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RESEARCH ARTICLE

Adult erectile functions and prepubertal role of vitamin C supplementation during crude oil-contaminated water ingestion

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ABSTRACT

Background: The benefits of vitamin C treatment for erectile functions during prepubertal crude oil-contaminated water (CCW) ingestion are unknown. We currently considered the import of vitamin C supplement during prepubertal crude oil-contaminated water ingestion on adult erectile mechanisms in Wistar rats.

Materials and methods: 18 prepubertal male rats were divided into three groups and given saline, 2.5 ml CCW, and 2.5 ml CCW + vitamin C (10 mg/kg), separately, until adulthood. Cavernosa tissue responses to phenylephrine, acetylcholine, potassium chloride, and responses after incubation with glibenclamide, nifedipine, methyl blue, and indomethacin were investigated. Serum testosterone and the cavernosa oxidative biomarkers were determined.

Results: Testosterone and catalase activity were significantly reduced in CCW group, while malondialdehyde activity was significantly increased. Potassium significantly increased cavernosa contraction in CCW-treated group. Incubation of the cavernosa with nifedipine and indomethacin reduced the percentage of relaxation in CCW group. When compared to the vitamin C-supplemented groups, incubating the cavernosa with methyl blue and glibenclamide markedly reduced relaxation in CCW.

Conclusions: Prepubertal CCW ingestion impaired adult cavernosa ATP-sensitive K+ channels, receptor and voltage-operated calcium channels, guanylate cyclase, and prostaglandin activity. Vitamin C administration reduced contractile impairments by increasing antioxidant activity in the cavernosa.

Keywords: vitamin C administration, prepubertal, antioxidants, crude oil-polluted water, erectile mechanism, corpus cavernosa

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INTRODUCTION

One of Africa's key crude oil producers is Nigeria. The country's Niger-Delta areas usually witness exploration and production activities that greatly impact on the freshwater bodies in the area. The region also houses many freshwater bodies that often provide the main drinking water source and food for the locals and the marine population [1]. Spillages occurring during exploration, production, refining, or sabotage of crude oil installations are known to adversely pollute the environment and water sources [2].

Oil spills, prevalent in oil-producing regions, are a source of heavy metal contamination in our environments [3]. According to available research, ingesting crude oil-contaminated water (CWC) is nephrotoxic, hepatotoxic, cardiotoxic, and a depressant of the central nervous system [4]. Hematological studies showed a significant reduction in hemoglobin and packed cell volume and a rise in white blood cells as a result of crude oil ingestion [5]. Moreover, contact with crude oil can be hemotoxic to seabirds and other avian species [6]. A significant impairment in biochemical.

blood parameters was reported in rabbits given crude oil-polluted foods [7].

A study by Yoshida et al. [8] showed that crude oil constituents triggered deterioration of the Leydig cell and reduced regular sperm production and seminiferous tubule numbers in the testis.

A recent report [9] suggested that offspring's procreative activity was impaired when there was gestational exposure to crude oil. A single dose of 800 mg/kg body weight of Bonny light crude oil (BLCO) has also been demonstrated to cause a reduction in testosterone level [10]. The epididymis of rats chronically exposed to crude oil for six weeks showed dose-dependent degeneration and a significant decrease in tubular content [11]. Polycyclic aromatic hydrocarbons (PAHs) are the dominant constituent of crude oil and have also been implicated as endocrine disruptors, modulating steroidogenesis and sexual functions in fish, rodents, and mammals [12].

Although several studies have investigated the impact of crude oil and crude oil-contaminated water on several body functions, a literature search reveals a paucity of information on crude oil-contaminated water ingestion during the prepubertal period in males specifically. Furthermore, previous studies have not adequately explored the contractile functions and activities of erectile tissues as indicators of impaired functions during CCW ingestion. The benefits of vitamin C supplementation on erectile functions during CCW ingestion during the prepubertal period in males are also unknown.

This study investigates the consequences of prepubertal CCW ingestion and vitamin C supplementation on the contractile functions of the adult corpus cavernosum in male Wistar rats.

MATERIALS AND METHODS

Drugs and Chemicals for the Study

Phenylephrine, calcium chloride, acetylcholine, and potassium chloride (KCl) were purchased from Tocris, UK. Unicure Pharmaceutical, Lagos and Jiangxi Pharmaceutical, China supplied the nifedipine and indomethacin, respectively.

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis of CCW

GC-MS analysis was carried out using a gas chromatograph (model 6890), equipped with a flame ionization detector (FID), and coupled with a HP 7683 series injector (Hewlett Packard). The oven temperature was initially held at 50 °C for five minutes and then raised to 250 °C at a rate of 2 °C/min, and helium was used as the carrier. 1.0 ml of CCW was injected at a split ratio of 1:30. An agilent technology network mass spectrometer (model 5973 series) equipped with the NIST08 library software database was used for the identification of compounds by comparing the mass spectra of unknown compounds with known compounds stored in the software database library.

Animals for the Study

18 prepubertal albino Wistar strain male rats (100-120 g) were housed in cages and acclimatized for two weeks in the laboratory. They were allowed free access to standard rat feed and water for the entirety of the study. NIH standard protocol for the safe use of laboratory animals was safeguarded in the study.

Experimental Design and the Treatment

18 prepubertal (three-week-old) male rats were divided into three groups of six rats each. The control group (group 1) was given distilled water. Group 2 and group 3 were treated with crude oil-polluted water (2.5 ml); however, group 3 was further supplemented daily with 10 mg/kg of vitamin C [13]. Treatments were via oral gavage until puberty (eight weeks). The crude oil-contaminated water for the study was obtained from a water source in the Ogbia Community, Bayelsa State, Nigeria.

Animal Sacrifice, Serum Collection, and Tissue Homogenization

When the treatment period was complete, the animals were killed through cervical dislocation under sodium pentobarbital anesthesia (30 mg/kg). The blood samples from the animals were collected via cardiac puncture into sample bottles. The samples were centrifuged using a Surgifield centrifuge (model SMBO-2, England) for 15 minutes at 3000 revolutions per minute. The serum obtained was put into an Eppendorf tube and refrigerated at -4 °C for the testosterone assay. A section of the corpus cavernosum was excised for the in vitro contractile experiments, while another section was homogenized to assay the SOD and malondialdehyde.

Corpus Cavernosa and the Tissue Chamber

The cavernosa tissue was suspended in a 50-ml chamber as described in [14], and the constituents of the chamber were also as reported [14].

Experimental Design for Contractile Activity

1. The cavernosa tissues were allowed to stabilize in PSS for a period of 90 minutes. The cavernosa tissues were stimulated (PHE, 10^{-7} M) three times at 30-minute intervals during the period of stabilization. The cumulative dose responses of the cavernosa tissue to acetylcholine (10^{-9}-10^{-5} M) were determined and recorded. Dose response to acetylcholine was done to investigate the influence of CCW treatment and vitamin C supplementation on the parasympathetic muscarinic receptor activity of the cavernosa tissues across treatment groups.
2. 10-60 mM of KCl were added to the potassium-free solution in the tissue chamber to assess the receptor-operated calcium channel activity of the cavernosa tissues across treatment groups.

3. Cavernosa tissues from all the groups were incubated in indomethacin (10^{-4} M), a cyclo-oxygenase inhibitor, for 15 minutes, and cumulative acetylcholine-mediated contractile responses were determined.
4. The activity of ATP-sensitive K^+ channels in the cavernosa tissues during CCW ingestion with vitamin C supplementation was investigated by incubating cavernosa tissues in glibenclamide (10^{-4} M) and cumulative acetylcholine-mediated contractile responses were determined.
5. The action of soluble guanyl cyclase activity in the cavernosa tissue across groups was investigated by incubating cavernosa tissues for 15 minutes in methylene blue (10^{-4}M) and cumulative acetylcholine-mediated contractile responses were determined.
6. Incubation of cavernosa tissues in nifedipine (10^{-4} M) for 15 minutes was used to investigate the effect of calcium channel blockage on the contractile activity of the cavernosa tissues across groups.

The contractile responses of the tissues were captured using the Ugo Basile data acquisition system (Italy). Between drug infusions, tissues were thrice washed.

Determination of Cavernosa MDA, Catalase, SOD, and Serum Testosterone Concentration

Malondialdehyde (MDA), an indicator of lipid peroxidation, was measured using the Buege and Aust technique [15]. It was assessed superoxide dismutase activity by its capacity to block epinephrine auto-oxidation [16]. The catalase activity was measured using the technique in [17]. ELISA kit (Monobind, USA) was used to measure testosterone levels.

Data Presentation and Analysis

Prism graph pad (version 8) statistical software was used to evaluate the data. The data were displayed as the mean with Turkey's multiple comparison, and a one- or two-way ANOVA was determined where suitable. The threshold for statistical significance was set at 0.05.

RESULTS

GC-MS of the CCW

Through gas chromatography and mass spectrometry analysis, twenty-one chemicals were detected as indicated in **Table 1**. Among the principal ingredients are methylene chloride (22%), decahydro-1, 1, 4a, 5, 6-pentamethylnaphthalene (6%), is3olongifolol (3%), and cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl (5%).

Serum Testosterone and Cavernosa Tissue SOD, Catalase, and MDA Concentrations

Serum testosterone and cavernosa tissue catalase activity significantly reduced in CCW group. MDA activity was significantly increased in CCW group when compared to the vitamin C-supplemented and control groups (**Table 2**).

Table 1. Bio-constituents found in CCW

S/N	P	R	Chemical name	W
1	21.41	3.139	Methylene chloride	83
2	6.023	9.422	Decahydro-1, 1, 4a, 5, 6-pentamethylnaphthalene	91
3	5.519	14.912	11, 13-dimethyl-12-tetradecen-1-ol acetate	83
4	4.855	14.642	Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-	94
5	4.374	16.012	Octadecane, 1-chloro-	74
6	3.494	10.486	Bicyclo [3.1.1] heptane, 2, 6, 6, trimethyl, [1R (1.alpha., 2.alpha., 5.alpha.)]-	83
7	3.288	10.299	7-pentadecyne	53
8	3.263	10.859	2, 4-di-tert-butylphenol	91
9	3.170	9.061	1-methylbicyclo [3.2.1] octane	46
10	3.106	16.254	1-octadecene	80
11	2.800	12.693	2, 6, 10-trimethyltridecane	83
12	2.668	9.234	Bicyclo [3.1.1] heptane, 2, 6, 6-trimethyl-, [1R-(1.alpha., 2.alpha., 5.alpha.)]-	60
13	2.663	11.369	Isolongifolol	55
14	2.481	14.966	Phthalic acid, neopentyl 2-propyl ester	72
15	2.395	12.978	6, 11-undecadiene, 1-acetoxy-3, 7-dimethyl-	47
16	2.351	16.314	1-docosene	95
17	2.310	15.507	D-Homoandrostane, (5.alph a., 13.alpha.)-	97
18	2.223	10.426	Bicyclo [3.1.1] heptane, 2, 6, 6-trimethyl-	38
19	2.211	11.313	Z-8-methyl-9-tetradecen-1-ol acetate	72
20	2.124	11.243	Bicyclo [2.2.1] heptane, 1, 3, 3-trimethyl-	38
21	2.018	16.498	1-Docosene	95

Note. P: % of total; R: Retention time (min); & W: Worth

There were no significant differences in the phenylephrine-mediated contractions in the cavernosa tissues across all the treatment groups (part a in **Figure 1**). Influx of cumulative doses of KCl resulted in a significant increase in cavernosa tissue contraction in CCW-treated (part b in **Figure 1**). In **Table 2**, no significant difference occurred in acetylcholine mediated relaxation in the cavernosa tissues across all treated groups. Incubation of cavernosa tissues in nifedipine reduced the relaxation (%) more in the CCW (20%, 35%) than the control (26%, 41%) and the vitamin C co-treated (28%, 41%) (part a in **Figure 2**). Incubation of cavernous tissues in methyl blue significantly reduced the acetylcholine-mediated relaxation in CCW (15%, 19%) than the control (21% 27%) and the vitamin C co-treated (25%, 34%) respectively (part b in **Figure 2**). Incubation of cavernosa tissues in glibenclamide significantly reduced ($p<0.01$) the percentage relaxation in CCW treated group (6%, 7%, 13%, 20%, and 26%) when compared to the control (12%, 21%, 31%, 48%, and 61%) and the vitamin C supplemented (14%, 22%, 35%, 46%, and 56%) groups (part c in **Figure 2**). After incubation in

Table 2. Cavernosa tissue oxidative biomarkers, serum testosterone, & dose response to acetylcholine

Groups/parameters	Control	CCW	CCW+VIT C
Biomarkers			
Testosterone (ng/ml)	3.60±0.41	1.68±0.42*	4.73±0.70
MDA (µmol/ml)	3.75±0.74	5.42±0.16*	4.41±0.13
Catalase (µmol/ml)	10.33±0.51	6.82±0.68*	10.47±0.11
SOD (µmol/ml)	2.43±0.06	2.33±0.10	2.49±0.07
Dose response to acetylcholine (10^{-9}-10^{-5} M)			
Acetylcholine (M)			
-9	17.78±2.22	18.99±2.32	16.78±0.92
-8	33.33±3.33	38.33±4.09	32.32±2.14
-7	46.11±3.17	56.67±4.08	45.67±4.61
-6	56.39±2.37	64.17±4.33	54.65±3.38
-5	65.14±4.46	68.58±3.78	61.66±2.33
Cumulative acetylcholine-mediated responses (10^{-9}-10^{-5} M) following indomethacin incubation (10^{-4} M)			
Acetylcholine (M)			
-9	21.9±1.2	19.9±1.8	17.6±1.2
-8	37.1±1.7	22.9±2.9*	30.1±2.9
-7	47.5±3.2	32.1±3.1*	43.0±2.7
-6	60.4±2.4	53.8±2.4	58.1±3.5
-5	69.2±3.6	60.0±2.0	66.1±2.9

Note. n=6; *p<0.05; Value expressed as mean SEM; CCW: Contaminated water treated; CCW+VIT C: Contaminated water & vitamin C treated

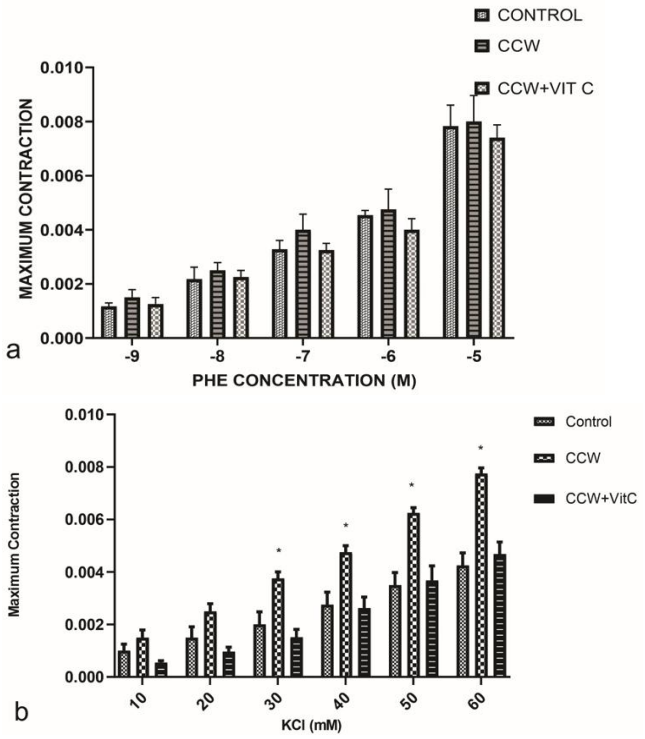

Figure 1. (a) Cavernosa tissue maximum contraction to phenylephrine (10^{-9} – 10^{-5} M). (b) Cavernosa tissue maximum contraction to cumulative dose of KCl (10-60 mM). N=6, * = p <0.05, Values expressed as mean SEM, CCW= contaminated water treated, CCW+VIT C= contaminated water and Vitamin C treated (Source: Authors' own elaboration)

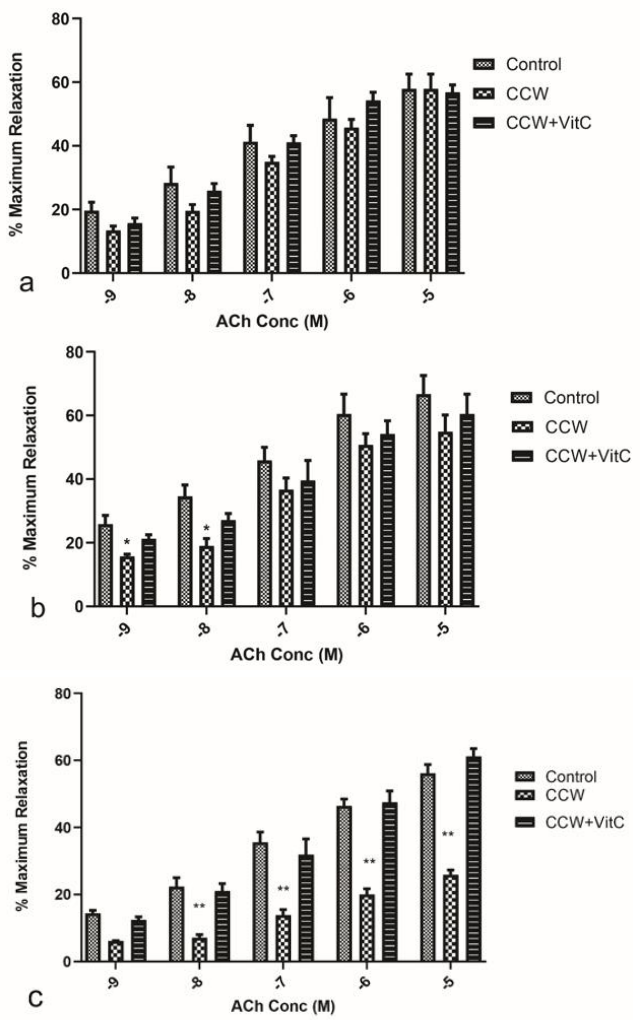

Figure 2. Cavernosa tissue relaxation response (%) acetylcholine (10^{-9}-10^{-5} M) following nifedipine incubation (10^{-4} M) (a); Cavernosa tissue relaxation response (%) acetylcholine (10^{-9}-10^{-5} M) following methylene blue incubation (10^{-4} M) (b); & Cavernosa tissue relaxation response (%) acetylcholine (10^{-9}-10^{-5} M) following glibenclamide incubation (10^{-4} M) (c) (n=6; *p<0.05; Values expressed as mean SEM; CCW: Contaminated water treated; & CCW+VIT C: Contaminated water & vitamin C treated) (Source: Authors' own elaboration)

indomethacin, the percentage relaxation response reduced significantly in CCW-treated than the control (**Table 2**).

DISCUSSION

In this study, the impact of prepubertal crude oil-contaminated water ingestion on the contractility of the cavernosa tissues was investigated in adulthood. Further, the influence of vitamin C supplementation on the contractile activity of the cavernosa tissues after the ingestion of CCW was also investigated. This study observed that prepubertal exposure to CCW impaired the contractile mechanism of the cavernosa tissues later at puberty and that vitamin C supplementation conferred an ameliorative impact on the cavernosa contractile impairments.

Ca^{2+} is a critical factor in the excitation-contraction coupling in smooth muscle cells [18]. Contraction can be mediated by the influx of extracellular Ca^{2+} through receptor-operated Ca^{2+} channels (ROCCs), voltage-dependent Ca^{2+} channels (VDCCs), and the release of Ca^{2+} from the sarcoplasmic reticulum by activation of 1, 4, 5-triphosphate inositol (IP3) and ryanodine receptors [19]. One of the cavernous contractile activities that was impaired in this study is the receptor-operated calcium channel. This study found that cumulative doses of potassium influx in the cavernosa tissues resulted in exaggerated contraction in the CCW group compared to that observed in the control and vitamin C-co-administered groups (part b in **Figure 1**). In addition, this study observed that other calcium channel-mediated contractile activity was greatly impaired with CCW administration as compared to the control and vitamin C-co-administered groups. Incubation of the cavernosa tissues with nifedipine (a calcium channel blocker) reduced relaxation mediated by acetylcholine in the CCW-treated group compared to the vitamin C-supplemented group.

Another cavernosa contractile mechanism that was impaired with CCW ingestion in this study is the ATP-sensitive K^+ channel. This study found that relaxation mediated by acetylcholine was significantly reduced in the cavernosa of the CCW-treated group after incubation with glibenclamide (**Figure 2**). However, cavernosa tissue in the vitamin C-supplemented group significantly ameliorated the reduction compared to that of the control.

In this study, the activity of the non-specific cyclo-oxygenase inhibitors and the soluble guanylyl cyclase was impaired in the cavernosa tissues of the CCW-treated group. This is made obvious when the incubation of the cavernosa tissues in indomethacin and methyl blue (an inhibitor of the guanylate cyclase enzyme according to [20]), respectively, reduced the relaxation response in the cavernosa tissue of the CCW-treated group compared to the vitamin C-co-treated group.

This study observed that phenylephrine and acetylcholine-mediated contractile activity were not significantly altered in the cavernosa tissues of all the groups. This signifies that the contractile mechanism mediated by the adrenoreceptor agonist (phenylephrine) and the parasympathetic muscarinic receptor agonist (acetylcholine) may have been greatly spared during CCW ingestion. Alternatively, the constituents of CCW in this study may not have impaired the contractile mechanism mediated by acetylcholine and phenylephrine. Phenylephrine causes contraction of the cavernosa tissue by calcium ion influx through the receptor-operated calcium channels and/or release of Ca^{2+} from the sarcoplasmic reticulum [21]. The fact that receptor-operated calcium channel activity was greatly impaired in CCW group suggests that the phenylephrine-mediated contraction seen in this study may be due to calcium release from the sarcoplasmic reticulum as opposed to calcium influx through receptor-operated calcium channels.

Generally, this study suggests that the amelioration in the impaired contractile activity of the cavernosa tissue of the vitamin C-supplemented group is due to the protection offered by the enhanced antioxidant supplementation in the group. This study found that lipid peroxidation (which causes tissue damage and impairs functions) significantly increased in CCW-exposed group compared to the group co-treated with vitamin C. Furthermore, antioxidant enzyme levels were increased in the group supplemented with vitamin C as compared to CCW-only treated group. Several studies have reported that tissue and organ damage are highly associated with exposure to crude oil and crude oil constituents [9, 11, 22].

In addition, GC-MS analysis revealed that some of the elements detected in CCW utilized in this investigation are chemicals linked to tissue damage and malfunction. It is reasonable to believe that they are jointly responsible for the decreased cavernosa tissue function seen in this investigation.

The serum testosterone concentration was considerably lower in CCW-treated group compared to the control and vitamin C-supplemented groups in this investigation (**Table 2**). The reduced testosterone concentration in CCW-treated group is predicated on CCW-induced testicular impairment. Few constituents of CCW water in this study have been reported to impair testicular steroidogenic functions, while others are known to be endocrine disruptors likely to disrupt the hypothalamic-pituitary-gonadal axis mediation of testosterone synthesis.

Although testicular histological assessments were not carried out on the testis in this study, a previous study had shown that crude oil and its constituents can bio-accumulate in the testis to impair testicular normal function [23].

CONCLUSIONS

Prepubertal CCW ingestion impaired adult corpora cavernosa activity of ATP-sensitive K^+ channel, receptor and voltage-operated calcium channels, guanylate cyclase activity, and prostaglandin activity. Vitamin C supplementation during ingestion attenuated the impaired contractile mechanisms by promoting antioxidant activity in the cavernosa tissue. The use of specific blockers of channels identified in this study and the estimation of their expression in cavernosa tissues will further elucidate the mechanisms by which vitamin C ameliorates impaired contractile activity after CCW ingestion.

Author contributions: **SAS:** conceptualization, supervision, methodology, software, validation, formal analysis, resources, data curation, writing of original draft, review, & editing; **GTO:** investigation, resources, & data curation; **MOA:** resources, data curation, reviewing, & editing; **HMS:** methodology, data curation, &

review; & **BAM:** investigation, data curation, & reviewing. All authors have agreed with the results and conclusions.

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Ethics declaration: Authors declared that this study was approved by the Lagos State University, College of Medicine, Animal Ethics Committee with reference number: AREC/2022/054 in July 2022. The authors declare that the procedures followed were in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Declaration of interest: No conflict of interest is declared by authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

REFERENCES

1. Lindén O, Pålsson J. Oil contamination in Ogoniland, Niger Delta. Ambio. 2013;42(6):685-701. doi:10.1007/s13280-013-0412-8
2. Maduka O, Ephraim-Emmanuel B. The quality of public sources of drinking water in oil-bearing communities in the Niger Delta Region of Nigeria. AAS Open Res. 2019;2:23. doi:10.12688/aasopenres.12964.1
3. Egbe RE, Thompson D. Environmental challenges of oil spillage for families in oil producing communities of the Niger-Delta Region. JHER. 2010;13:24-34.
4. Kponee KZ, Chiger A, Kakulu II, Vorhees D, Heiger-Bernays W. Petroleum contaminated water and health symptoms: A cross-sectional pilot study in a rural Nigerian community. Environ Health. 2015;14:86 doi:10.1186/s12940-015-0073-0
5. Eyong EU. Heamatoxic effect following ingestion of Nigeria crude oil polluted shellfish by rats. Niger J Physiol Sci. 2005;19:1-6. doi:10.4314/njps.v19i1.32627
6. King MD, Elliott JE, Williams TD. Effects of petroleum exposure on birds: A review. Sci Total Environ. 2021;755(Pt 1):142834. doi:10.1016/j.scitotenv.2020.142834
7. Ovuru SS, Berepubo NA, Nodu MB. Biochemical blood parameter in semi-adult rabbits experimentally fed crude oil contaminated diet. Afr J Biotechnol. 2004;3:343-5. doi:10.5897/AJB2004.000-2063
8. Yoshida S, Takeda K. The effects of diesel exhaust on murine male reproductive function. J Health Sci. 2004;50:210-4. doi:10.1248/jhs.50.210
9. Jeje SO, Ukwenya VO, Anita AB, Daramola OM. Maternal exposure to bonny light crude oil altered reproductive indices in male and female offspring of Wistar rats. Niger J Physiol Sci. 2021;36(1):49-55. PMid: 34987248
10. Ebokaiwe AP, D'Cruz SC, Jubendradass R, Amala Rani JS, Mathur PP, Farombi EO. Nigerian bonny-light crude oil induces alteration in testicular stress response proteins and caspase-3 dependent apoptosis in albino wistar rats. Environ Toxicol. 2015;30(2):242-52. doi:10.1002/tox.21902
11. Raji Y, Hart VO. Influence of prolonged exposure to Nigerian bonny light crude oil on fertility indices in rats. Niger J Physiol Sci. 2012;27(1):55-63. PMid: 23235309
12. Truter J, Van Wyk J, Oberholster PJ, Botha A, Mokwena L. An evaluation of the endocrine disruptive potential of crude oil water accommodated fractions and crude oil contaminated surface water to freshwater organisms using in vitro and in vivo approaches. Environ Toxicol Chem. 2017;36(5):1330-42. doi:10.1002/etc.3665
13. McEvoy CT, Schilling D, Clay N, et al. Vitamin C supplementation for pregnant smoking women and pulmonary function in their newborn infants: A randomized clinical trial. JAMA. 2014;311(20):2074-82. doi:10.1001/jama.2014.5217
14. Salami SA, Adesina DV, Ajayi GO, Akinsanya MA, Allen MO, Salahdeen HM. Possible mechanisms in Ficus exasperate ethanol root fraction amelioration of lead-induced corpus cavernosal contractile impairments in male Wistar rats. J Res Trad Med. 2022;8(2):55-63. doi:10.5455/jrtm.2022/ 46087
15. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978;52:302-10. doi:10.1016/s0076-6879(78)52032-6
16. Sun M, Zigman S. An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. Anal Biochem. 1978;90(1):81-9. doi:10.1016/0003-2697(78)90010-6
17. Sinha KA. Colorimetric assay of catalase. Anal Biochem. 1972;47:389-94. doi:10.1016/0003-2697(72)90132-7
18. Hafen BB, Burns B. Physiology, smooth muscle. Treasure Island, FL: StatPearls Publishing; 2022.
19. Berridge MJ. Inositol trisphosphate and calcium signaling mechanisms. Biochim Biophys Acta. 2009;1793(6):933-40. doi:10.1016/j.bbamcr.2008.10.005
20. Lenglet S, Mach F, Montecucco F. Methylene blue: Potential use of an antique molecule in vasoplegic syndrome during cardiac surgery. Expert Rev Cardiovasc Ther. 2011;9(12):1519-25. doi:10.1586/erc.11.160
21. Brozovich FV, Nicholson CJ, Degen CV, Gao YZ, Aggarwal M, Morgan KG. Mechanisms of vascular smooth muscle contraction and the basis for pharmacologic treatment of smooth muscle disorders. Pharmacol Rev. 2016;68(2):476-532. doi:10.1124/pr.115.010652
22. Adedara IA, Ebokaiwe AP, Ehwerhemuepha T, Teberen R, Farombi EO. Nigerian bonny light crude oil induces irreversible reproductive toxicity in adult male rats. Afr J Med Med Sci. 2012;41 Suppl:65-74. PMid: 23678639
23. Afonne OJ, Onyiaorah IV, Orisakwe OE. Toxicity of chevron escravos crude oil and chemical dispersant on guinea pig testicular function. J Basic Clin Physiol Pharmacol. 2013;24(4):321-9. doi:10.1515/jbcpp-2012-0060

