include from one to sixty carbons (Petroleum Handbook, 1996). It contains trace amount of sulphur containing chemicals, such as sulfides, mercaptans, thiophenes, and other more complex compounds. Although the chemical composition of the crude oil varies by source, crude oil and petroleum products share certain toxic characteristics and are toxic to biological life (Walkinson and Holt,1987)). The oil industry is the backbone of the Nigerian economy, accounting for over 90% of the total foreign exchange revenue. Nigeria's marker crude oil on the international market is the Bonny Light Crude Oil (BLCO). It is preferred to others because of its low specific gravity and low corrosiveness to refinery infrastructure. Industrialization, through exploitation and exploration of crude oil or total petroleum hydrocarbon (TPH), has introduced into the ecosystem substances that are potentially toxic to life and environment (Dede and Kagbo, 2002).
The magnitude of oil pollution and damage occasioned by multinational oil companies operating in the Niger delta region of Nigeria is not only disturbing but incredible (Akpofure *et al*., 2000). Bioaccumulation of crude oil in marine life possess potential health hazards to terrestrial species (Shore and Douben, 1994). There is available evidence suggesting changes in chemical properties of soil following contamination by crude oil (Ifeadi and Nwankwo, 1987). The devastating consequences of the spill of crude oil and its products in the Niger delta region with its eventual hazards on both aerial and terrestrial environs tantamount to an irreversible chain effect both on biodiversity and human safety (Akpofure *et al*., 2000).
Many studies on the toxic effects of crude oil of different geological formation in laboratory and non-laboratory animals have been carried out (Payne *et al*., 1987; 1983; Rice *et al*., 1977). Epidemiological data and results of toxicity studies in experimental animals consistently report that there is significant health risk due to prolonged exposure to petroleum (Didia *et al.*, 2003; Sheepers and Bios, 1992). The chemical composition of crude oil differs widely and these chemicals are capable of mimicking the inherent actions of reproductive hormones and hence have the ability to disrupt the neuro-endocrine system or the functions of the gonads directly (Colborn *et al*., 1993).
Some people (Kalabari's, Ijaw's, Ogoni's) ingest crude oil for various medicinal purposes; as laxative, anti-poisoning agent, anti-convulsion agent, used for treatment of arthritis, snake antidote, especially in rural areas where the conventional antidotes are not available (Dede *et al*., 2002). Animal studies have shown cyto toxic effects on some organs from exposure to crude oil compounds (Didia *et al.*, 2003, Eyong *et al*.,2004; Eyong, 2000; Khan *et al*., 1999) and gonads contain rapidly proliferating cells that are probably susceptible to damage by PAH's (ASTDR, 2004).
This research work was therefore undertaken to investigate the histological changes and antifertility effects associated with ingestion of BLCO on male wistar rats.

2. Materials And Methods
2.1 Bonny Light Crude Oil
The bonny light crude oil used for this study was obtained from Shell Petroleum Development Company, Port

Harcourt, Rivers state with authority from the Department of Petroleum Resources, NNPC, Lagos Nigeria.

2.2 Experimental Animals

Sixty (60) male albino wistar rats obtained from the animal house of the Department of pharmacology, University of Calabar were used for this study. The animals whose weight ranged from 150 – 200g were acclimatized for three (3) weeks before the commencement of the experiment in the animal house of the Department of Human Anatomy. The animals were housed in well-ventilated cages and kept under controlled environmental conditions of temperature (25±5°c), relative humidity (50±5%) and 12-hour light/dark cycle. The animals were fed with grower's marsh and tap water *ad libitum*

2.3 Experimental Design

The animals were assigned into four (4) parallel groups A, B, C, and D consisting of ten (10) experimental animals and five (5) control animals, the doses used were based on predetermined LD50 values obtained from previous studies (Eyong, 2000). The experimental animals in groups A, B, C, and D received 0.5, 1.5, 2.5 and 3.5ml/kg B.W of BLCO respectively, the control animals in the corresponding groups received 0.5, 1.5, 2.5 and 3.5ml/kg B.W of normal saline respectively both by oral gastric intubation. The treatment was done once a day on alternate days for a period of sixty (60) days. At the end of the sixty (60) days, the animals were euthanized under chloroform vapor and sacrificed. The spermatozoa was expressed out, a homogenate was made and then used for semen analysis. The testes were surgically removed, and suspended in bouins fluid fixation, preparatory to histological processing.

Table 1. Experimental design

Group	No. of Animals'	Treatment with BLCO ml/kg b.w	Group	No. of Animals'	Treatments with (normal) saline ml/kg b.w
		(control)			
A	10	0.5	A	5	0.5
B	10	1.5	B	5	1.5
C	10	2.5	C	5	2.5
D	10	3.5	D	5	3.5

2.4 Determination Of Sperm Count

The sperm count was determined using the new improved neubauer's haemocytometer. The homogenate (dilute semen) was used. Five primary squares were counted; the counting was performed with the help of a hand counter.

2.5 Statistical Analysis

The results were analyzed for statistical significance by one way and two-way analysis of variance (ANOVA). All data were expressed as mean ±SEM. P values <0.05 were considered significant.

3. Observations And Results

3.1 Sperm Count

The table below (table 2) show data obtained from caudal epididymal sperm count following oral administration of BLCO. The sperm count was significantly reduced in the treated groups A, B, C, and D; 21.80±0.57, 19.00±0.85, 16.20±0.00, 12.90±0.99 respectively when compared with their corresponding control groups; 26.30±1.27, 25.50±0.71, 26.70±2.69 and 25.60±0.57 respectively at a probability level of P<0.05. The reduction in the number of sperm cells was also seen to be dose dependent.

Table 2. Effect of BLCO treatment on sperm count(10)

Group	Control	Treated
A	26.30±1.27	21.80±0.57*
B	25.50±0.71	19.00±0.85*
C	26.70±2.69	16.20±0.00*
D	25.60±0.57	12.90±0.99*

*Values are expressed as Mean±SEM, *=significant at P<0.05 vs. control*

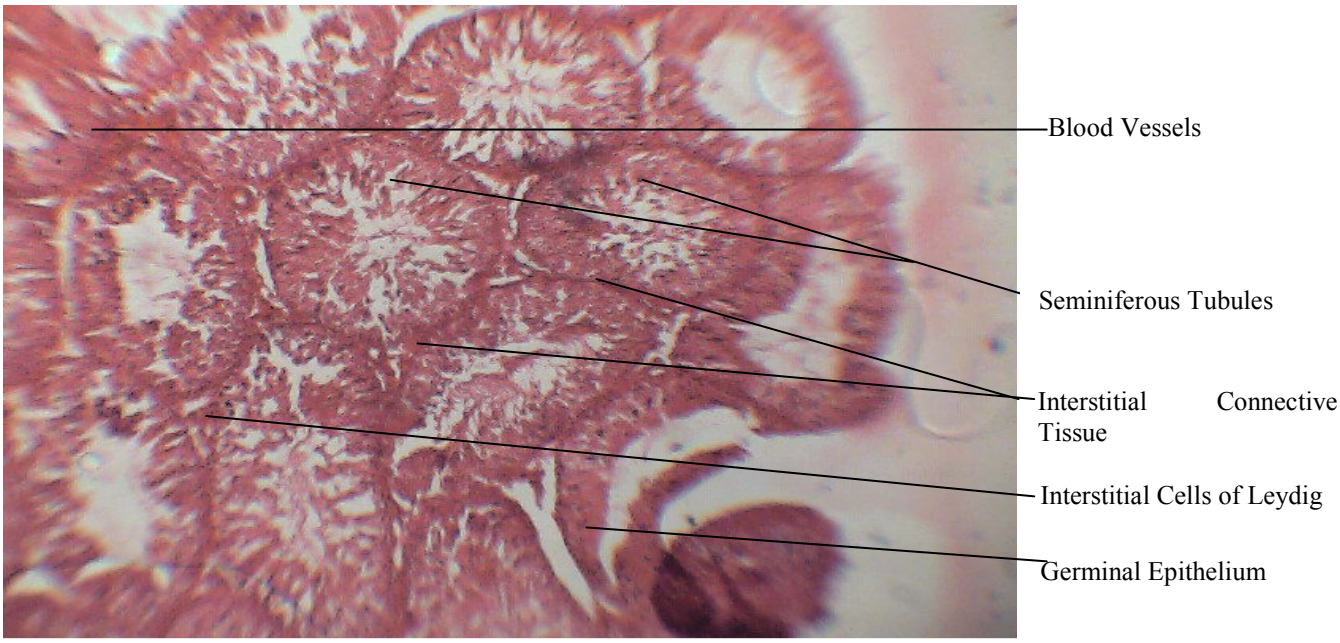
3.2 Histology Of The Testes

The cellular architecture and integrity of the testes were examined in this study, results in the control groups (shown in micrographs 1, 3, 5 and 7), revealed several layers of spermatogenic cells such as primary spermatocytes, spermatids, spermatozoa and sertoli cells.

On the other hand, testes of treated rats (micrographs 2, 4, 6 and 8) showed minimal to marked disintegration of

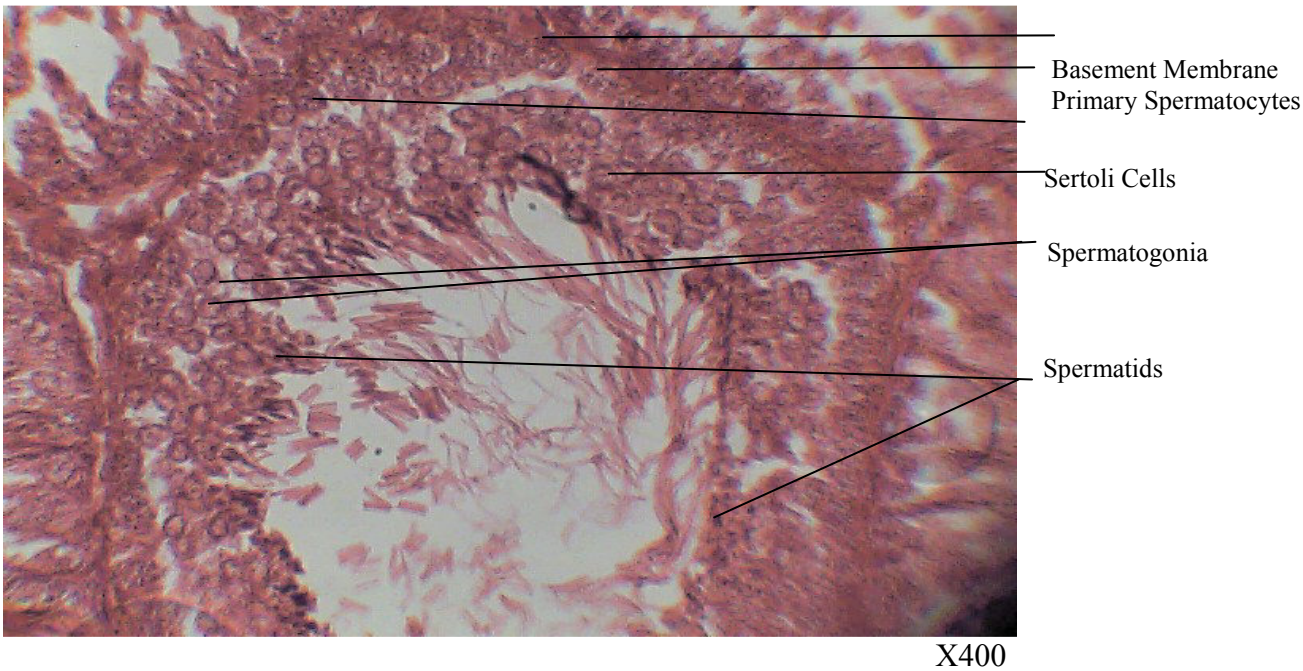
the basic cellular outline and histology of the seminiferous tubules.
The testes of the treated group A animals showed similar features with the control but fewer spermatids in the seminiferous tubules. The seminiferous tubules of treated group B rats showed less interstitium and interstitial cells (of leydig), sertoli cells were found singly. This suggests degenerative and necrotic changes. The testes of group C animals were similar to those of group B, but the interstitium was scantier and lesser spermatids were observed. The germinal epithelium of the group D animals were distorted, tubular lamina was scanty with few spermatids seen.

H AND E STAIN



X100

PLATE 1a Photomicrographs of testes of control rats in Group A



X400

PLATE 1b Photomicrographs of testes of control rats in Group A

H AND E STAIN

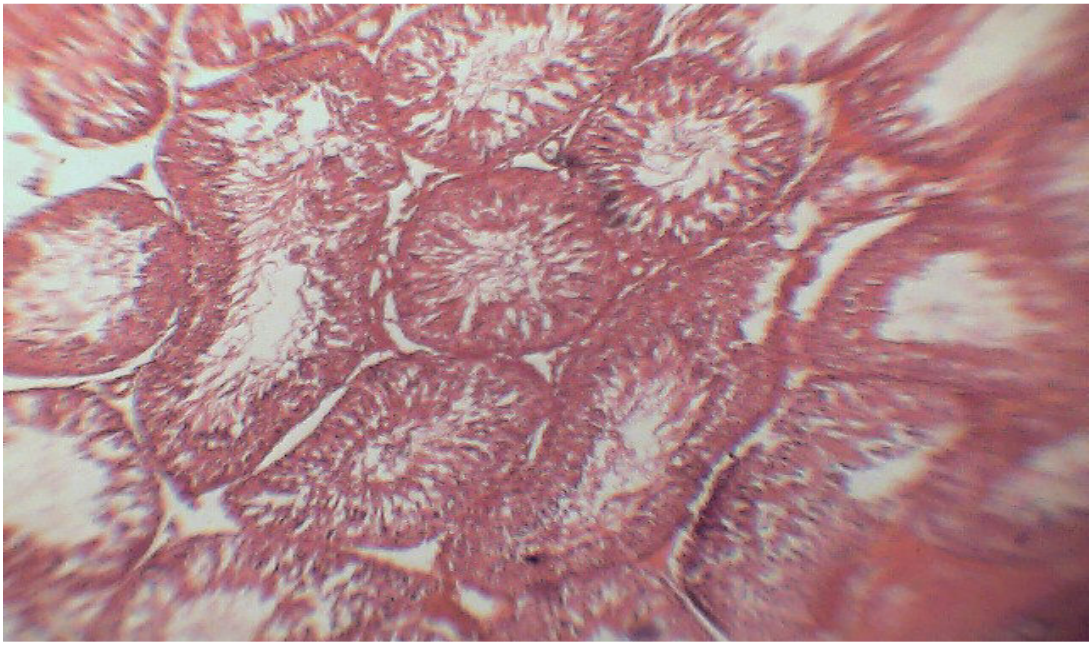


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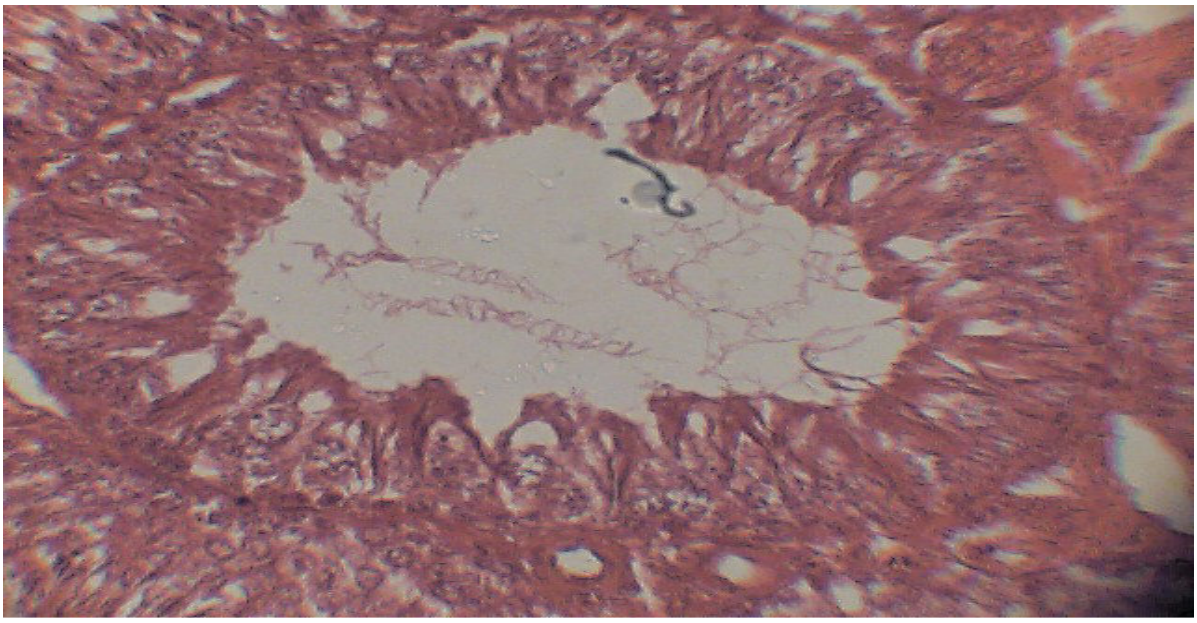
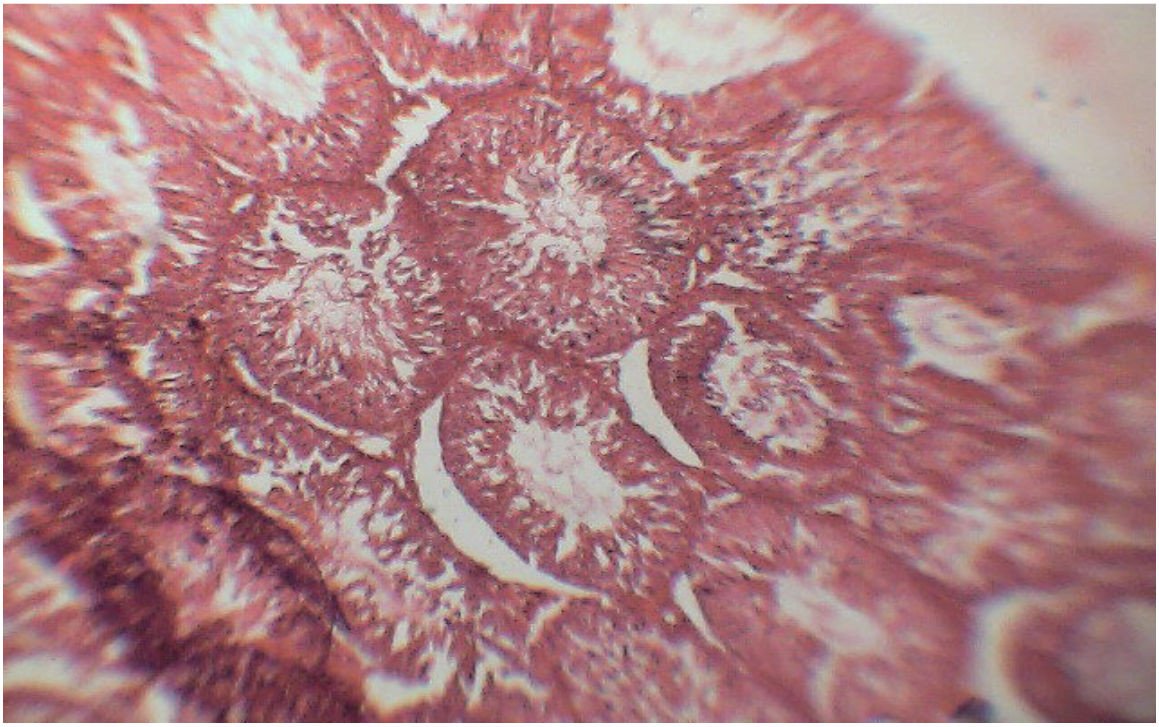
PLATE 2a Photomicrographs of testes treated with 0.5ml/kg b.w of BLCO rats in Group A_1



X400

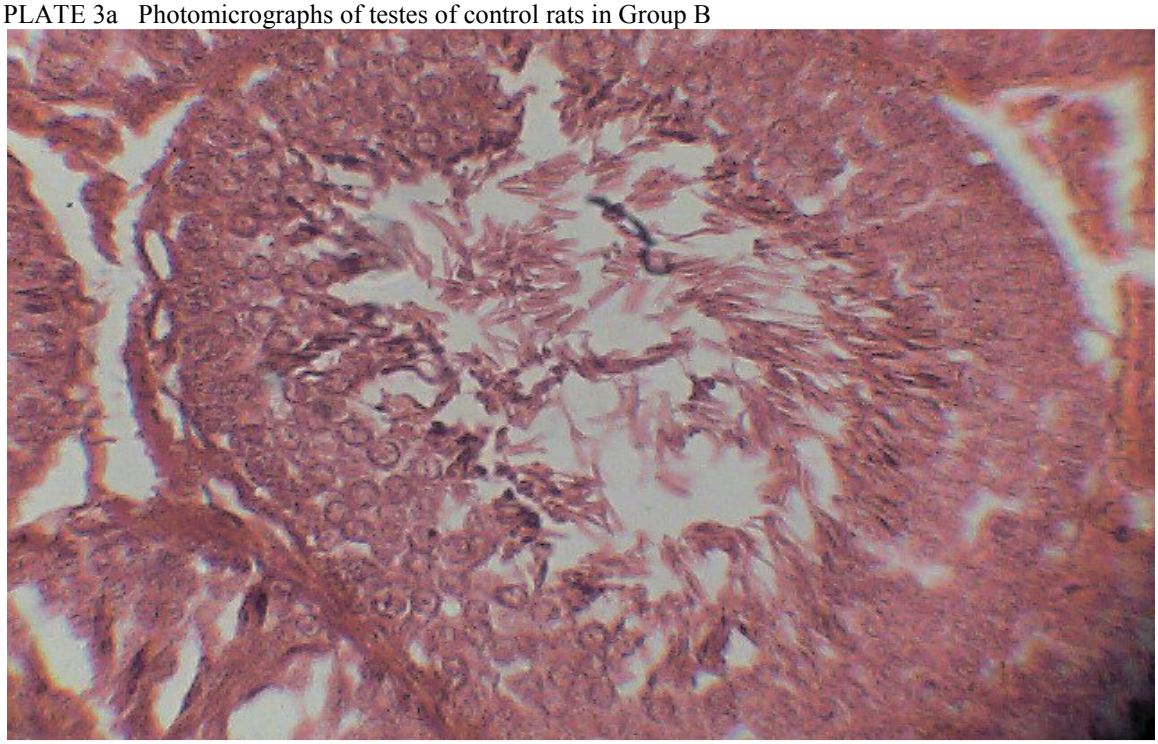
PLATE 2b Photomicrographs of testes treated with 0.5ml/kg b.w of BLCO rats in Group A_1

H AND E STAIN



X100

PLATE 3a Photomicrographs of testes of control rats in Group B



X400

PLATE 3b Photomicrographs of testes of control rats in Group B

H AND E STAIN

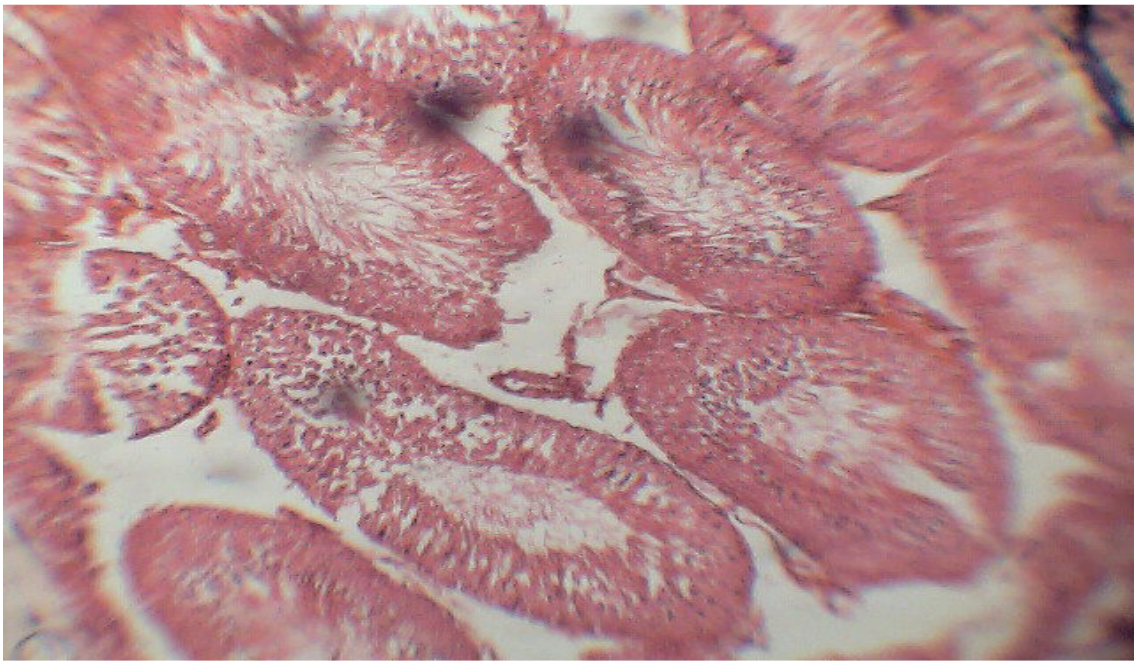


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PLATE 4a Photomicrographs of testes treated with 1.5ml/kg b.w of BLCO rats in Group B_1

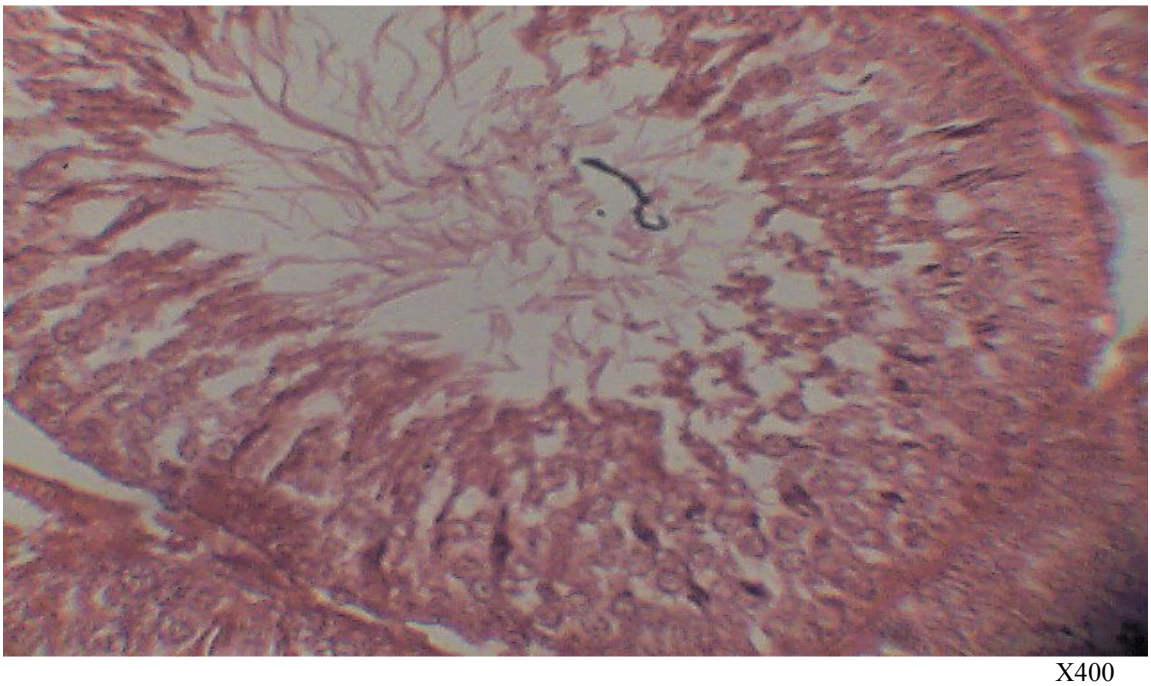

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PLATE 4b Photomicrographs of testes treated with 1.5ml/kg b.w of BLCO rats in Group B_1

H AND E STAIN

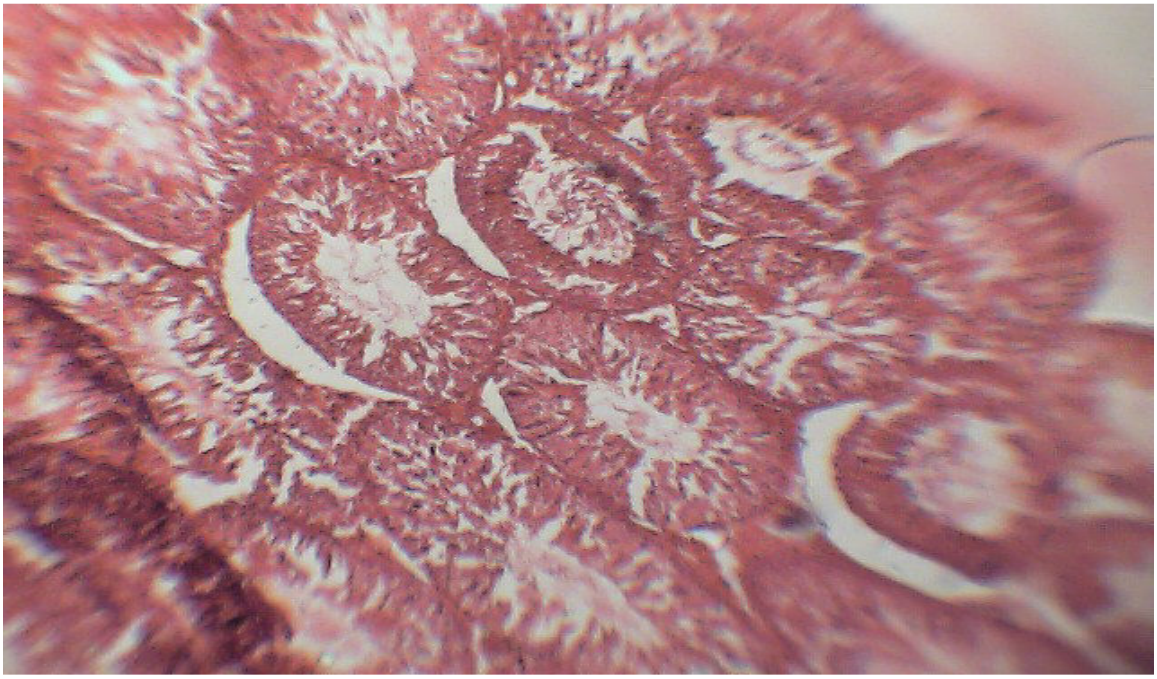

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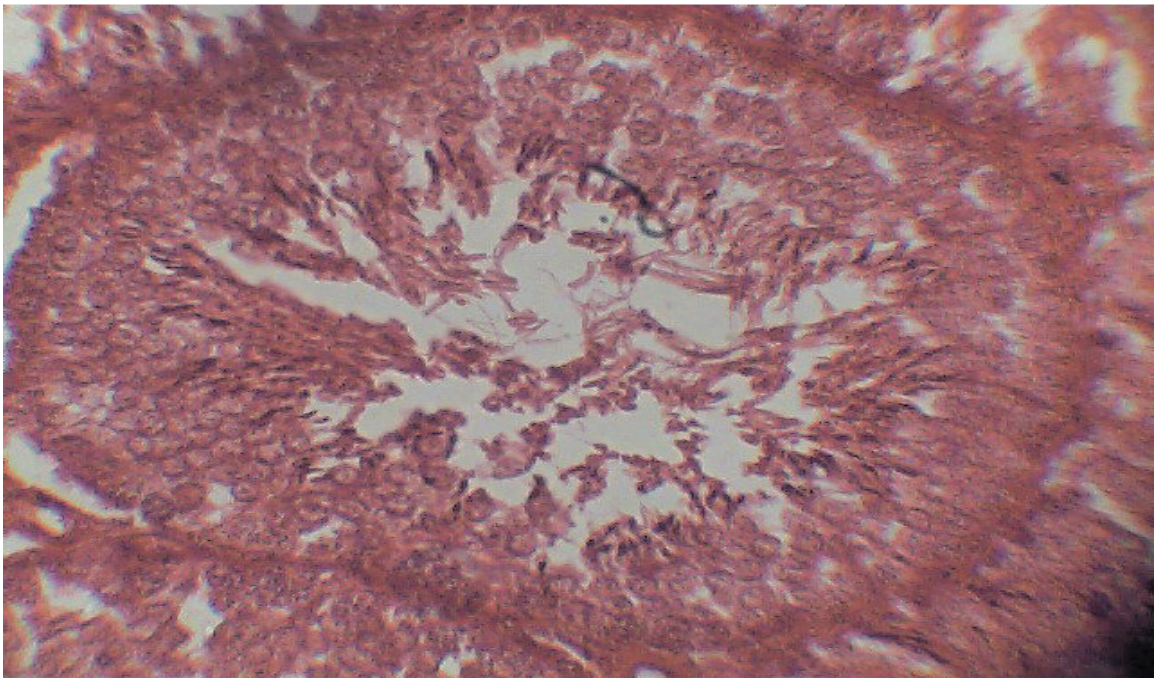
PLATE 5a Photomicrographs of testes of control rats in Group C



X400

PLATE 5b Photomicrographs of testes of control rats in Group C

H AND E STAIN

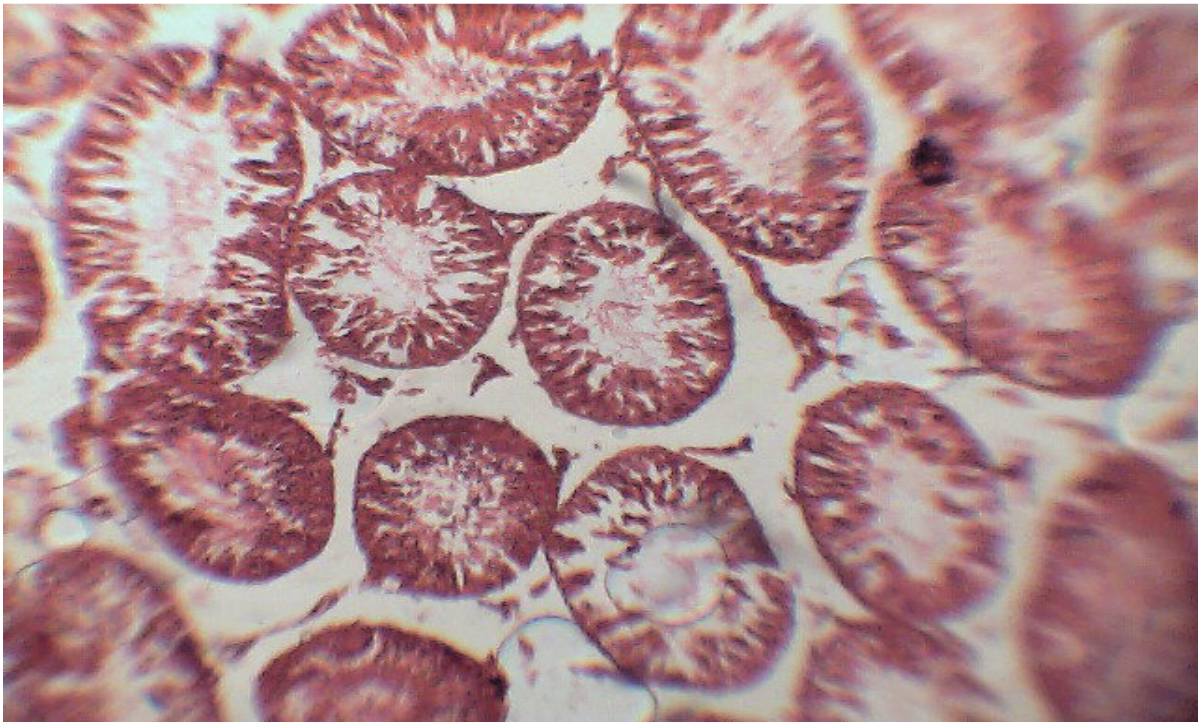


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PLATE 6a Photomicrographs of testes treated with 2.5ml/kg b.w of BLCO rats in Group C_1

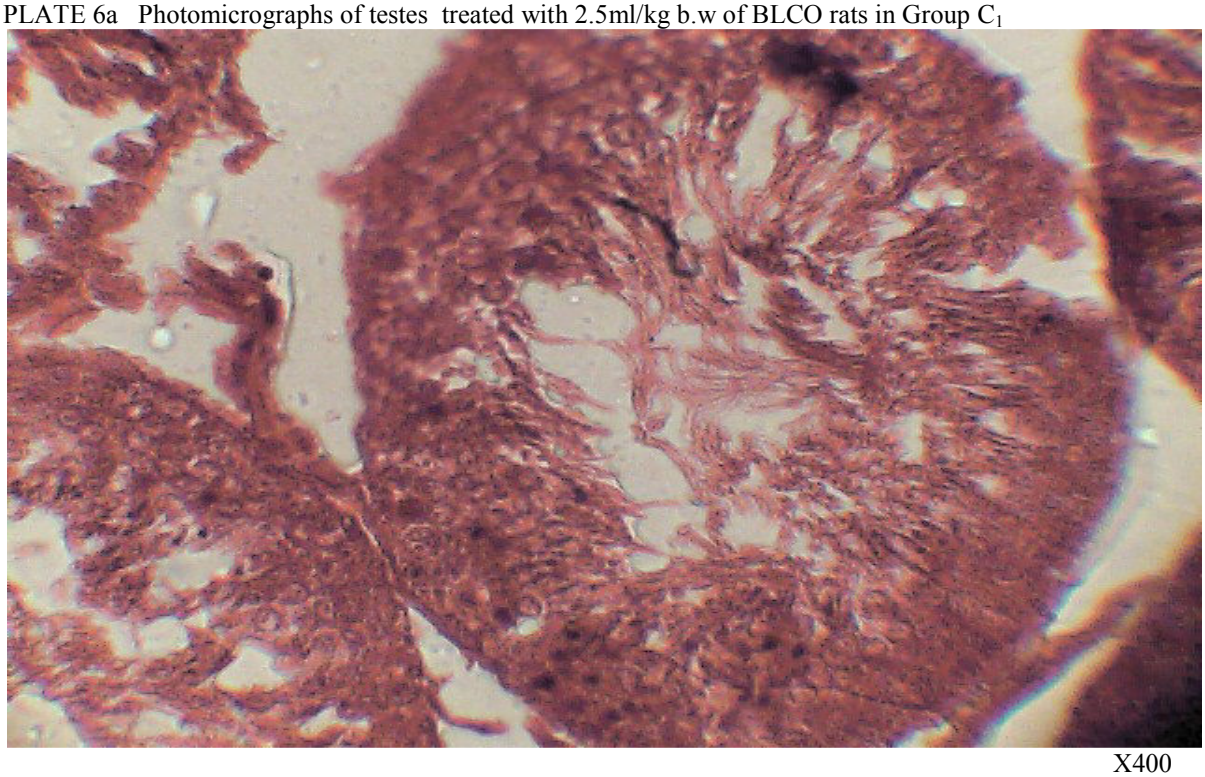

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PLATE 6b Photomicrographs of testes treated with 2.5ml/kg b.w of BLCO rats in Group C_1

H AND E STAIN

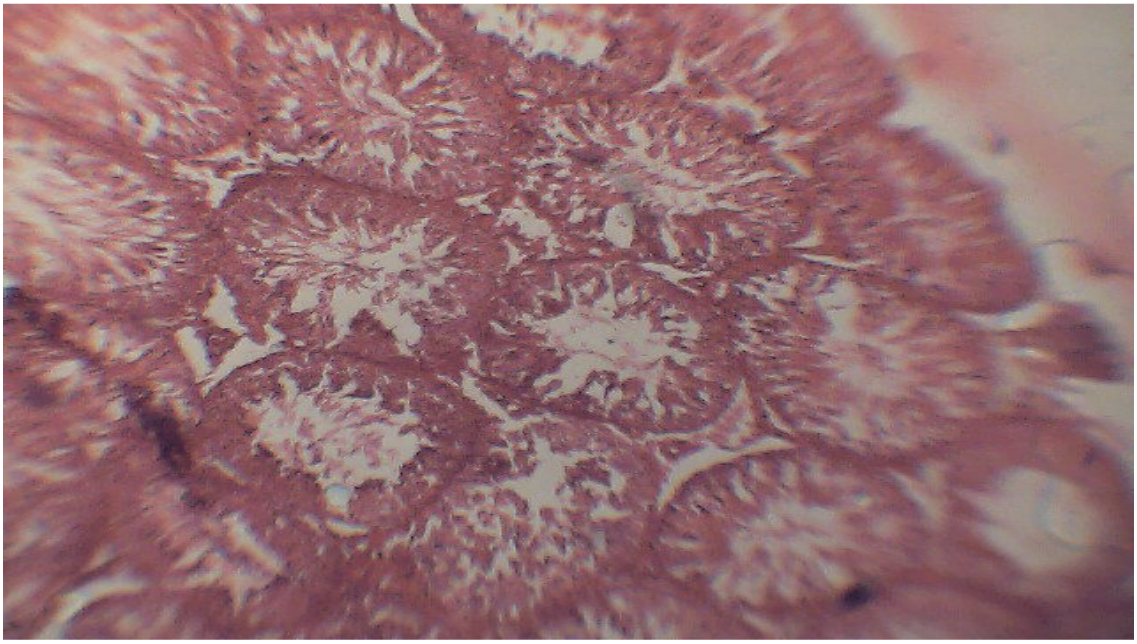

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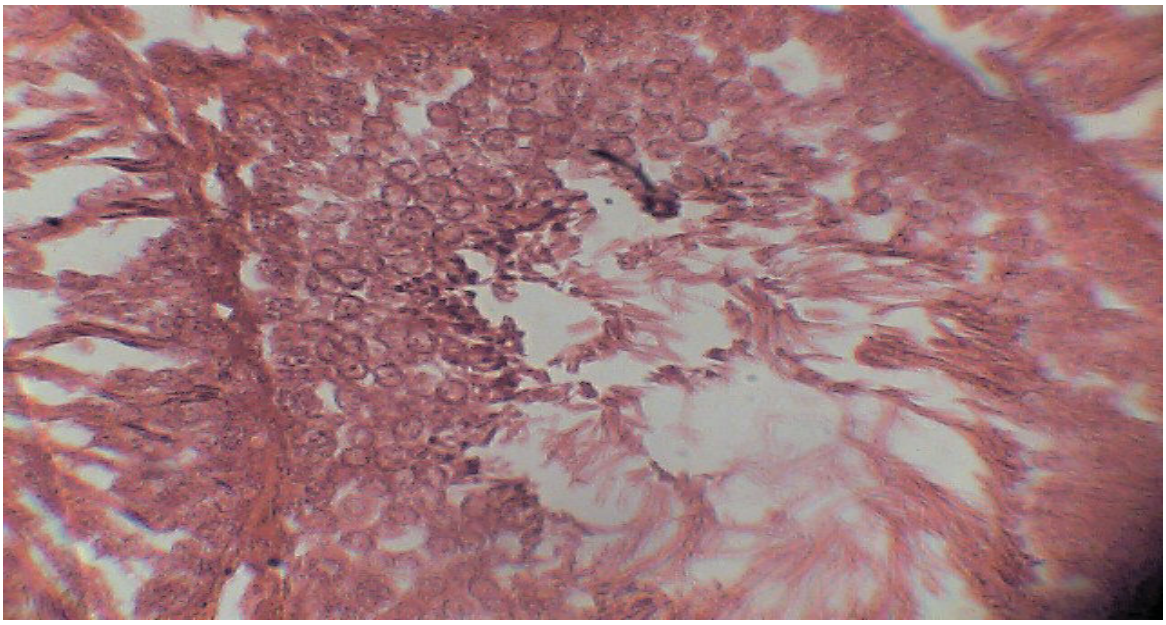
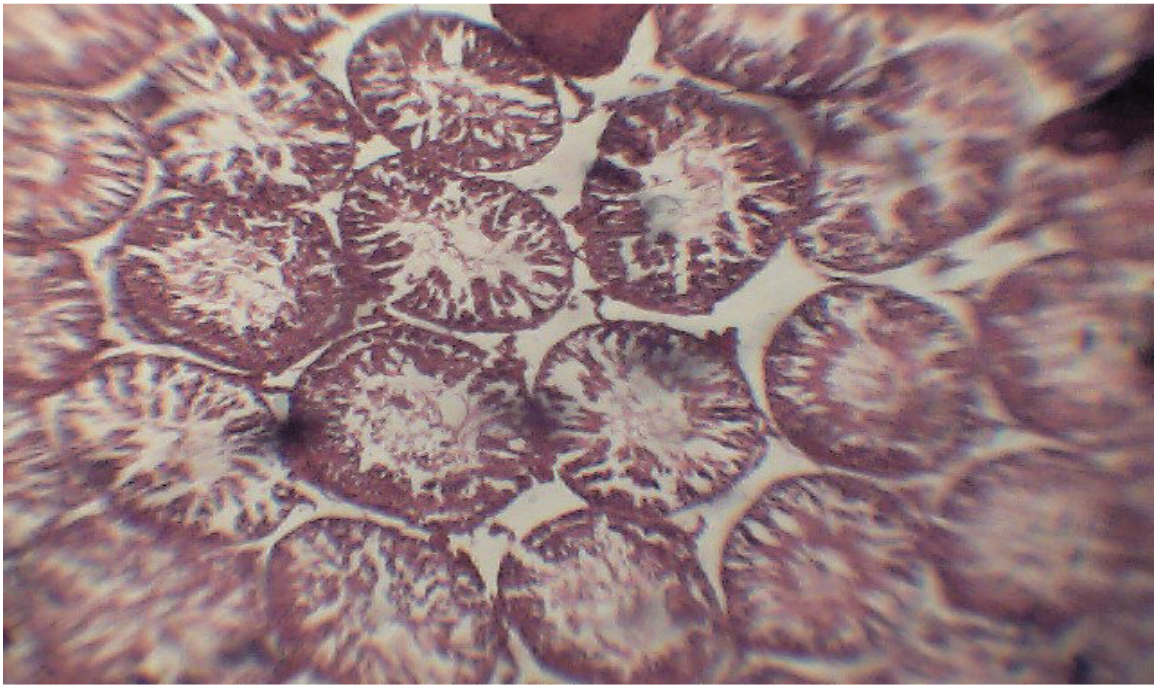
PLATE 7a Photomicrographs of testes of control rats in Group D



X400

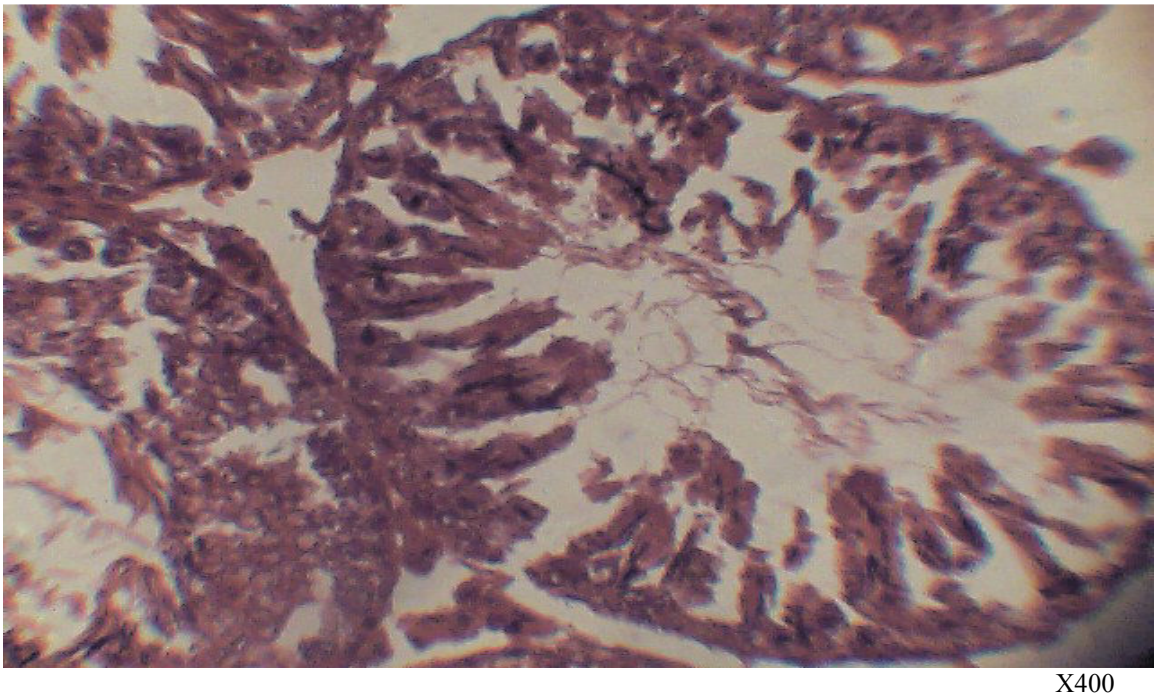
PLATE 7b Photomicrographs of testes of control rats in Group D

H AND E STAIN



X100

PLATE 8a Photomicrographs of testes treated with 3.5ml/kg b.w of BLCO rats in Group D_1



X400

PLATE 8b Photomicrographs of testes treated with 3.5ml/kg b.w of BLCO rats in Group D_1

4. Discussion

This study investigated the effect of ingested BLCO on the sperm count of male wistar rats. Several researches (Ellenton and Hallet, 1981; Mckee *et al.,* 1994; Eyong, 2000; Didia *et al.,*2003) carried out in both laboratory and non-laboratory animals with crude oils of different geologic origins have pointed out the toxic effects of this important commodity. The observed dose dependent reduction in the number of caudal epididymal sperm cells of BLCO treated rats is in accordance with earlier reports by Obidike *et al*., 2007 and Orisakwe *et al.*, 2004. The observed reduction in the number of sperm cells of the BLCO treated rats suggests depression of spermatogenic activity which probably indicates a reduction in the number of developing germ cells. These effects can be

attributed to polyaromatic hydrocarbons (PAH's) contained in BLCO which has the potential to induce adverse developmental effects such as termination of pregnancy, malformations, sterility in offsprings, testicular changes such as lack of sperm, immunosuppression, and tumors (lyons, 1998; Fischer *et al.,* 2007; 2006; 2005).

This study revealed marked changes in the histology of the testes. The degeneration and necrosis of spermatogenic cells observed in this study corroborates reports in previous studies (Obidike *et al*., 2007 and Orisakwe *et al.*, 2004). In addition, the sertoli cells which appeared to lose their connections to the developing germ cells, probably did not support the maturation and differentiation of these germ cells (Orth *et al.* 1988). This probably indicates that BLCO damages the sertoli cells and their number decreases, impairing spermatogenesis. This leads to a decrease in the number of germ cells with complete differentiation (Steger *et al.,* 1999). In this study, degeneration and necrosis of the leydig cells following exudation into the interstices was observed in the testes of the rats that received BLCO.

In conclusion, results from this investigation indicated that BLCO administration can cause deterioration in semen quality, degenerative and necrotic changes of cells in the seminiferous tubules and interstitium of the testes as evidenced in the histology of the testes observed. Therefore, haven shown reproductive cyto -toxicity in rats, BLCO likely poses reproductive risks to animals and humans in areas where continual oil spillage occurs.

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