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Research Article

Impact of Crude Oil-Induced Hyper-Secretion of Thyroid Hormones on Some Reproductive Hormones and Spermatozoa Quality in Wistar Rats: The Interventionist Role of *Ageratum conyzoides*

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Abstract

Restorative potentials of *Ageratum conyzoides* against Nigerian Bonnylight crude oil-induced (NBLCO) thyroid toxicity, reproductive hormonal imbalance and spermatozoa quality in Wistar rats were investigated. Twenty male Wistar rats (150-180g body weight) were randomly divided into four groups of five rats each. Group I served as the control and received 3ml/kg body weight of distilled water, group II received 748.33 mg/kg body weight of extract of *A. conyzoides*, being 20% of 3741.66 mg/kg (the LD_{50}), group III received 3ml/kg body weight of NBLCO; being 20% of 14.14 ml/kg of (LD_{50}) and group IV animals received 748.33 mg/kg and 3ml/kg body weight of extract of *A. conyzoides* and NBLCO respectively through oral gavage for 28 days. The results showed that NBLCO to group III animals significantly ($P < 0.05$) increased triiodothyronine (T_3), thyroxin (T_4), thyroid stimulating hormone (TSH), Estrogen and SHBG compared to groups I and II, but intervention with the co-administration of *A. conyzoides* extract with NBLCO to group IV significantly ($P < 0.05$) reduced T_3, T_4, TSH, Estrogen and SHBG levels. On the other hand, NBLCO to group III animals significantly ($P < 0.05$) reduced luteinizing hormone, follicle stimulating hormone, testosterone levels, sperm concentration and motility compared to groups I and II; but the co-administration of *A. conyzoides* with NBLCO to group IV significantly ($P < 0.05$) reversed the aforementioned parameters. It is evidently demonstrated in this study that altered thyroid physiology by the NBLCO administration impacted negatively on male reproduction and fertility which ethanol leave extract of *Ageratum conyzoides* intervention has demonstrated ameliorative potential.

Keywords: *Ageratum conyzoides;* Male Infertility; Crude Oil; Thyroid Hormones; Reproductive Hormones; Spermatozoa Quality

Introduction

The endocrine system is one of the known regulators of the body's physiological processes, which exerts its influence through the release of hormones. These hormones are in turn involved in signaling operation through the second messenger systems to influence organs and system functions. The aforementioned operation can be altered by the activities of hormone disruptors. Therefore, evaluation of endocrine-mediated toxicological effects of xenobiotic agents which are common in the environment due to human explorative and technological activities is important for understanding their relevance to health of human and other animals. Thyroid gland is one of the endocrine glands in the body that play

cardinal role in metabolic activities, basically by the synthesis and release of triiodothyronine (T_3) and thyroxin (T_4). These metabolic hormones are essential for growth, as well as the development and differentiation of many cells in the body [1]. Considering the important roles of these hormones in the maintenance of normal physiology, interference by chemical substances with endocrine-disrupting tendencies therefore can impact negatively on thyroid gland function with adverse consequences on developmental events, metabolism, and general physiology of tissues, organs and systems of the body, including the reproductive system [2]. Such adverse effects on the reproductive system may have deep consequences on fecundity.

One of the major health challenges facing human society is infertility, which is the inability of a sexually active, non-contraceptive couple to achieve spontaneous pregnancy in one year [2,3]. Although, infertility in male may have a multifactorial etiology. Hormonal balance and semen quality are vital to the male reproductive health, as initiation, growth, development and indeed maintenance of male reproductive function depended so much on hormonal balance in the body. Evaluations of the positive associations between circulating hormone levels and semen quality have been largely conducted on fertile and infertile men [4-8].

Triiodothyronine (T_3) and thyroxine (T_4) act on nearly every cell in the body including testicular cells, where they are primarily responsible for regulation of the metabolism, protein synthesis and growth within the testicular tissues. These hormones were previously believed not to have any significant role in the process of sperm formation and indeed male fertility, but are now being recognized as having important role in male reproductive functions [1,2,9]. In fact, the link between thyroid hormones and reproductive hormones has been clearly demonstrated by studies where distortions in thyroid gland physiology in animal models were shown to have decreased sexual activity and fertility [2,9]. This link is further strengthened by the fact that both primary hyperthyroidism and hypothyroidism have been documented to produce varied degrees of gonadal dysfunction in both sexes [10-12].

Hypothyroidism by induction or occurring soon after birth has been demonstrated to be associated with significance sexual maturation and developmental delays in animals [13], indicated by decrease testicular size, retardation in Sertoli cell differentiation, and prolongation of Sertoli cell proliferation time [13], decreased testosterone concentration as well as an absence of *libido* and ejaculation [14,15]. Among the studies on human subjects, Hernandez., *et al*. (1990) [16] reported that hypothyroidism adversely affected semen quality by compromising semen volume, sperm morphology and sperm motility [17].

Hyperthyroidism on the other hand, has also been reported to adversely impact spermatogenesis by altering sex steroid levels [18], reduce sperm concentrations and decreased motility in patients. These workers postulated that there could be a possible synergy between thyroid hormones and sperm quality as the aforementioned abnormalities were corrected upon treatment.

In the Niger Delta region of Nigeria, the rate of occurrence of environmental contamination by crude petroleum and its derivatives due to petroleum exploitation and exploration is alarming. Humans in this region may be inadvertently exposed to crude petroleum and its refined products reaching them through spillages, pipeline vandalization and other Petroleum industrial related activities. Such exposure is associated with free radical producing agents resulting in oxidative stress, as a major injury causative agent to the spermatozoa, and ultimately lowering fertility ability of male [19-23]. There are myriads of documented proofs that some xenobiotic agents like the NBLCO causes injuries to cells, organs and tissues by induction of oxidative stress, lipid peroxidation and inflammation resulting in membrane damages, denaturing of protein, destruction of DNA and RNA [24].

Research has shown that such damages can be mitigated by efficient antioxidant interventions. Where such damages can be minimized or prevented or even eliminated by bioactive antioxidant compounds. These antioxidant compounds are readily obtainable from natural plant sources [25], which, *Ageratum conyzoides* is one of such. *Ageratum conyzoides* has long been known in herbal or folk medicine as a remedy for diverse ailments in Africa [26]. Numerous phytochemical screenings of *A. conyzoides* have shown the presence of alkaloids, resins, saponins, tannins, glycosides and flavonoids [26]. Pharmacological investigations have verified that its antioxidant efficacy is associated with its high flavonoid content [27].

The present study was designed to evaluate the impact of crude oil-induced thyroid hormones imbalance on some reproductive

hormones and spermatozoa quality in Wistar rats and the role of *Ageratum conyzoides* intervention.

Materials and Methods

The crude petroleum used in this study was obtained from the Nigerian National Petroleum Corporation (NNPC), Port Harcourt, Nigeria. Chemical and reagents used were purchased directly from the manufacturers through their accredited outlets. Such manufacturing companies are Sigma Aldrich, USA Sigma Aldrich and Merck Germany, Juhel Nigeria Limited, Taj Pharmaceutical Mumbai, India and Jiaxhu-Shaghaou Shanghal, China. Distilled water used was prepared by distillation at Derindam Research Institute of Biotechnology, Uyo, Akwa Ibom, Nigeria.

Collection of Plant material

The whole plant was obtained from the Botanical farm of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo, Nigeria. Specimen of the leaves was identified and authenticated by an expert in the Department of Botany and Ecological Studies, University of Uyo, Uyo. A voucher specimen (UUH 3517) was deposited at the Herbarium.

Preparation of Leave extract

The leaves of *A. conyzoides* were rinsed with distilled water and dried under shade. The dried leaves were ground into powder with an electric blender. Four hundred grammes of the blended leaves sample was macerated in 700ml of 70% ethanol agitated for 10 minutes with an electric blender and left overnight in a refrigerator at 4°C. The mixture was filtered with No. 1 filter paper and the filtrate concentrated under reduced pressure using a rotary evaporator (at 37°C) to about 10% of original volume. The concentrate was then allowed in a water bath at 37°C for complete evaporation to dryness yielding 40.64g (10.15%) of the extract.

Acute toxicity test

Acute toxicity study (LD_{50}) was estimated using Lorke's method [28]. A total of 25 mice weighing between 15-22g were divided into five groups with five mice each. Mice in the five groups were administered 3000 mg/kg, 3500 mg/kg, 4000 mg/kg, 4500 mg/kg and 5000ml/kg of body weight respectively (intraperitoneally). All experimental animals were observed for physical signs of toxicity such as gasping, palpitation, writhing, decreased respiratory rate, and death after 24 hours.

The median lethal dose of *Ageratum conyzoides* was calculated as geometrical means of the maximum (most tolerable) dose producing 0% mortality (a) and the minimum (least tolerable) dose producing 100% mortality (b) using the formula:

$LD_{50} = \sqrt{ab}$

$LD_{50} = \sqrt{3500 \times 4000}$

$= 3741.66$ mg/kg

The acute toxicity test for the NBLCO also involved 25 mice weighing between 15-22g were divided into five groups with five mice each. Mice in the five groups were administered intraperitoneally 10ml/kg, 15ml/kg, 20ml/kg, 25ml/kg and 30ml/kg of body weight respectively.

$LD_{50} = \sqrt{10 \times 20}$

$= 14.14$ml/kg

Experimental animals

Twenty male Wistar rats (150-180g body weight) were obtained from the Animal House of the Faculty of Basic Medical Sciences University of Uyo, Uyo, Nigeria and were kept in a well-ventilated section of the Animal House. They were allowed access to feed (Chow: vital feeds, Grand Cereals Ltd, Jos) and water *ad libitum*. The animals were kept in separate experimental room and allowed to acclimatize for a period of one week before commencement of studies.

Experimental design and treatment of animals

A total of twenty male Wistar rats (150-180g body weight) were randomly divided into four groups of five rats each. The rats in group I served as the control and were oral gavage 3ml/kg body weight of distilled water, group II animals were oral gavage 748.33 mg/kg body weight of the extract of *A. conyzoides*, being 20% of 3741.66 mg/kg (the LD_{50}), group III was gavaged 3ml/kg body weight of NBLCO; this dose was calculated as 20% of the lethal dose of 14.14 ml/kg and group IV animals were co-administered 748.33 mg/kg and 3ml/kg body weight of *A. conyzoides* and NBLCO respectively. In all cases, doses were applied int the morning hours daily for 28 days according to animal's most recent body weight. The experimental procedures involving the animals and their care were conducted in conformity with the approved guidelines by the

Research and Ethical Committee of the Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria.

Collection of blood sample for analysis

After 28 days of administration, the rats were anaesthetized with sodium pentobarbital at 50 mg/kg of body weight intraperitoneally. Blood was collected by cardiac puncture with a 5 ml sterile syringe and needle. The total volume of blood collected was 3.5 ml, which was transferred into plain sample bottles. This was allowed to stand for 2 hours to clot after which the serum was separated by centrifugation (RM-12 micro centrifuge, REMI, England) at 3000 rpm for 10 minutes at 25°C. The serum obtained was stored at –20°C until required for analysis.

Triodothyronine Elisa assay

The assay was performed at room temperature (18-25◦C). The Sandwich-ELISA method was adopted as all reagents, working standards and samples were prepared according to the manufacturer's instructions. The optical density (OD) was measured spectrophotometrically using microplate reader at a wavelength of 450 nm. The OD value was proportional to the concentration of T_3.

Thyroxine Elisa assay

The assay was performed at room temperature (18-25◦C). The Sandwich-ELISA method was adopted as all reagents, working standards and samples were prepared according to the manufacturer's instructions. The optical density (OD) was measured spectrophotometrically using microplate reader at a wavelength of 450 nm. The OD value is proportional to the concentration of T_4.

Thyroid stimulating hormone elisa assay

The assay was performed at room temperature (18-25◦C). The Sandwich-ELISA method was adopted as all reagents, working standards and samples were prepared according to the manufacturer's instructions. The optical density (OD) was measured spectrophotometrically using microplate reader at a wavelength of 450 nm. The OD value is proportional to the concentration of TSH.

Sex hormone binding globulin elisa assay

The assay was performed at room temperature (18-25◦C). The Sandwich-ELISA method was adopted as all reagents, working standards and samples were prepared according to the manufacturer's instructions. The optical density (OD) was measured spectrophotometrically using microplate reader at a wavelength of 450 nm. The OD value is proportional to the concentration of SHBG.

Seminal analysis using computer aided system (CASA)

The cauda epididymis from each side of the testes was dissected out and several small cuts of about 1mm made and the tissue suspended in 1m of buffered formal saline to allow the spermatozoa to swim up. The assessment of sperm motility was done using Computer-Assisted Semen Analysis (CASA) in accordance with WHO criteria and the method described by Breanna Tilley [29,30]. The following measurements were obtained from the Total cell detected and was used for the computation of other patterns of motility, these included Total cell in sample (10^6/ml), Concentration (10^6/ml). Motile sperm, Motile sperm rate %, Concentration of motile sperm (10^6/ml), Amplitude of Lateral Hunting (ALH) µm, Wobbling (WOB) rate %, Beating Cilia Frequency (BCF) in Hz, Linearity (LIN) in %, Straight forward line (SFR) %, Progressivity (PR), Non-progressive, Immotility (IM), Velocity of Active Path (VAP) µm/s, Velocity of Curve Line (VCL) in µm/s, Velocity of Straight Line (VSL) in µm/s, Sperm of Curve moving, Sperm of Line moving, Sperm of Line moving fast, Concentration of curve moving (x10^6/ml), Concentration of line moving (106/ml), Concentration of line moving fast (10^6/ml), Percentage of curve movement (%), Percentage of curve movement (%), Percentage of line moving (%), Percentage of line fast (%), Rate of line moving (%), Rate of line moving fast (%) Morphology count: Normal Cell, Normality Rate (%), Anomality Rate (%), Defects, Anomality of Head, Head Anomality Rate (%), Anomality of Body, Body Anomality Rate (%), Anomality of Tail, Tail Anomality Rate (%), Mixed Anomality, Mixed Anomality Rate (%),Red Blood Cell (RBC), White Blood Cell (WBC), Epithelium, Spermatocyte.

Statistical analysis

Data were expressed as the mean ± standard error of the mean. Statistical analysis was carried out using window Statistical Package for Social Science (SPSS 23.00 version). The statistical method adopted was One way Analysis of Variance (ANOVA), results obtained were further subjected to test for least significant difference (LSD). Values of $P < 0.05$ were considered significant.

Results

Thyroid hormones following treatment with ethanol leave extract of *Ageratum conyzoides* and Nigerian Bonnylight crude oil

The mean value of serum thyroid stimulating hormone (TSH) in (ng/ml) obtained were: 0.58 ± 0.04, 0.46 ± 0.04, 5.60 ± 0.15 and 3.43 ± 0.24 for groups I, II, III and IV respectively. The administration of NBLCO to group III animals significantly ($P < 0.05$) increased serum level of TSH compared to groups I and II (control and extract). But co-administration of *Ageratum conyzoides* with NBLCO significantly ($P < 0.05$) reduced serum TSH level compared to group III, as presented in Figure 1.

The mean values of serum triiodothyronine in (ng/ml) obtained were: 55.20 ± 2.67, 69.80 ± 0.86, 147.25 ± 1.11 and 104.75 ± 1.55 for groups I, II, III, and IV respectively. The administration of NBLCO to group III animals significantly ($P < 0.05$) increased serum T_3 level compared to groups I and II (controlled and extract). But the co-administration of ethanol extract of *Ageratum conyzoides* with NBLCO significantly ($P < 0.05$) reduced T_3 level compared to group III, as presented in Figure 2.

The mean value of serum thyroid hormone (T_4) in (ng/ml) obtained were: 1.32 ± 0.06, 1.18 ± 0.11, 6.83 ± 0.26 and 3.40 ± 0.23 for groups I, II, III and IV respectively. The administration of NBLCO to group III animals significantly ($P < 0.05$) increased T_4 level compared to groups I and II (control and extract). But the co-administration ethanol extract of *Ageratum conyzoides* with NBLCO significantly ($P < 0.05$) reduced T_4 level compared to group III, as presented in Figure 3.

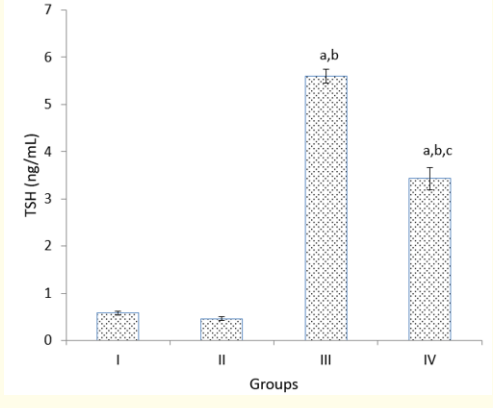

Figure 1: Comparing thyroid stimulating hormone (TSH) concentration in NBLCO and extract of *Ageratum conyzoides*.

a = versus group I, b = versus group II and c = versus group III at $p < 0.05$.

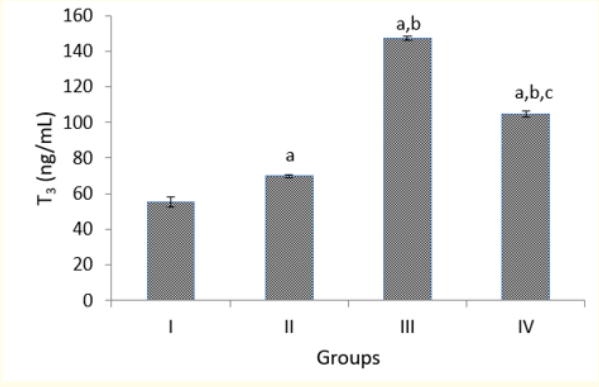

Figure 2: Comparing triiodothyronine (T3) concentration in NBLCO and extract of Ageratum conyzoides.

a = versus group I, b = versus group II and c = versus group III at $p < 0.05$.

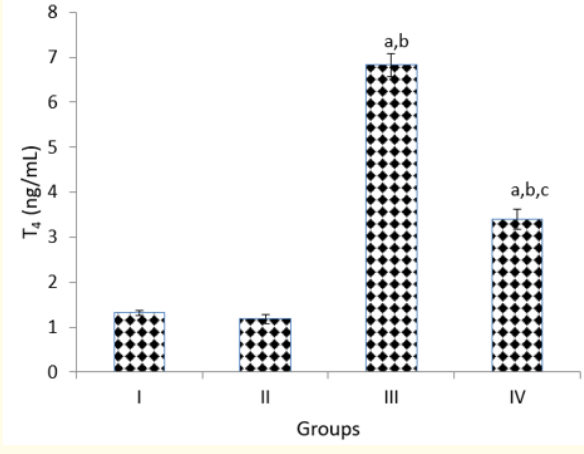

Figure 3: Comparing tetraiodothyronine (T4) concentration in NBLCO and extract of *Ageratum conyzoides*.

a = versus group I, b = versus group II and c = versus group III at $p < 0.05$.

Estrogen following treatment with ethanol leave extract of *Ageratum conyzoides* and Nigerian Bonnylight crude oil

The mean values of serum estrogen in (ng/mg) obtained were: 91.00 ± 1.05, 88.20 ± 1.46, 191.00 ± 2.42 and 101.75 ±1.8 for I, II, III and IV respectively. The administration of NBLCO to group III animals significantly ($P < 0.05$) increased serum estrogen level compared to groups I and II. But co-administration of ethanol extracts of *Ageratum conyzoides* with NBLCO to group IV animals significantly ($P < 0.05$) reduced estrogen level compared to group III, as presented in Figure 4.

The sex hormone binding globulin (SHBG) following treatment with ethanol leave extract of *Ageratum conyzoides* and Nigerian Bonnylightcrude oil

The mean value of serum sex hormone binding globulin (SHBG) in (ng/ml) obtained were: 127.00 ± 1.05, 104.00 ± 1.52, 359.00 ± 3.45 and 141.5 ± 0.65 for groups I, II, III and IV respectively. The administration of NBLCO to group III animals significantly($P < 0.05$) increased serum SHBG compared to groups I and II respectively. But co-administration of ethanol extract of *Ageratum conyzoides* with NBLCO to group IV animals significantly ($P < 0.05$) reduced the SHBG level compared to group III, as presented in Figure 5.

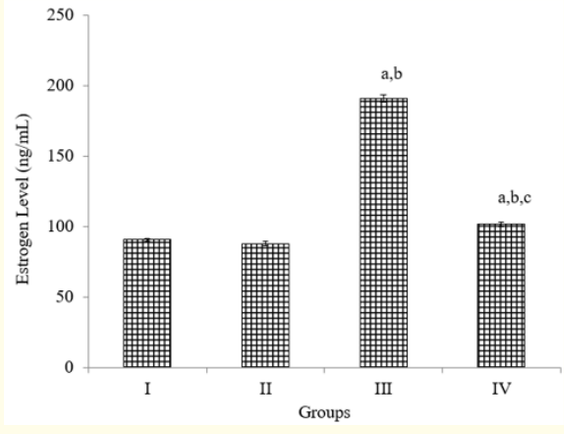

Figure 4: Comparing estrogen levels in NBLCO and extract of *Ageratum conyzoides*.

a = versus group I, b = versus group II and c = versus group III at $p < 0.05$.

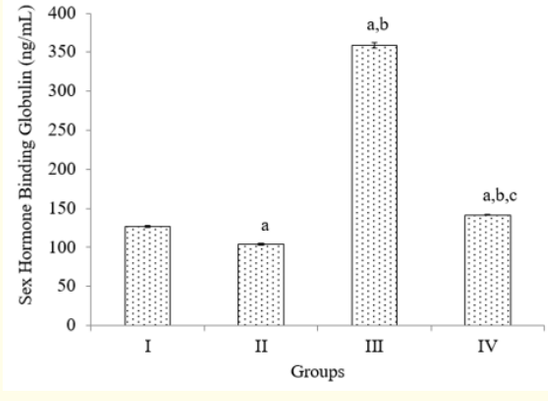

Figure 5: Comparing sex hormone binding globulin (SHBG) concentration in NBLCO and extract of *Ageratum conyzoides*.

a = versus group I, b = versus group II and c = versus group III at $p < 0.05$.

Luteinizing hormone following treatment with ethanol leave extract of *Ageratum conyzoides* and Nigerian Bonnylight crude oil

The mean values of serum luteinizing hormones (LH) in (ng/ml) obtained were: 5.74 ± 0.19, 5.62 ± 0.18, 2.76 ± 0.27 and 5.70 ± 0.24 (ng/ml) for groups I, II, III and IV respectively. The administration of NBLCO to group III animals significantly ($P < 0.05$) reduced LH level compared to groups I and II (control and extract) but co-administration of ethanol extract of *Ageratum conyzoides* with NBLCO to group IV animals significantly ($P < 0.05$) increased LH level compared to group III, as presented in Figure 6.

Follicle stimulating hormone (FSH) following treatment with ethanol leave extract of *Ageratum conyzoides* and Nigerian Bonnylight crude oil

The mean values of serum follicle stimulating hormone (FSH) in (ng/ml) obtained were: 71.10 ± 1.26, 80.20 ± 1.18, 28.25 ± 1.60 and 97.50 ± 1.71 (ng/ml) for groups I, II, III and IV respectively. Administration of NBLCO to group III animals significantly ($P < 0.05$) reduced FSH level compared to groups I and 2. But co-administration of ethanol extract of *Ageratum conyzoides* with NBLCO to group IV animals significantly ($P < 0.05$) increased FSH level compared to group III, as presented in Figure 7.

Testosterone following treatment with ethanol leave extract of *Ageratum conyzoides* and Nigerian Bonnylight crude oil.

The mean values of serum testosterone in (ng/ml) obtained were: 1.20 ± 0.06, 1.70 ± 0.15, 0.18 ± 0.01 and 0.99 ± 0.03 for groups I, II, III and IV respectively. Administration of NBLCO to group III animals significantly ($P < 0.05$) reduced testosterone level. But the co-administration of *Ageraturm conyzoides* extract with NBLCO to group IV animals significantly ($P < 0.05$) increased testosterone level compared to group III, as presented in Figure 8.

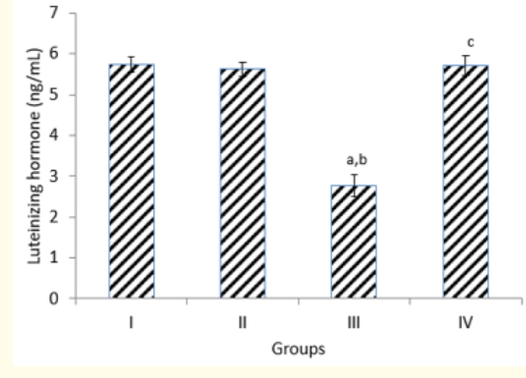

Figure 6: Comparing the luteinizing hormone in NBLCO and extract of *Ageratum conyzoides*.

a = versus group I, b = versus group II and c = versus group III at $p < 0.05$.

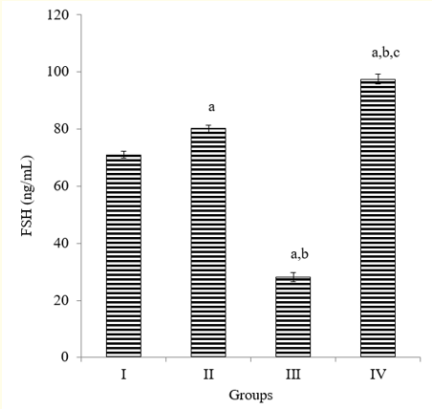

Figure 7: Comparing follicle stimulating hormone (FSH) concentration in NBLCO and extract of *Ageratum conyzoides*.

a = versus group I, b = versus group II and c = versus group III at p < 0.05.

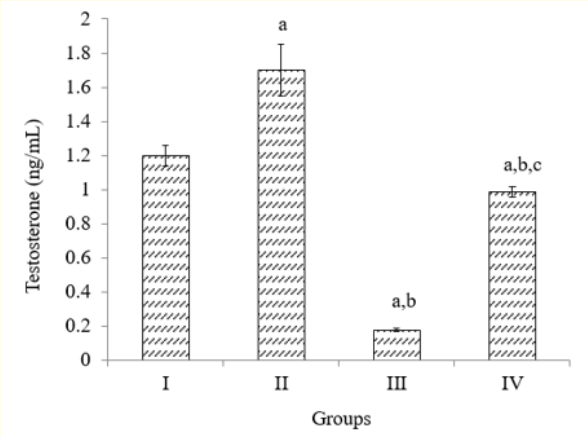

Figure 8: Comparing testosterone concentration in NBLCO and extract of *Ageratum conyzoides*.

a = versus group I, b = versus group II and c = versus group III at p < 0.05.

Sperm concentration following treatment with ethanol leave extract of *Ageratum conyzoides* and Nigerian Bonnylight crude oil

The results obtained following administration of ethanol leave extract of *Ageratum conyzoides* and Nigerian Bonnylight crude oil are presented in Table 1. The results showed that administration NBLCO to group III animals significantly (P < 0.05) reduced the percentage of total sperm cells detected (TSCD) and total sperm cell count (TSCC). This trend was reversed as co-administration ethanol leave with extract of *Ageratum conyzoides* to group IV animals significantly (P < 0.05) increased TSCD and TSCC.

Spermatozoa movement following treatment with ethanol leave extract of *Ageratum conyzoides* and Nigerian Bonnylight crude oil

The results as presented in Table 2 showed that administration of NBLCO to group III rats significantly (P < 0.05) reduced all indices for motility and progression of sperm cells, as TMS, PMS, PR, VAP, VCL, VSL, BCF, PLM, LIN and SFL were all significantly (P < 0.05) reduced. While indices for non-motility and non-progression of sperm cells; NP, ALH, WR, MAD, and IM were significantly (P < 0.05) elevated. These trends were significantly (P < 0.05) reversed following co-administration of ethanol leave extract of *A. conyzoides*.

Groups	TSCD (10^6/ml)	TSCC (10^6/ml)
I (Control)	82.60 ± 0.93	74.80 ± 1.39
II	85.20 ± 1.32	78.80± 1.59
III	$42,50 \pm 1.66^{a,b}$	$52.00 \pm 1.68^{a,b}$
IV	$62.00 \pm 1.47^{a,b,c}$	$71.50 \pm 1.44^{b,c}$

Table 1: Spermatozoa concentration following treatment with of ethanol leave extract of *Ageratum conyzoides* and NBLCO.

Legend: a, b and c = significantly different from groups I, II, III and IV respectively (P < 0.05). TSCD = total sperm cell detected, TSCC = total sperm cell concentrated.

Groups Parameters	I	II	III	IV
TMS (%)	66.80 ± 1.39	78.80 ± 1.59^{a}	$52.00 \pm 1.68^{a,b}$	$71.50 \pm 1.44^{a,b,c}$
PMS (%)	82.60 ± 1.29	71.60 ± 1.03^{a}	$44.25 \pm 1.31^{a,b}$	$59.50 \pm 0.50^{a,b,c}$
PR (%)	70.80 ± 0.86	77.40 ± 1.63^{a}	$35.25 \pm 1.25^{a,b}$	$71.50 \pm 1.44^{a,b,c}$
NP	15.00 ± 0.84	13.60 ± 0.60	$36.50 \pm 1.19^{a,b}$	$64.75 \pm 1.93^{a,b,c}$

VAP(um/s)	1.52 ± 0.10	2.44 ± 0.11[a]	0.45 ± 0.01[a,b]	1.25 ± 0.09[a,b,c]
VCL(µm/s)	0.41± 0.06	0.83 ± 0.03[a]	0.10 ± 0.00[a,b]	0.77 ± 0.03[a,b,c]
VSL(µm/s)	1.25 ± 0.02	1.21 ± 0.05[a]	0.22 ± 0.04[a,b]	1.01 ± 0.02[a,b,c]
ALH (µm/s)	0.05 ± 0.01	0.03 ± 0.01[a]	0.41 ± 0.04[a,b]	1.05 ± 0.13[a,b,c]
BCF (Hz)	1.20 ± 0.08	1.10 ± 0.24[a]	0.40 ± 0.07[a,b]	11.50 ± 0.65[a,b,c]
PLM (%)	15.20 ± 0.37	19.80 ± 0.80[a]	3.00 ± 0.41[a,b]	58.75 ± 0.95[a,b,c]
LIN (%)	67.60 ± 1.29	74.60 ± 1.47[a]	36.50 ± 2.33[a,b]	14.75 ± 0.48[a,b,c]
SFL (%)	23.00 ± 1.14	38.80 ± 0.80[a]	24.25 ± 1.38[a,b]	6.75 ± 0.75[a,b,c]
WR (%)	4.60 ± 0.51	2.80 ± 0.37[a]	20.75 ± 1.11[a,b]	5.50 ± 0.29[a,b,c]
MAD	4.80 ± 0.37	3.60 ± 0.68[a]	6.50 ± 0.65[a,b]	5.75 ± 0.85[a,b,c]
IM (%)	5.00 ± 0.55	4.60 ± 0.40[a]	15.00 ± 0.58[a,b]	4.50 ± 0.96[a,b,c]

Table 2: Spermatozoa movement after treatment with varied doses of ethanol leave extracts of *A. conyzoides* and NBLCO.

Legend: a, b and c = significantly different from groups II, III and IV respectively ($P < 0.05$).

TMS = total motile sperm, PMS = percent of motile sperm, PR = progressivity, NP = non-progressivity, VAP = velocity of active path, VCL = velocity of curve line, VSL = velocity of straight line, ALH = amplitude of lateral head, BCF = beating cilia frequency, PLM = percent of line moving, LIN = linearity, SFL = straight forward line, WR = wobbling rate, MAD = mean move angle degree, IM = immobility.

Discussion

Environmental pollution by non-biodegradable contaminants such as crude oil has become a source of concern to health experts all over the world including Nigeria, as the Niger Delta region of the country is constantly exposed to crude petroleum and petroleum products [31].

Evidences abound that crude oil has the capacity to induce oxidative stress as well as apoptosis by inducing formation of reactive oxygen species (ROS) and inhibiting the efficiency of endogenous antioxidant defense mechanism [32]. ROS at physiological level is intimately involved in regulation of sperm capacitation, and yet their continued generation can overwhelm the limited antioxidant defense capacity to protect these sex gametes, giving rise to a state of oxidative stress [33].

The results of the study showed significant elevations in the levels of TSH, T_3, T_4, estrogen and sex hormone binding globulin (SHBG) following oral administration of Nigerian Bonnylight crude oil (NBLCO). Though, the mechanisms behind such elevations were not investigated in the present study, it appears to be operating through oxidative reaction process as administration of crude oil or its refined products have been reported to induce oxidative stress in the testis by elevating free radical formation while suppressing endogenous antioxidant defense system [34].

Thyroid hormones within physiological limit are essential for the normal functioning of the reproductive system, but distortion in the concentration of these hormones is associated with adverse effects on the health and general physiology of the testes. This will affect particularly spermatogenesis, spermatozoa quality and therefore semen quality with adverse effect on the male fertility [35,36]. This is even more worrisome as crude oil administration was actually observed in this study to have significantly reduced levels of LH, FSH and testosterone in circulation. The reduction in levels of the circulated sex hormones (LH, FSH and testosterone) reported in this study is suggestive of NBLCO-induced endocrine disruptive tendency which might cause reproductive dysfunctions regarding the significant role of these sex hormones in spermatogenic process.

The reported elevated estrogen level in this study corroborates documented evidence of estrogenic tendency of NBLCO reported by Ita., *et al.* [34]. The NBLCO appeared to have a stimulatory effect on the thyroid gland with elevated T_3 and T_4 and estrogen production. It appeared NBLCO may have exhibited a thyrotoxic effect on thyroid gland resulting in elevated thyroid hormones as reported in this study, which may probably be responsible for the elevated

estrogen levels also reported here. This observation therefore corroborates the postulation by Ridgway., *et al*. [37], where these workers reported enhanced production rate of estrogen as well as increment of peripheral conversion of androgen to estrogen to increase circulating estrogen level in thyrotoxic men.

The administration of NBLCO is reported in this study to increase SHBG level in circulation. Other than the direct effect of NBLCO on the production of testosterone, there could also be an indirect effect acting through SHBG which may account for the significantly low testosterone level recorded in this study. SHBG is a binding protein with high affinity for dihydrotestosterone and testosterone; and thus regulates their biological availability in the circulation [38]. In addition to the hormonal imbalance recorded, the results of this study also showed NBLCO administration could impact adversely on male fertility as it reduces both sperm quality and motility indices. These findings agree with previous documented findings in literature by Ita., *et al*. [34].

The significantly low total sperm detected (TSCD) and low total sperm cell concentrated (TSCC) recorded in this study could probably be attributed to the elevated SHBG level. It is possible to suggest that SHBG may have indirectly suppressed sperm production as the majority of testosterone is locked up in the binding protein SHBG resulting in insufficient testosterone available for biological action. It therefore follows that elevated SHBG levels could imply less bioavailability of testosterone with less bioactive effect. This could possibly lead to a reduction in the involvement of testosterone in sperm production and androgenization, which may have serious implication for developing male fetus in particular.

It will not be out of point to speculate that NBLCO administration act as a thyrotoxic agent and could induce excessive thyroid hormones production and secretion (hyperthyroidism), particularly when there is evidential proof that elevation of thyroid hormones may increase SHBG levels [38].

This study reported that when *Ageratum conyzoides* was co-administered with NBLCO, the observed negative trend on hormonal values was reversed. The ethanol leaves extract of *Ageratum conyzoides* intervention significantly reversed the hormonal imbalances as well as the sperm quality. The phytochemistry of *Ageratum conyzoides* showed the plant to be a good reservoir of antioxidant; polyphenol and flavonoid. These are good scavengers of free radicals and many documented reports in literature have demonstrated the effects of flavonoid on various super-oxide generating systems [39,40]. These effects may depend on the inhibition of the enzymes responsible for free radical production [39,40].

Conclusion

It is evidently demonstrated in this study that administration of NBLCO caused hormonal imbalance of both thyroid and some reproductive hormones; reduced sperm quality, which could probably impact adversely on male fertility. The aforementioned effects were ameliorated with the intervention of ethanol leaves extract of *Ageratum conyzoides*.

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