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Histopathological, Hemochromatotic, Hypercholesterolemic, and Androgenic Effects of Escravos Crude oil on the Testis in Male Chinchilla Rabbits

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Authors' contributions

This work was carried out in collaboration between all authors. Author JOO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TU, SNI and CJA managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: This study aimed to investigate the effects of Escravos crude oil on serum concentrations of testosterone, total cholesterol, body weight, relative weight and micro-anatomical architecture of the testis using male Chinchilla Rabbits.
Place and Duration of Study: This study was carried out at the Department of Medical Laboratory Science, Nnamdi Azikiwe University, Nnewi Campus between May and June 2013 (28 days).
Methodology: A total of thirty male Chinchilla Rabbits aged 12 to 14 weeks and weighing 1.2kg to 1.45kg was used. Crude oil was administered orally at the doses of 15, 20, 25 and 30mg/kg body weight to groups designated B, C, D and E respectively for 28 days, while group A was given normal saline. Serum concentration of testosterone and total

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cholesterol were estimated using the microplate enzyme immunoassay and enzymatic end point methods, respectively. The SPSS software (version 20) was used for the statistical analysis and the result expressed in mean ± SD.
Result: The results showed dose dependent effects on the hormone and biochemical assays, especially at the concentration of 25mg/kg body weight of the administered crude oil. Significant increases in serum concentrations of testosterone (0.32+/-0.05 to 0.46+/-0.14) and total cholesterol (1.35+/-0.17 to 1.76+/-0.15) concentrations ($p \leq .05$), and insignificant increase ($p \geq .05$) in the relative weight of the testis (4.80+/-0.40 to 6.50+/-0.90) were observed. The histology of the testes revealed hypertrophy of the seminiferous tubules, atrophy of the basal lamina and interstitial cells, and hemochromatosis. The histological findings agree with the hormonal and biochemical findings.
Conclusion: These findings suggest that Escravos crude oil might be a potential endocrine disruptor and toxic substance which can affect the micro-anatomical architecture of the testis.

Keywords: Escravos crude oil; total cholesterol; testosterone; hemochromatosis; histopathology; testis; male chinchilla Rabbit.

1. INTRODUCTION

Crude oil is claimed to be an antidote to poisoning and a cure for various gastrointestinal disturbances [1]. According to Dede et al. [2], cases of misuse of this substance by individuals have been reported, as it is known to be liberally used by some of the indigenes living in the Niger Delta area who believe that it can repel witches when applied either topically, or orally given to afflicted individuals. Other countries such as Kenya, Tanzania, Zimbabwe, Ghana and Tunisia depend on crude oil for unorthodox treatment of ailments such as stomach ache, diarrhoea, respiratory distress and convulsion.

The impact of crude oil spillage and discharge on the ecosystem as a result of oil exploration activities is an obvious problem of environmental concern [3,4]. Petroleum hydrocarbons have been reported to affect reproductive and development processes including hormonal synthesis [5], and increase the incidence of developmental abnormalities [6]. Benzone arene oxide, produces destructive and mutagenic effects on various organ systems of test group animals and is implicated in the etiology of cancer due to crude oil exposure [7]. Polycyclic aromatic hydrocarbons (PAHs), have also been implicated as endocrine disruptors in fish, especially as modulators of steroidogenesis [8].

Chronic exposure of animals to crude oil produces signs and symptoms of toxicity in the central nervous system [9], the gastro-intestinal tract, the reproductive system [10], as well as genetoxicity [11,12]. According to Aslani et al. [13], female goats exposed to West Texas intermediate crude oil suffered ulcer, cough, constipation and infertility, while studies conducted by Igwebuike et al. [14] revealed that exposure of male rats to Nigerian Qua-iboe brent resulted in reduced packed cell volume, increased total leukocytes count and reduced epididymal sperm reserves. Similar studies have shown that a single oral dose of crude oil causes growth retardation, decrease in accessory sex organs weights, altered sexual behaviour and delayed puberty in wild animals especially the sea nestling gull (*Larus argentatus*) [15,16]. Cholesterol is an unsaturated steroid alcohol, used by the liver, testes, and adrenal gland as a major metabolic precursor for the biosynthesis of bile acids, and steroid hormones which include male and female sex steroids (androgens and oestrogens), and adrenal steroid hormones [17]. According to the reports by Udeme and Etim [18], the

Nigerian crude oil blends have been observed to contain some trace metals such as Pb, Cd, Cr, Mn, Zn, Cu, and Co at a low concentration but with high values of Ni, V and Fe.

Secondary haemochromatosis can be caused by severe chronic haemolysis of any cause, intravascular haemolysis and ineffective erythropoiesis (haemolysis within the bone marrow), excess parenteral iron supplements, such as what can acutely happen in iron poisoning. Some disorders do not normally cause haemochromatosis on their own, but may do so in the presence of other predisposing factor which includes cirrhosis [19]. Cirrhosis is a result of advanced liver disease, characterized by replacement of liver tissue by fibrosis (scar tissue), regenerative nodules; lumps that occur due to attempted repair of damaged tissue, elevated cholesterol and haemochromatosis among others. Iron overload can also manifest in other medical conditions such as inflammation, liver disease and renal disease. Escravos crude oil has also been known to cause serum increase in C-reactive protein (A marker for monitoring inflammatory process) and inflammation (glomerulonephritis; Marked by increased lymphocytic infiltrations and stromal proliferation) [20]. According to CDC [21], haemochromatosis occurs when the body absorbs too much iron. This disease causes extra iron to gradually build up in the body's tissues and organs. Hence, if this iron build up is not treated, it can damage the body organs. The latter is supported by previous findings which reported that exposure to Escravos crude oil causes iron deposits in the liver and liver cirrhosis [22]. The aim of this study was to investigate the effect of Escravos crude oil on serum concentrations of total cholesterol, testosterone, body weight and architecture of the testis.

2. MATERIALS AND METHODS

2.1 Test Sample

The Escravos crude oil (with reference number 863) used in this study was provided by Warri Refining and Petrochemical Company Effurun, Delta State, Nigeria. The crude oil was exposed to sunlight in shallow pans (25cmx25cmx5cm) for 24hours at the site of the study to allow the extremely light and volatile fractions to evaporate leaving behind the stable components. Its exposure to sunlight simulates the naturally occurring condition following spillage [23].

2.2 Experimental Design

A total of 30 male Chinchilla Rabbits aged 12 to 14 weeks and weighing 1.2 to 1.45kg was used for this study. The animals were examined, treated for ectoparasites using Lymectin (Hebei New Century Pharmaceutical C0. Ltd) by a veterinarian and allowed to acclimatize for two weeks. The animals were randomly divided into five groups, each containing 6 rabbits.

The experimental group consisted of five groups, designated Group A (control), B, C, D and E. Group B to E were orally administered the doses of 15, 20, 25 and 30mg/kg body weight of the Escravos crude oil, respectively. Group A was given normal saline (00mg/kg body weight of Escravos crude oil). Due consideration was given to their body weight (those with greater body weights have their dose divided into two; one in the morning one at evening). The different doses of the liquid Escravos crude oil were measured in weight on an electronic weighing balance (210/0.1mg digital balance ESJ-210-4) and given orally (oral gavage) for 28 days. The control group was given normal saline.

2.3 Animal Treatment

The animals were kept under standard and good laboratory conditions (12hour light and 12hour darkness, temperature (30ºC±4.5ºC), humidity and ventilation). Prior to exposure, the animals were starved overnight for solid food and their body weights taken. This was also done weekly, for the duration of the study to check for weight loss or gain which is associated with toxicity. The animals were fed grower pallets (from vital feed Ltd, Jos, Plateau State, Nigeria) and water *ad libitum* for 28 days.

2.4 Animal Sacrifice, Sample Collection and Histology

On the 29th day (morning)**,** the animals were anaesthetized using cotton wool damped in chloroform with due consideration of their body weights. The blood samples, obtained by marginal ear vein puncture, were drawn into tubes using 22 gauge sterile needles. For biochemical analyses, blood samples collected into plain test tubes were centrifuged (Rotofix 32®-Hettich) at 3000g for 10 min; the serum was collected and kept at –20ºC until analysis. Animals were sacrificed; the testes excised, blotted dry to remove traces of blood and weighed using an electronic weighing balance (using 210/0.1mg digital balance ESJ-210-4). The testes were excised, grossed, fixed in 10% formal saline, processed through paraffin wax, sectioned and slices of 3µm thickness were stained using Haematoxylin and Eosin (H&E) technique [24]. Micrographs of the stained tissue sections were taken (by a microscope which had a camera attached to it) for comparison and documentation. The processing of the testes was carried out at Histopathology Unit in the Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria.

2.5 Biochemical Analysis

Serum concentration of testosterone was determined using the microplate enzyme immunoassay method (MONOBIND Inc., USA). The serum total cholesterol level was estimated using the enzymatic end point method (RANDOX Laboratories, United Kingdom) [25]. Serum concentrations of the sex hormones and total cholesterol were measured using, Mindray MR 96 ELISA machine (USA), and spectrophotometer, respectively. The experiment was carried out using the facilities of Reene Laboratories Onitsha, Anambra State, Nigeria.

2.6 Statistical Analysis

Mean values (±SD) of the sex hormones, total cholesterol, body and testicular weights were taken for analysis. The data was tested for homogeneity of variance and significantly different results were established by one-way ANOVA using the SPSS software application (version 20). Pair-wise comparisons were made using the Post hoc test. The accepted level of significance was set at $P \leq .05$. The Pearson's correlation was made to correlate the serum concentrations of testosterone and total cholesterol level, with the accepted level of significance set at 0.01.

3. RESULTS AND DISCUSSION

The insignificant increase in testes weight (Table 1) observed when the control group was compared to the treated groups could be attributed to the different histopathological findings (Fig. 3). More so, the iron deposits observed could have been the most contributing factor to the increase in testicular weight (Figs. 2, 3). This is in accordance with the findings of

Adesanya et al. [26] who reported a reduction in sperm count and motility, but with no significant difference in the relative testicular weight and sperm density in male Swiss albino rats given Bonny light crude oil (BLCO) via oral garvage. After 7days of administration, the animals in the treated groups lost their appetite, especially group D animals. Considering the fact that all the groups received approximately the same amount of food, it was observed that the treated groups had reduction in food consumption (with remnants which was approximately one-third of the entire food given) when compared to that consumed by the control group. The significant decrease in body weight observed in the treated groups (Fig.1, Table 2) could be linked to the reduction in appetite caused by the crude oil exposure and possibly the mechanism of crude oil metabolism.

Table 1. Mean ± SD and pair-wise comparison of testosterone, total cholesterol and weight of testes of the control and treated groups

Parameters	Groups					F-value	*P*-value
	Group A (control)	Group B (15mg/kg)	Group C (20mg/kg)	Group D (25 mg/kg)	Group E (30mg/kg)		
Cholesterol (mmol/l)	1.35±0.17	1.54±0.13 (0.095)	1.62±0.06 (0.025)	1.85±0.20 (0.000)	1.76±0.15 (0.001)	6.778	.003
Testosterone (ng/ml)	0.32±0.05	0.32±0.10 (0.976)	0.43±0.11 (0.161)	0.57±0.12 (0.004)	0.46±0.14 (0.066)	4.233	.017
Weight of Testis (kg)	4.80±0.40	5.40±0.80 (0.345)	6.10±0.90 (0.046)	6.30±0.90 (0.019)	6.50±0.90 (0.010)	3.042	.051

P –value is significant at P≤0.05, N=30, n=6

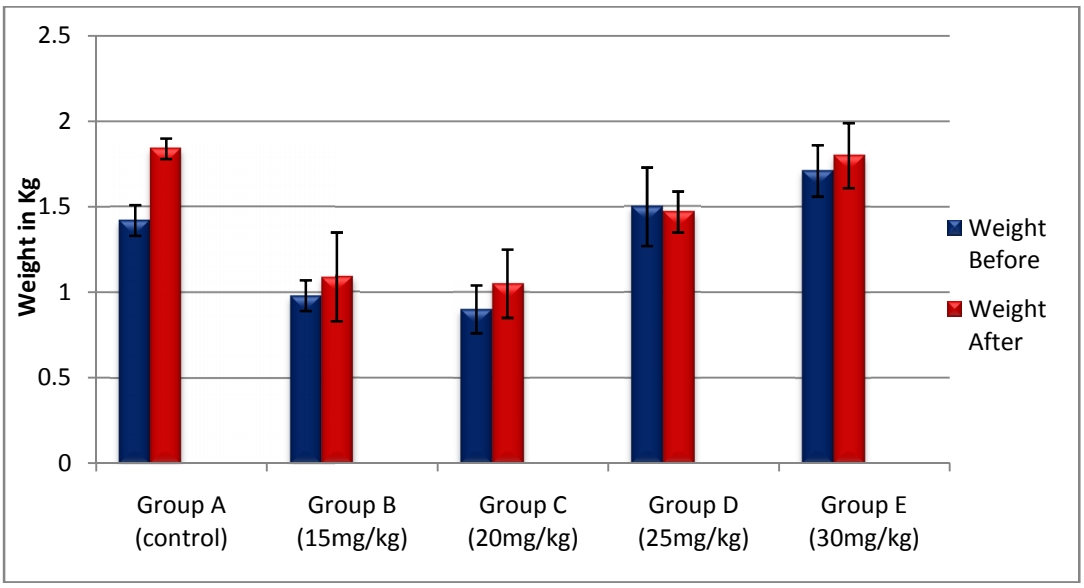

Fig. 1. A graphical comparison of the mean (±SD) weight of animals in the control and treated groups before and after the experiment (One-way ANOVA)
Mean ± SD = Mean values ± Standard Deviation of means of five experimental groups

The table above shows significant increase in the serum concentrations of total cholesterol and testosterone (*P*≤0.05) and insignificant increase in the weight of the testes (*P*≥0.05), in a

dose dependent manner when the control group was compared to the treated groups. One-way ANOVA and Post Hoc Test.

Table 2. Paired sample T-test of the weight of the animals before and after the experiment

Body Weight of animals(kg)		Mean±	SD	t	Sig.
Pair 1 (control: 00 mg/kg)	Weight After A - Weight Before A	0.41±	0.14	5.745	.01
Pair 2 (15 mg/kg)	Weight After B - Weight Before B	0.11±	0.31	0.728	.52
Pair 3 (20 mg/kg)	Weight After C - Weight Before C	0.15±	0.28	1.042	.37
Pair 4 (25 mg/kg)	Weight After D - Weight Before D	-0.03±	0.34	-0.178	.87
Pair 5 (30 mg/kg)	Weight After E - Weight Before E	0.08±	0.25	0.658	.56

P is significant at P≤0.05, N=30, n=6

The table above shows a significant increase in the weight of the control group (P≤0.05) with insignificant increases in weight of the treated groups (P≥.05). Group D actually had an insignificant decrease in weight when compared to the other groups (P≥0.05). This means that the crude oil exposure affected the weight acquisition of the treated animals since the animals were still at the developmental stage.

In this study, a significant increase in serum cholesterol concentration was observed in the treated groups (Table 1). This result is in accordance with the findings of Ngokere et al. [22] but discordant with the reports of Otitoju et al. [27] who recorded a significant decrease in serum cholesterol concentration following the administration of Bonny-light Crude oil to male albino Wistar rats.

The result of this study also showed significant increase in testosterone concentration (Table 1) which is in agreement with the result reported by Afonne et al. [28] in male Albino Wistar rats administered Chevron Escravos crude oil, but discordant with the findings of Otitoju et al. [27] who reported a decrease in serum testosterone concentrations following Bonny light crude oil administration to Albino Wistar rats. Since there was a positive correlation of testosterone with the serum concentration of cholesterol in this study (Table 3), it could be adduced that testosterone might have been abundantly synthesized from the increased concentration of cholesterol in the serum.

Table 3. Correlation between the biochemical parameters within the test groups

Variables	r-value	*p*-value	Remark
Cholesterol correlated with Testosterone	0.707**	.000	Positive correlation

***. Correlation is significant at 0.01 level (2-tailed), N=30, n=6: Group A (control: 00 mg/kg body weight Escravos crude oil), Group B (15mg/kg body weight of Escravos Crude oil), Group C (20mg/kg body weight of Escravos crude oil), Group D (25mg/kg body weight of Escravos crude oil) and Group E (30 mg/kg body weight of Escravos crude oil)*

The table above shows a significant strong positive correlation between testosterone and cholesterol concentration at 0.01 level of confidence interval. This means that serum total

cholesterol concentration increased simultaneously with testosterone concentration ($p \leq 0.01$). Pearson's correlation.

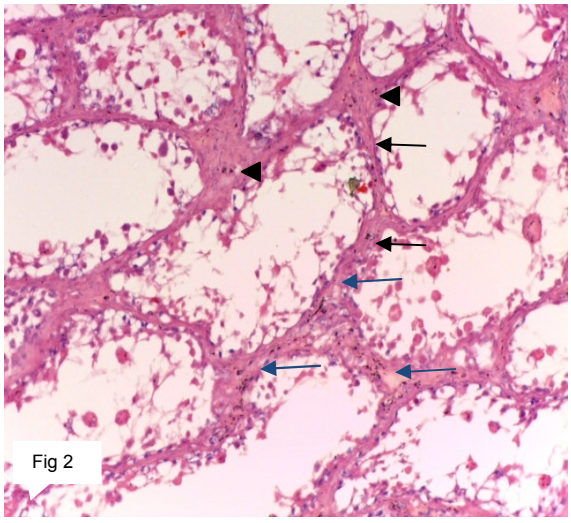

Fig. 2. Group D: A section of the testis featuring the interstitial cells (Leydig cells; marked by black arrow head) and basal lamina (marked by black arrows) with evidence of iron deposits on the basal lamina (marked by blue arrows). H&E stain. X200

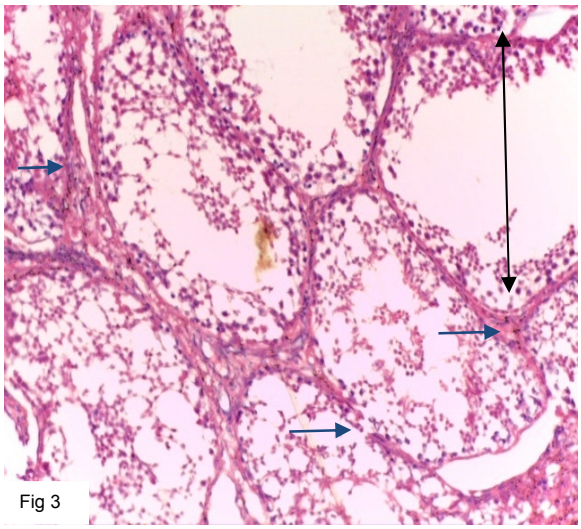

Fig. 3. Group E: A section of the testis featuring the seminiferous tubules with signs of hypertrophy (increased diameter of the seminiferous tubules-marked by the double headed arrow), necrotic basal lamina (marked by black arrow heads), iron deposits on the interstitial cells (hemochromatosis; marked by blue arrows) and generalized necrosis. H&E stain. x200

Sertoli cells are known to produce inhibin B which inhibits the production of follicle stimulating hormone (FSH) by the hypophysis [29]. Hence, a possible mechanism for the in observed increase in testosterone in this study could have been that the decrease in the number of Sertoli cells (as a result of damage) by iron deposits may have resulted in the production of more FSH leading to an increase in testosterone production. Histology of the treated groups D and E (Figs. 2 and 3), when compared with the control group and treated groups A, B and C which had no obvious pathological change, revealed hemochromatosis (iron deposits). The iron deposits could have cause the observable change in the testis (seminiferous tubules and interstitial cells) resulting in decreased spermatogenesis (Fig. 3). Hence, it could be adduced that at the concentration of 30 mg/kg body weight, the Escravos crude oil became toxic to the testes.

4. CONCLUSION

Hormones act at extremely low levels (part per trillion), therefore exposure to low levels of hormonally active agents as found in the crude oil may be of major health concern, particularly during sensitive periods of development and reproduction. Crude oil(s) extracted from different wells and locations have different chemical compositions, which may finally determine their toxicity [23]. The latter could be the reason why the effect caused by Escravos crude oil exposure seem to differ from the reported effects caused by other variants of Nigerian crude oil. Thus, Escravos crude oil is suggested to be a potential endocrine disruptor and can affect the micro-architectural integrity of the testis which can predispose exposed males to infertility if not checked.

ETHICAL APPROVAL

The experiment was conducted in accordance with the Guidelines of the U.S. National Institute of Health (NIH Publication No. 85-23, Revised 1985) and Animal Welfare Act on the care and use of laboratory animals. All procedures were examined and approved by the Faculty's ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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