Nigerian Journal of Pharmaceutical and Biomedical Research
Vol. 10 Issue.2 August, 2026. p-ISSN: 2579-1419 e-ISSN: 2814-1423

NAUCLEA LATIFOLIA ETHANOL STEM BARK EXTRACT PROTECTS AGAINST CRUDE OIL-INDUCED MALE REPRODUCTIVE DYSFUNCTION AND RENAL DAMAGE IN WISTAR RATS

Ugwuanyi, Kingsley Chijioke[1], Amoke, Ofoma Cornelius[1], Uroko, Robert Ikechukwu[2], Oji, Amarachukwu Ezinne[1], Nwauzoma, Augustine Promise[3]

[1]Department of Zoology and Environmental Biology, College of Natural Sciences, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.
[2] Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.
[3]Department of Human Physiology, Basic Medical Sciences, University of Uyo, Akwa-Ibom State, Nigeria.
Correspondence Email: ugwuanyi.kingsley@mouau.edu.ng, kingugwuanyicj@gmail.com
Telephone: 09157533381, DOI 10.5281/zenodo.24221514

ABSTRACT

Exposure to Nigerian Bonny Light Crude Oil (NBLCO) is associated with male reproductive dysfunction and renal damage. This study investigates the protective effect of *Nauclea latifolia* stem bark extract (*NLSB-extract*) against NBLCO-induced male reproductive dysfunction and renal damage in rats. The plant extract and NBLCO were subjected to seven days lethal dose (LD_{50}) test. Twenty-five Wistar rats were randomly divided into five groups (n=5 per group). Group I was the control and received 3.5 mL/kg distilled water (DS), group II was administered 500 mg/kg *NLSB-extract*, group III received 2.8 mL/kg NBLCO (20% LD_{50}) only, group IV was co-administered 2.8 mL/kg NBLCO + 500 mg/kg, and group V co-administered 2.8 mL/kg NBLCO + 1000 mg/kg *NLSB-extract* orally for 28 days via oral gavage. LD_{50} values for the extract and NBLCO were found to be 6708.20 mg/kg and 14.14 mL/kg respectively. Group III had significantly ($P < 0.05$) reduced levels of testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH), sperm count, sperm motility and morphology compared with groups I and II, but co-administration of NBLCO and *NLSB-extract* to groups IV and V significantly ($P < 0.05$) increased the levels of aforementioned parameters in a dose-dependent manner relative to group III. Administration of NBLCO to group III rats significantly ($P < 0.05$) elevated the levels of serum blood urea nitrogen (BUN), creatinine (Cr), sodium ion (Na^+), potassium ion (K^+), and calcium ion (Ca^{2+}) compared with groups I and II, but co-administration of NBLCO and *NLSB-extract* to groups IV and V significantly ($P < 0.05$) reduced the levels of the above-mentioned renal markers compared with group III. In this study, it has evidently been demonstrated that NBLCO exposure impairs male reproductive potential, and causes renal damage, which were ameliorated by the administration of *Nauclea latifolia* ethanol stem bark extract.

Key words: *Nauclea latifolia*, crude oil, male reproductive hormones, renal markers, semen quality, Wistar rat

INTRODUCTION

Semen quality parameters including sperm motility and counts have been steadily decreasing for over three decades globally. In fact, five in every one hundred sexually matured males are currently facing the problem of compromised fertility. Hence, increased application of assisted reproductive means to about nine percent in every year (Ravitsky *et al*., 2019). Male fertility is heavily dependent on the levels of male hormones and semen quality as hormonal balance is needed for the development and sustenance of male reproductive potential. Chemical compounds including crude oil impair male reproductive ability by destroying the leydig and sertoli cells, and renal system via several pathological processes including lipid peroxidation, inflammation and oxidative damage (Ita *et al*., 2026).

NBLCO is a naturally occurring source of premium products that is formed under earth crust and composed of hydrocarbons including paraffins and naphthene. It is undoubtedly a very important naturally occurring product due to its economic benefits and has over the years been Nigerian's number one commercial product. Crude oil occupies the central position among all naturally occurring products in terms of sustaining the nation's financial integrity, and remains the most important source of foreign exchange earnings in Nigeria (Hebrew *et al.*, 2026). However, in the Niger Delta region of Nigeria where crude oil exploration and exploitation predominantly take place, the extent at which environmental assault by this xenobiotic and its derivative occur is frightening (Ita *et al.*, 2026).

Exploration and exploitation activities of Nigerian bonny light crude oil over the years have been associated with serious environmental degradation including contamination of water bodies necessary for agricultural activities and consumption. This occurs as a result of illegal oil refining activity, lack of proper waste disposal, destruction of buried pipelines and gas flaring by local and foreign companies within the local communities (Hebrew *et al.*, 2026). People living in the communities where environmental pollution by NBLCO is common especially those in the Niger Delta region of Nigeria constantly face serious health risks. Exposure to chemical components of crude oil including polycyclic aromatic hydrocarbon, xylene, hydrogen sulfide and benzene has been implicated in many pathophysiological conditions such as cancer, cardiovascular diseases, diabetes (Krajnak *et al.*, 2023; Jayaraman *et al*., 2025).

In the body system of an animal, xenobiotic agents including NBLCO undergo metabolism through cytochrome-dependent oxidation or glutathione conjugation pathway producing highly reactive and unstable metabolites that bind biological macromolecules and trigger oxidative stress, inflammatory reactions as well as apoptosis (Dorsey *et al*., 2023). Despite the growing reports on the systemic toxicity associated with crude oil exposure, environmental degradation by NBLCO majorly through gas flaring by petroleum companies, and pipeline destruction by bunkers is still a common practice in the oil-rich due to its enormous economic benefits (Elehinafe *et al*., 2022). Therefore, there is serious need for the identification of natural therapeutic agents that have the potentials to protect against the male

reproductive system damaging effect associated with NBLCO.

Findings from previous researches have shown that pathophysiological process triggered by crude oil can be prevented, reduced to a minimum level and possibly eradicated through the use of bioactive compounds that possess anti-inflammatory and antioxidant potentials (Altanam *et al*., 2025; Ita *et al*., 2026). These bioactive compounds including phenols and flavonoids are heavily present in natural plant products (Nwozo *et al*., 2023).

Among natural plants of great medicinal importance due to the presence of antioxidant and anti-inflammatory bioactive is *Nauclea latifolia* (Adepoju *et al*., 2024). *Nauclea latifolia*, which is commonly referred to as African peach, is a tree or shrub of Rubiaceae family, and can grow up to 198 meters tall (Balogun et al., 2013). *Nauclea latifolia* plant is commonly available in Nigeria where it is locally referred to as egbesi by the western, tafashiya by the northern and ubulu inu by the eastern people (Adepoju *et al*., 2024).

It has been reported that *N. latifolia* has numerous medicinal application, and has been used against organ damage due to environmental pollutants (Mordi *et al*., 2014), inflammation (Iheagwam *et al.,* 2022), high blood pressure (Adepoju *et al*., 2024), diabetes and respiratory diseases (Oke *et al.*, 2021; Anarado *et al*., 2022), obesity (Busari *et al*., 2021). Based on Pharmacological studies, these effects of *N. latifolia* are associated with its high levels of antioxidant and anti-inflammatory compounds including Phenols and flavonoids (Adepoju *et al*., 2024). This study was designed to determine the protective effect of *Nauclea latifolia* stem bark extract against NBLCO-induced male reproductive and renal damage in Wistar rats.

MATERIALS AND METHODS

Materials

Gavage, refrigerator, Weighing balance, Surgical scissors, forceps, Beakers, Spatula, Spectrophotometer, Oven, Sample bottles, Test tubes, Filter papers, Hand gloves, Conical flasks, Masking tape, Centrifuge, Microplate reader, Iron cages, Nose mask, Micropipette.

Plant material

Nauclea latifolia stem bark was obtained from the forest reserve of Michael Okpara University of Agriculture Umudike (MOUAU), Abia State, Nigeria. Identification and authentication were done by an expert in the Department of Plant Science and Biotechnology, MOUAU, Abia State. A voucher specimen (MOUAUH 3847) was deposited at the Herbarium.

Experimental animals

Twenty-five rats (130-160 g body weight) were obtained from the Animal House of the College of Veterinary Medicine, MOUAU, Abia State, Nigeria and were transferred to a separate and well-ventilated section of the experimental animal breeding house where they were allowed access to pelleted animal feed (Chikun, Olam Nigeria Ltd, Kaduna) and water *ad libitum*. The rats were thereafter allowed to acclimatize for a period of 14 days before commencement of studies.

Chemicals and reagents

Nigerian Bonny Light Crude Oil (NBLCO) used in this study was obtained from the

Nigerian National Petroleum Corporation (NNPC) Warri, Nigeria. Other Chemicals and reagents were procured directly from the certified outlets and distributors for the manufacturing companies including Randox laboratories UK, BDH chemical London, Sigma-Aldrich USA and Taj Pharmaceuticals India. Distilled water (DS) used in this study was prepared at the Department of Chemistry, Michael Okpara University of Agriculture Umudike (MOUAU), Abia State, Nigeria.

Preparation of plant extract

Fresh stem bark of *N. latifolia* was collected, rinsed with distilled water, air-dried and ground into fine powder with an electric blender. The powdered material was subjected to extraction by weighing 600g and macerating it in 1 liter of 70% ethanol in a sealed container with continuous stirring at regular intervals for 3 days at room temperature. The mixture was filtered with Whatman No. 1 filter paper and the filtrate concentrated under reduced pressure using a rotary evaporator at 37^0C. The concentrate was then allowed in a water bath at 37^0C to completely evaporate the extraction solvent, and the obtained dry extract weighed 34.2g (5.7% of powdered material) and was kept in a refrigerator at 4^0C for future use.

Acute toxicity study

Acute toxicity (LD_{50}) was evaluated using Lorke method (1993), as reported by Ita *et al*. (2026). For the plant extract, a total of 24 rats weighing 130 to 160 g were randomly selected into eight (I-VIII) groups of three rats each. The first seven groups were administered 100, 1000, 2000, 3900, 5000, 7000 and 9000 mg/kg body weight respectively through oral route. The rats were observed for seven days for physical signs of toxicity and a possible mortality. Following 100 percent mortality recorded in group VII within the period of LD_{50} test, group VIII rats were administered the same 9000 mg/kg body weight (confirmatory test) and were also observed for 7 days. 100 percent mortality was also recorded and thereafter, the lethal dose was calculated as geometrical means of the highest (most tolerable) dose producing 0% mortality (x) and the lowest (least tolerable) dose producing 100% mortality (y) using Lorke's expression:

$LD_{50} = \sqrt{xy}$. $LD_{50} = \sqrt{5000 \times 9000} = 6708.20$ mg/kg.

For the NBLCO, 18 rats (130-160g) were randomly selected into six groups of three rats each, and were administered 5, 10, 15, 20, 25, and 30 mL/kg body weight respectively through oral route. Following mortality in some groups, the lethal dose was calculated as geometrical means of the highest (most tolerable) dose producing 0% mortality (x) and the lowest (least tolerable) dose producing 100% mortality (y) using Lorke's expression:

$LD_{50} = \sqrt{xy}$. $LD_{50} = \sqrt{10 \times 20} = 14.14$ mL/kg.

Experimental design and treatment of animals

Twenty five Wistar rats (130-160 g body weight) were randomly selected into five groups of five rats each. Rats in group I served as the control and were orally administered 3.5 mL/kg body weight of distilled water, group II rats received 500 mg/kg body weight of *NLSB-extract*, group III received 2.8 mL/kg body weight of NBLCO (20 % of its LD_{50}), while groups IV and V rats received co-administration of NBLCO (2.8 mL/kg) plus 500 mg/kg and

1000 mg/kg body weight of *NLSB-extract* respectively. Treatments were done orally using gavage during morning hours and lasted for 28 days based on the most recent body weight of each animal. Guidelines for the use and care of experimental animals as approved by the Research and Ethical Committee of the College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria were fully observed in all procedures involving animals. The ethical approval number is MOUAU/CNS/HREC/VOL.1/113.

Collection of blood sample for analysis

After 28 days of treatment, the rats were sacrificed using 100 mg/kg body weight of ketamine hydrochloride as anesthetic agent through intraperitoneal route. Blood sample was collected by cardiac puncture using 5 mL sterile syringe and needle, and was transferred into sample bottles containing no anticoagulant. The blood sample was allowed to stand for about 2 hours and thereafter centrifuged (BKC-TH21RM, BIOBASE, China) at 3000 revolution per minute (rpm) for 15 minutes at 25 degree Celsius to obtain the serum, which was stored in a refrigerator at -20 degree Celsius till needed for biochemical analysis.

Determination of the levels of serum testosterone

Testosterone levels were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) method as described by Wang *et al.* (2014).

Follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels assay

Follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were determined by enzyme-linked immunosorbent assay (ELISA) method using their respective test Kits according to the manufacturers' (Biosource, Califonia, USA) instructions as reported by Burow *et al*. (2019).

Seminal analysis using computer aided system (CASA)

From the testes, caudal epididymis was dissected out and 0.1cm sections made and suspended in 100cm of buffered normal saline to allow the sperm cells to swim up. The assessment of sperm motility was carried out with the use of Computer-Assisted Semen Analysis (CASA) in accordance with World Health Organization procedures and the method described by Ita *et al.*, (2026). From the total cell detected, other patterns of motility including sperm concentration (10^6/mL), motility: percentage motile and non-motile sperm cells, morphology: percentage normal and abnormal sperm cells were evaluated.

Determination of the levels of serum urea and creatinine

Measurement of blood urea nitrogen (BUN) and creatinine (Cr) was carried out using the urease kinetic and Jaffe's reaction methods respectively with automated analyzer (Cobas 6000/c311) as described by Ekwempu *et al*. (2019).

Determination of the levels of serum electrolyte

The soduim, potassium, and calcium ion concentrations were determined using flame atomic absorption and visible spectroscopy as described by Murphy (1987).

Statistical analysis

The data generated were subjected to one way analysis of variance (ANOVA) using a Statistical Product and Service Solutions (SPSS) version 23. The means were compared with a Duncan Multiple Range Comparison Test and the levels of statistical significance were obtained at 95 % confidence level ($p < 0.05$). The results were presented as mean ± standard deviation.

RESULTS

Median lethal dose (LD_{50}) of *Nauclea latifolia* stem bark extract in male rats

Mortality was observed in all the tested groups. Therefore, the oral LD_{50} value of *Nauclea latifolia* stem bark extract in male rats was above 5000 mg/kg (Table 1A).

Table 1A: Median lethal dose (LD_{50}) of *Nauclea latifolia* stem bark extract in male rats

Groups	Number of Rats	Dose (mg/kg)	Mortality
I	3	100	0/3
II	3	1000	0/3
III	3	2000	0/3
IV	3	3900	0/3
V	3	5000	0/3
VI	3	7000	1/3
VII	3	9000	3/3

LD_{50} = 6708.20 mg/kg

Median lethal dose (LD_{50}) of NBLCO in male rats

Mortality was observed in the groups administered 15 mL/kg, 20 mL/kg, 25 mL/kg and 30 mL/kg body weight. Thereafter, the oral LD_{50} value of NBLCO in male rats was calculates as $\sqrt{10 \times 20}$ = 14.14 mL/kg. (Table 1B).

Table 1B: Median lethal dose (LD_{50}) of NBLCO in male rats

Groups	Number of Rats	Dose (mL/kg)	Mortality
I	3	5	0/3
II	3	10	0/3
III	3	15	1/3
IV	3	20	3/3
V	3	25	3/3
VI	3	30	3/3

LD_{50} = 14.14 mL/kg

Effects of *Nauclea latifolia* stem bark extract and NBLCO on the testosterone, follicle- stimulating hormone and luteinizing hormone in rats

The results in Table 2 showed that oral administration of *Nauclea latifolia* stem bark extract to group II rats did not alter the levels of testosterone, follicle-stimulating hormone and luteinizing hormone significantly compared to rats in group I, but NBLCO administration to group III rats significantly ($P < 0.05$) reduced the concentrations of the aforementioned hormones relative to groups I and II rats. However, Co-administration of NBLCO with *Nauclea latifolia* extract to groups IV and V rats significantly ($P < 0.05$) increased the levels of testosterone, follicle-stimulating hormone and luteinizing hormone in a dose-dependent manner compared to rats in group III.

Table 2: Testosterone, follicle stimulating and luteinizing hormone concentrations in rats administered NBLCO and *Nauclea latifolia* stem bark extract.

Treatment groups	Testosterone (ng/mL)	FSH (ng/mL)	LH (ng/mL)
I (3.50 mL/kg DS)	3.63±0.16	4.37±0.50	5.98±0.46
II (500 mg/kg *NLSB-extract*)	3.63±0.09	3.98±0.66	6.26±0.61
III (2.80 mL/kg NBLCO)	0.94±0.10^{a,b}	1.26±0.52^{a,b}	1.71±0.52^{a,b}
IV (2.80 mL/kg of NBLCO + 500 mg/kg *NLSB-extract*)	1.52±0.34^{a,b,c}	1.31±0.12^{a,b}	2.54±0.57^{a,b,c}
V (2.80 mL/kg NBLCO + 1000 mg/kg *NLSB-extract*)	2.86±0.13^{a,b,c,d}	3.50±0.32^{c,d}	4.06±0.63^{a,b,c,d}

Values are presented as mean ± standard deviation (n = 5); a = significantly different from group I ($P < 0.05$), b = significantly different from group II ($P < 0.05$), c = significantly different from group III ($P < 0.05$), d = significantly different from group IV ($P < 0.05$).

Effects of *Nauclea latifolia* stem bark extract and NBLCO on the semen concentration, spermatozoa motility and spermatozoa morphology in rats

The results in Table 3 showed that oral administration of *Nauclea latifolia* stem bark extract to group II rats did not induce significant alteration in the concentration, motility and morphology of spermatozoa relative to group I rats, but administration of NBLCO to group III rats significantly ($P < 0.05$) reduced the concentration, motility and morphology of spermatozoa compared to the rats in groups I and II. However, following co-administration of NBLCO and *N. latifolia* extract to groups IV and V rats, the concentration, motility and morphology of spermatozoa significantly ($P < 0.05$) increased in a dose-dependent manner relative to rats in group III.

Table 3: Sperm concentration, motility and morphology in rats administered NBLCO and *Nauclea latifolia* stem bark extract.

Treatment groups	Sperm Concentration ($x10^6$/mL)	Motile Sperms (%)	Non-motile Sperms (%)	Sperms with Normal Morphology (%)	Sperms with Abnormal Morphology (%)
I (3.50 mL/kg DS)	82.72±3.24	84.06±4.80	15.94±4.80	83.87±6.27	16.13±6.27
II (500 mg/kg *NLSB-extract*)	81.48±0.84	79.02±2.00	20.98±2.00	82.98±4.89	17.02±4.89
III (2.80 mL/kg NBLCO)	45.69±1.24^{a,b}	36.76±4.69^{a,b}	63.24±4.69^{a,b}	37.31±2.71^{a,b}	62.69±2.71^{a,b}
IV (2.80 mL/kg NBLCO +500 mg/kg *NLSB-extract*)	53.53±3.10^{a,b}	56.68±1.28^{a,b,c}	43.32±1.28^{a,b,c}	46.47±0.74^{a,b,c}	36.87±2.02^{a,b,c}
V (2.80 mL/kg NBLCO + 1000mg/kg *NLSB-extract*)	64.42±8.48^{a,b,c,d}	62.17±2.39^{a,b,c}	37.83±2.39^{a,b,c}	60.49±2.39^{a,b,c,d}	39.51±2.39^{a,b,c}

Values are presented as mean ± standard deviation (n = 5); a = significantly different from group I ($P < 0.05$), b = significantly different from group II ($P < 0.05$), c = significantly different from group III ($P < 0.05$), d = significantly different from group IV ($P < 0.05$).

Effects of *Nauclea latifolia* stem bark extract and NBLCO on the serum urea, creatinine and electrolyte concentrations in rats

The results in Table 4 showed that oral administration of *Nauclea latifolia* stem bark extract to group II rats did not induce significant alteration in the levels of BUN, Creatinine, Na^+, K^+ and Ca^{2+} compared to the rats in group I, but administration of .

NBLCO to group III rats significantly ($P < 0.05$) elevated the levels of BUN, Creatinine, Na^+, K^+ and Ca^{2+} compared to the rats in groups I and II. However, Co-administration of NBLCO with *Nauclea latifolia* stem bark extract to groups IV and V rats significantly ($P < 0.05$) reduced the levels of the aforementioned renal parameters in a dose-dependent manner compared to rats in group III

Table 4: Serum concentrations of urea, creatinine and electrolytes in rats administered NBLCO and *Nauclea latifolia* stem bark extract.

Treatment groups	BUN (mg/dL)	sCr (mg/dL)	Na^+ (mmol/L)	K^+ (mmol/L)	Ca^{2+} (mmol/L)
I (3.50 mL/kg DS)	39.70±1.09	0.67±0.05	128.37±0.57	3.92±0.27	2.32±0.22
II (500 mg/kg *NLSB-extract*)	39.20±0.87	0.70±0.06	129.24±1.07	4.05±0.66	2.32±0.13
III (2.80 mL/kg NBLCO)	58.84±1.90[a,b]	1.60±0.05[a,b]	163.41±5.64[a,b]	7.81±0.25[a,b]	3.76±0.11[a,b]
IV (2.80 mL/kg NBLCO + 500 mg/kg *NLSB-extract*)	47.19±0.43[a,b,c]	1.12±0.20[a,b,c]	150.35±2.38[a,b,c]	6.49±0.64[a,b,c]	2.78±0.12[a,b,c]
V (2.80 mL/kg NBLCO + 1000 mg/kg *NLSB-extract*)	43.80±2.29[a,b,c,d]	0.89±0.05[a,b,c,d]	147.64±1.01[a,b,c]	5.97±0.23[a,b,c]	2.74±0.11[a,b,c]

Values are presented as mean ± standard deviation (n = 5); a = significantly different from group I ($P < 0.05$), b = significantly different from group II ($P < 0.05$), c = significantly different from group III ($P < 0.05$), d = significantly different from group IV ($P < 0.05$).

DISCUSSION

Environmental degradation by xenobiotics including crude petroleum contributes immensely to the rising global disease burden, especially in oil-rich nations including Nigeria. In the Niger-Delta region of Nigeria, humans and animals are faced with the danger of crude oil-associated systemic toxicity due to constant exposure through inhalation, ingestion or absorption. Exposure to crude oil and its components has been reported to induce several pathophysiological conditions including male infertility and organ damage through lipid peroxidation by promoting excessive generation of reactive species of oxygen (ROS) (Ita *et al*., 2026).

The OECD guidelines 423 and 425 for acute toxicity test had stipulated that mortality is the primary endpoint for the intake of toxic dose levels of substances, and that failure to produce mortality even at high doses may indicate that the extract is safe (Lipnick *et al.,* 1995). An LD_{50} value above 5000 mg/kg body weight obtained for stem bark extract suggests that the extract may be safe for oral use. Similar conclusion has been drawn in a previous acute toxicity study involving plant extracts (Coulidiaty *et al*., 2024). NBLCO was found to be toxic as mortality was recorded in groups administered 15 mL/kg, 20 mL/kg, 25 mL/kg and 30 mL/kg body weight following oral administration. These findings agree with the report of a previous study (Ita *et al*., 2026).

This study investigated the protective effect of *Nauclea latifolia* ethanol stem bark extract against NBLCO-induced male reproductive and renal damage in male Wistar rats. Results showed that exposure to NBLCO caused significant reduction in the levels of testosterone, follicle-stimulating hormone and luteinizing hormone, with reduced sperm count, motile and morphology. These observed effects of NBLCO in this study clearly suggest that it is capable of compromising male reproductive health, as hormonal balance and semen quality are among the key important factors in male fertility (Midzak *et al*., 2009; Dorostghoal *et al*., 2019). When the balance of the aforementioned male reproductive hormones is compromised as observed in this study, it may be an indication of compromised male reproductive potential (Ita *et al*., 2026).

Reduced levels of male sex hormones and semen quality following exposure to NBLCO may have occurred through oxidative damage, as crude oil has been previously reported to be capable of causing oxidative stress in testicular tissues by inducing excessive generation of reactive oxygen species and suppression of endogenous antioxidant defenses (Ita *et al*., 2018). Excessive generation of ROS has been implicated in compromised semen quality, male infertility Agarwal *et al*. (2014). Reduced levels of male hormones associated with NBLCO exposure may be a clear indication that it is a powerful disruptor to hypothalamic-pituitary–adrenal-axis, a physiological mechanism that regulate androgen levels.

In this study, it was observed that NBLCO exposure significantly increased the levels of serum urea, creatinine, sodium, potassium and calcium ions. The build-up of metabolic wastes including urea and creatinine, alongside the accumulation of electrolytes evidenced by high levels of sodium, potassium and calcium as observed in this study points to nephrotoxic effect of NBLCO. These findings are in line with Ita

and Nsikan (2016), who reported nephrotoxic potential of NBLCO due to serum accumulation of urea and creatinine, and distorted distribution of electrolytes in rats.

Exposure to environmental chemical pollutants such as crude oil has in previous studies been associated with excessive generation of ROS and subsequent induction of lipid peroxidation, which causes cytolysis (Ita *et al*., 2013). When the membrane of a renal cell is destroyed, its physiological roles including regulation of metabolic wastes and maintenance of electrolyte balance in serum are compromised leading to their abnormal levels as observed in this study. High levels of serum urea, creatinine and electrolytes following NBLCO administration as observed in this study indicated renal damage which may have been induced through tissue oxidation (Ita *et al.,* 2016). It is not out of place to say that under significant renal impairment, the principal function of the Bowman's capsule is negatively affected leading to reduced rate of glomerular filtration, which may possibly be the reason why urea, creatinine and electrolytes accumulated in serum as observed in this study.

It was observed in this study that co-administration of *Nauclea latifolia* extract with NBLCO reversed the effects of crude oil on male sex hormones, semen quality and renal parameters. *N. latifolia* ethanol stem bark extract significantly reversed the imbalance in male sex hormones, and improved semen quality and renal function. The effect produce by *N. latifolia* extract in this study may be attributed to its phytochemical composition. *N. latifola* has been reported to contain high amounts of phytochemical compounds including phenols and flavonoids, which have demonstrated in a previous study to possess antioxidant and free radical scavenging effects (Egbung *et al.,* 2013). These compounds protect endogenous antioxidant defense system, thus preventing oxidative stress and tissue damage (Ita *et al*., 2026).

CONCLUSION

Findings from this study have demonstrated that NBLCO administration remarkably distorted the hormonal imbalance including negative alteration of testosterone, FSH and LH levels, and similarly impaired the semen parameters in rats, which could possibly compromise male fertility. The NBLCO administration adversely impaired renal functions, which were substantially restored by the administration of *Nauclea latifolia* stem bark extract.

Study limitation: Tissue oxidative stress assay was not carried out in this study.

Conflict of interest

Authors have declared no conflict of interest.

Funding

The authors received zero financial support for the research, or publication of this original manuscript.

Acknowledgement

We wholeheartedly acknowledge everyone who contributed to the success of this work.

REFERENCES

Adepoju, A. J., Esan, A. O., Olawoore, I. T., Ibikunle, G. J. and Adepoju, V. O. (2024). Nauclea latifolia stem bark extracts: potentially effective source of antibacterial, antioxidant, antidiabetic and anti-inflammatory compounds. *Journal o Applies Science and Environmental Management*, 28(1): 49-59.

Agarwal, A., Sharma, R. K., Sharma, R., Assidi, M., Abuzenadah, A. M., Alshahrani S., Durairajanayagam, D. and Sabanegh, E. (2014). Characterizing semen parameters and their association with reactive oxygen species in infertile men. *Reproductive Biology and Endocrinology,* 12: 33. http://www.rbej.com/content/12/1/33 .

Altanam, S. Y., Darwish, N. and Bakillah, A. (2025). Exploring the interplay of antioxidants, inflammation, and oxidative stress: mechanisms, therapeutic potential, and clinical implications. *Diseases*, 13(9): 309. doi: 10.3390/diseases13090309.

Anarado, C. E., Ejimofor, N., Chiadikobi, O. M. and Obumselu, O. F. (2022). Comparative Phytochemical, Proximate, and in vitro Antihyperglycemic analyses of the root bark extracts of Nauclea latifolia S. and the stem bark extracts of Azadirachta indica A. *Bulletin of the Chemical Society of Nigeria,* 47(4): 776-797.

Balogun, M; Oji, J; Besong, E; Ajah, A; Michael, E (2013). Anti-ulcer activity of aqueous leaf extract of Nauclea latifolia (rubiaceae) on indomethacininduced gastric ulcer in rats. Afr. *Journal of Biotechnology*, 12(32): 5080–5086.

Burow S., Fontaine, R., Krogh, K., Mayer, I., Nourizadeh-Lillabadi, R., Hollander-Cohen, L., Cohen, Y., Shpilman, M., Levavi-Sivan, B. and Weltzien, F. (2019). Establishment of specific enzyme-linked immunosorbent assay (ELISA) for measuring Fsh and Lh levels in medaka (*Oryzias latipes*), using recombinant gonadotropins. *MethodsX*, 6: 1473-1479.

Busari, M. B., Hamzah, R. U., Muhammad, H. L., Yusuf, R. S., Madaki, F. M., Adeniyi, J. O., Ibrahim, Y. O. and Berinyuy, E. B. (2021). Phenolic rich-extracts from *Nauclea latifolia* fruit restored Lead acetate-induced liver and kidney damaged in Wistar rats. *Clinical Phytoscience*, 7: 87. https://doi.org/10.1186/s40816-021-00322-z.

Coulidiaty, A., Boni, S. I., Ouedraogo, R., Koama, B. K., Soré, H., Meda, R. N., Yaméogo, T. M. and Youl, E. (2024). Acute and chronic oral toxicity of hydroethanolic extract of *Sclerocarya birrea* (Anacardiaceae) in wistar rats. *Journal of Experimental Pharmacology*, 16: 231-242.

Dorostghoal, M., Seyyednejad, S. M. and Nejad, M. (2019). Beneficial effects of *Cichorium intybus* L. extract on oxidative status and reproductive parameters in male Wistar rats: an experimental study. *International Journal of Reproductive Biomedicine*, 17(6): 425-434.

Dorsey, E. R., Zafar, M., Lettenberger, S. E., Pawlik, M. E., Kinel, D., Frissen, M., Schneider, R. B., Kieburtz, K., Tanner, C. M., De Miranda, B. R. and Goldman, S. 166 M. (2023). Trichloroethylene: an invisible cause of Parkison's disease? *Journal of Parkison's Disease,* 13(2): 203-218.

Egbung, G. E., Atangwho, I. J., Iwara, I. A., Odey, M. O. and Ebunf, P. E. (2013). Chemical composition of root and stem bark extracts of *Nauclea latifolia*. *Archives of Applied Science Research,* 5(3): 193-196.

Ekwempu, A. I., Shindang, J., Nnanna, O. U., Sariem, C., Davou, G. and Danaan, E. N. (2019). Serum creatinine and urea levels in wistar rats exposed to glacial acetic acid. *Tropical Journal of Health Sciences*, 26(1): 1-6.

Elehinafe, F. B., Nwizu, C. I., Olayemi B. Odunlami, O. B. and Ibukun, F. D. (2022). Natural gas flaring in Nigeria, its effects and potential alternatives – a review. *Journal of Ecological Engineering*, 23(8): 141–151.

Hebrew, J. Audu, M. and Umaru, I. J. (2026). Protective effects of ethanol extract of *Chrysophyllum albidum* stem bark on antioxidant enzymes and lipid peroxidation induced kidney toxicity in albino rats Exposed to Bonny Light Crude Oil. *Journal of Biochemistry and Molecular Biology Research*, 3(2): 222-235.

Iheagwam, F. N., Israel, E. N., Kayode, K. O., DeCampos, O. C., Ogunlana, O. O. and Chinedu, S. N. (2022). "*Nauclea latifolia* leaf extracts extenuate free radicals, infammation, and diabetes-linked enzymes. *Oxidative Medicine and Cellular Longevity*, 2020: e2020.

Ita S. O., Chinagoro, U. S., Ugwuanyi, K. C., Akpan, E. I., Jacob, J. Ben, E. E., Abia, E. R., Essoh, S. P., Udom, M. S. and Edem, P. B. (2026). 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. Acta Scientific Women's Health*, 8(5): 19-29..

Ita, S. O., Effiong, E. F., Okon, M. S., Robert, A. S. and Francis, U. E. (2018). Impact of crude oil-induced oxidative stress and lipid peroxidation on spermatogenesis: the anti-oxidative role of Ageratum conyzoides. *Journal of Reproductive Endocrinology and Infertility*, 3(1): 26.

Ita, S. O. and Nsikan, J. N. (2016). The ethanol leaf extract of *ageratum conyzoides* ameliorates nigerian bonny light crude oil-induced nephrotoxicity in female wistar rats. *International Journal of Health, Safety and Environments*, 2(1): 09-15.

Ita, S. O. and Edagha, I. A. (2016). Renal protective effect of antioxidant vitamins C and E against crude oil-induced nephrotoxicity. *Merit Research Journal of Medicine and Medical Sciences,* 4(9): 425- 431.

Ita, S. O., Aluko, E. O., Atang, D. E., Antai, A. B. and Osim E. E. (2013). Vitamin C or E supplement ameliorates Nigerian bonny light crude oil-induced erythrocyte haemolysis in male Wistar rats. *Biochemistry and Molecular Biology*, 1(3): 44-51.

Jayaraman, S., Eswaran, A., Veeraraghavan, V., Fazal, M., Al-Rahbi, A.and Sirasanagandla, S. R. (2025). Implications of petrochemical exposure and epigenetic alterations on human health. *Frontiers in Toxicology,* 13(7): 1542871. doi: 10.3389/ftox.2025.1542871.

Krajnak, K., Russ, K. A., McKinney, W., Waugh, S., Zheng, W., Kan, H., Kashon, M. L., Cumpston, J. and Fedan. J. S. (2023). Biological effects of crude oil vapor. IV. Cardiovascular effects. *Toxicology and Applied Pharmacology*, 15(447): 116071. doi: 10.1016/j.taap.2022.116071.

Lipnick, R. L, Cotruvo, J. A., Hill, R. N., Bruce, R. D., Stitzel, K. A., Walker, A. P., Chu, I., Goddard, M., Segal, L., Springer, J. A. and Myers, R. C. (1995). Comparison of the up-and-down, conventional LD_{50} and fixed dose acute toxicity procedures. *Food and Chemical Toxicology*, 33: 223-231.

Midzak, A. S., Chen, H. Papadopoulos, V. and Zirkin, B. R. (2009). Leydig cell aging and the mechanisms of reduced testosterone synthesis. *Molecular and Cellular Endocrinology*, 299: 23–31.

Mordi, J. C., Ojieh, A. E., Uzuegbu, U. E., Onyesom, I. and F. Onokurafe, F. (2014). Changes in ocular oxidative indices in *plasmodium berghei* infected mice treated with aqueous leaf extract of *Nauclea latifolia*. *Bioscience Biotechnology Research Communications*, 7(1): 1-6.

Murphy, V. A. (1987). Method for determination of sodium, potassium, calcium, magnesium, chloride, and phosphate in the rat choroid plexus by flame atomic absorption and visible spectroscopy. *Analytical Biochemistry*, 161(1): 144-151.

Nwozo, O. S., Effiong, E. M., Aja P. M. and Awichi, C. G. (2023). Antioxidant, phytochemical, and therapeutic properties of medicinal plants: a review. *International Journal of Food Properties*, 26(1) 3509-388.

Oke, E.O., Okolo, B. I., Adeyi, O., Agbede, O. O., Nnaji, P., Adeyi, J., Osoh, K. and Ude, C. (2021). Black-box modelling, bi-objective optimization and ASPEN batch simulation of phenolic compound extraction from *Nauclea latifolia* root. *Heliyon*, 7(1): e05856.

Ravitsky, V. and Kimmins, S. (2019). The forgotten men: rising rates of male infertility urgently require new approaches for its prevention, diagnosis and treatment. *Biology of Reproduction*, 101(5): 872-874. doi: 10.1093/biolre/ioz161.

Wang, Y., Gay, G., Botelho, J. C., Caudill, S. P. and Vesper H. W. (2014). Total testosterone quantitative measurement in serum by LCMS/MS. *Clinica Chimica Acta*, 436: 263–267. doi:10.1016/j.cca.2014.06.009.