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Toxicological impacts of crude oil-contaminated and uncontaminated *Mangifera indica* stem bark aqueous extracts on hormonal and lipid metabolism in female wistar rats

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Abstract
This study investigated the biochemical effects of aqueous extracts from crude oil-contaminated (C) and uncontaminated (F) *Mangifera indica* stem bark on female Wistar albino rats. Stem barks were collected from oil-polluted agricultural land in Umuechem, Rivers State (C), and an uncontaminated site at the University of Benin, Edo State (F). Aqueous extracts were prepared by soaking 100 g of powdered bark in 1000 ml distilled water for 72 hours, followed by filtration, concentration via rotary evaporator, and freeze-drying. Eighty rats were divided into five groups: a control group received distilled water, while four treatment groups were administered 250 mg/kg or 3500 mg/kg body weight of C or F extracts orally via gavage for 90 days. Progesterone levels significantly decreased ($p<0.05$) in animals treated with C and F extracts, with the greatest reduction in animals in group 3 (C, 3500 mg/kg), likely due to crude oil's endocrine-disrupting properties and phytoestrogens, flavonoids, and terpenes in F extracts. Lipid profiles showed significant increases ($p<0.05$) in total cholesterol (TC), LDL-C, VLDL-C, and triglycerides in all treated groups compared to the control, with animals in group 3 exhibiting the highest elevation, attributed to liver damage from crude oil metabolism and free radical-induced membrane disruption by F extract phytochemicals, such as saponins, tannins, and quercetin. HDL-C levels significantly decreased ($p<0.05$) in animals in groups treated with C and F extracts, linked to hepatocyte damage from crude oil metabolites and polyphenol-induced oxidative stress in F extracts. These dose-dependent effects highlighted the toxicological impact of crude oil and phytochemicals on hormonal and lipid metabolism, underscoring environmental and health risks in oil-polluted regions like the Niger Delta, where crude oil spills annually released approximately two million barrels, severely impacting agriculture, marine life, and human health.

Keywords: *Mangifera indica*, phytoestrogens, Umuechem, flavonoids and terpenes

Introduction
Oil spill pollution increasingly threatens global agricultural sustainability and human health, potentially worsening food shortages if not addressed promptly. These spills, occurring on land and in water, disrupt fishing and farming activities. The Department of Petroleum Resources (DPR) and studies report that around two million barrels of crude oil are released annually into Nigeria's Niger Delta offshore and coastal waters, a region heavily impacted by unrefined petroleum spills, raising significant global climate concerns (Ite *et al*., 2013) [7]. Coastal oil spills also deplete oxygen levels, reducing dissolved oxygen and impairing marine life support (Smith, 2011) [8]. Common spill sources include underground storage tank leaks, oil truck accidents, pipeline vandalism, and other incidents, making crude oil a major pollutant of surface and groundwater. In regions with limited healthcare, communities use traditional remedies like *Mangifera indica* (mango) stem bark extracts for medicinal purposes. The mango tree, from the Mangifera genus in the *Anacardiaceae* family, has been utilized in traditional medicine for over 4,000 years due to its diverse medicinal properties (Na *et al*., 2015) [6]. Mangiferin, a key xanthone in *Mangifera indica*, present in leaves and fruits, mitigates dyslipidemia in mice and hamsters on high fat diets and shows similar effects in humans, while also aiding weight loss (Imran *et al*., 2017; Matkowski *et al*., 2013; Guo *et al*., 2011) [3, 5, 2]. Studies on high-fat diet-fed rats show mangiferin upregulates liver proteins linked to mitochondrial bioenergetics and downregulates those involved in de novo

lipogenesis, suggesting its role in regulating lipid metabolism and energy homeostasis (Niu *et al*., 2012) [4]. In this study, we administered extracts from both oil-contaminated and uncontaminated *Mangifera indica* to Wistar albino rats, evaluating the biochemical effects on their health and well-being.

Materials and Methods

Chemicals

All chemicals and reagents, of analytical grade, were sourced from M/s Merck India, Ltd., Bombay. Distilled water and acid-washed glassware were used throughout the analysis.

Plant Collection

The stem bark of *Mangifera indica* (C) was collected from oil-polluted agricultural land in Umuechem, Ikwere Local Government Area, Rivers State. In contrast, uncontaminated *Mangifera indica* stem bark (F) was obtained from the University of Benin, Edo State, Nigeria, a site free from oil pollution. Both samples were sealed, labeled, and sent to the Department of Plant Biology and Biotechnology for identification, where Dr. Akinnibosun Henry assigned them the voucher number UBHM 0249.

Crude Drug Powder Preparation

The stem barks of *M. indica* C and *M. indica* F were collected, cut into smaller pieces, and air-dried for two weeks. The dried barks were ground, sieved into a coarse powder, and further air-dried indoors in a well-ventilated area for three weeks. Subsequently, they were milled into a fine powder using a milling machine at the Department of Pharmacy, University of Benin, for phytochemical and proximate analyses.

Aqueous Extract Preparation

A 100 g sample of the powdered herb was soaked in 1000 ml of distilled water for 72 hours at room temperature, with periodic stirring. The mixture was filtered through sintered glass to remove cellulose fibers, and the filtrate was concentrated using a rotary evaporator, then freeze-dried. The resulting extract was stored in clean plastic bottles and refrigerated at 4 °C.

Experimental Design and Extract Administration

The study was conducted at the animal facility of the Biochemistry Department, Faculty of Life Sciences, University of Benin, Edo State, Nigeria. Spanning four weeks, including acclimatization, experimental protocols, and data analysis, the administration of plant extracts in aqueous form lasted three months. Eighty female Wistar albino rats were randomly divided into five groups (16 rats each): four treatment groups and one control. Treatment groups received oral doses of 250 mg/kg or 3500 mg/kg body weight of the extract daily via gavage for 90 days, while the control group received normal saline. Rats were monitored for mortality and behavioral changes. Dosages were based on prior studies. The treatment groups were as follows:

- **Group 1 (Control):** Female Wistar albino rats received distilled water via gavage.
- **Group 2 (C-250 mg/kg bwt):** Female Wistar albino rats were given 250 mg/kg body weight of crude oil-contaminated aqueous extract of *Mangifera indica* stem bark via gavage.
- **Group 3 (C-3500 mg/kg bwt):** Female Wistar albino rats received 3500 mg/kg body weight of crude oil-contaminated aqueous extract of *Mangifera indica* stem bark via gavage.
- **Group 4 (F-250 mg/kg bwt):** Female Wistar albino rats were administered 250 mg/kg body weight of crude oil-free aqueous extract of *Mangifera indica* stem bark via gavage.
- **Group 5 (F-3500 mg/kg bwt):** Female Wistar albino rats received 3500 mg/kg body weight of crude oil-free aqueous extract of *Mangifera indica* stem bark via gavage.

Biochemical Sample Collection and Preparation

On the 90th day, following an overnight fast, the rats were sacrificed via cervical dislocation. Blood samples were collected from the heart using a syringe and stored in sterile bottles for serum analysis.

Assessment of serum biochemical parameters

Estimation of lipid profile

The plasma concentration of TC, TG, HDL-cholesterol and LDL-cholesterol were measured using spectrophotometric methods. Laboratory kit reagents (Randox Laboratory Ltd, UK) were used for all biochemical analysis and their absorbance was read using a UV-Vis spectrophotometer (DREL 3000 HACH).

Estimation of progesterone levels

Hormonal assay in the serum was carried out using an enzyme immunoassay kit (EIA) by Immunometrics, UK. Duplicate analyses of the samples were performed for accuracy.

Statistical Analysis

All data obtained were subjected to statistical analysis using Student's t-test using Statistical Package for Social Sciences (SPSS for windows, version 12.0). Data were expressed as mean± standard error of mean (SEM). Values of $p< 0.05$ were considered significant.

Result

Table 1.0 reveals significant changes in progesterone levels in specific treated groups compared to the control ($p<0.05$). Rats in groups 2, 3, and 5, which received aqueous extracts of C and F, showed a notable decrease in progesterone concentrations relative to the control. Animals in group 3, treated with 3500 mg/kg body weight of C extract, exhibited the most pronounced reduction among the treated groups, likely due to the high dosage and the presence of crude oil and its metabolites in the extract. Conversely, animals in group 4, administered F extracts, showed no significant decrease in progesterone levels compared to the control ($p<0.05$).

Table 2.0 highlights significant alterations in lipid profiles, including total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol, following administration of aqueous extracts of C and F to rats ($p<0.05$). Animals in groups 2, 3, 4, and 5, treated with 250 mg/kg and 3500 mg/kg body weight of the extracts displayed a significant increase in total cholesterol, triglycerides, VLDL cholesterol, and LDL cholesterol compared to the control ($p<0.05$). Animals in

group 3, receiving 3500 mg/kg body weight of C extract, showed the most substantial increase in these lipid parameters compared to other groups (p<0.05). Additionally, Table 2.0 indicates a significant reduction in HDL cholesterol levels in animals in groups 2, 3, and 5, with a non-significant decrease in animals in group 4, when treated with varying doses of C and F extracts, compared to the control (p<0.05).

Table 1: Effects of plant extracts on progesterone in treated Wistar albino

Treatment	Hormonal balance
	Progesterone (nmol/L)
Control	0.200±0.020[a]
250 mg/kg body weight (C)	0.143±0.033[b]
3500 mg/kg body weight (C)	0.093±0.006[c]
250 mg/kg body weight (F)	0.200±0.015[a]
3500 mg/kg body weight (F)	0.137±0.006[b]

Values are progesterone levels of rats and are expressed as means± SEM. Values with different superscripts different from the control across the columns in each group are significantly (p< 0.05) different.
F = *Mangifera indica* obtained from an uncontaminated environment
C= *Mangifera indica* obtained from a crude oil-contaminated environment

Table 2: Lipid profile indices of female rats treated with various doses of aqueous extract of *Mangifera indica* LINN stem bark gotten from uncontaminated and crude oil contaminated environments

Treatment			Serum Lipid profile (mg/dl)		
	Total Cholesterol	HDL-Cholesterol	LDL-Cholesterol	VLDL-Cholesterol	Triglyceride
Control	59.700±1.986[a]	30.000±2.161[a]	20.340±0.271[a]	9.362±0.427[a]	46.810±0.976[a]
250 mg/kg body Weight (C)	110.160±1.189[b]	12.500±0.479[b]	75.190±1.287[b]	22.468±0.727[b]	112.340±2.182[b]
3500 mg/kg bodyweight (C)	344.250±1.842[c]	4.000±0.404[c]	207.040±2.356[c]	133.210±1.703[c]	351.080±1.128[c]
250 mg/kg body Weight (F)	68.850±0.733[d]	20.000±0.739[a]	34.810±0.860[d]	14.040±0.525[d]	70.220±0.520[d]
3500 mg/kg bodyweight (F)	88.840±0.993[e]	15.500±0.623[b]	55.220±1.657[e]	19.880±0.162[e]	90.600±1.368[e]

Values are lipid profile of rats and are expressed as means± SEM. Values with different superscripts different from the control across the columns in each group are significantly (p< 0.05) different.
F = *Mangifera indica* obtained from an uncontaminated environment
C= *Mangifera indica* obtained from a crude oil-contaminated environment

Discussion

This study revealed a significant decrease (p<0.05) in progesterone levels in rats treated with aqueous extracts of C and F compared to the control, as shown in Table 1.0. This reduction may result from crude oil's toxic effects, acting as an anti-progesterone compound and endocrine disruptor, potentially causing abortions and reproductive dysfunction. This aligns with Achuba and Osakwe (2003) [9], who reported crude oil's disruption of the rat endocrine system, leading to hormonal imbalances and impaired reproductive and physiological functions. Additionally, the notable decrease in progesterone in rats treated with 3500 mg/kg body weight of F extracts may be linked to phytoestrogens, terpenes, and flavonoids. Phytoestrogens, plant compounds mimicking estrogen, can disrupt the endocrine system and affect reproductive health in rats, particularly at high doses (Cassidy, 2003) [10]. Flavonoids and polyphenols, despite their antioxidant benefits, may interfere with hormone signaling at high doses (Marques *et al*., 2015) [11], while terpenes can influence hormone levels and nervous system function, potentially causing hormonal disturbances (Wilson *et al*., 2018) [12]. Notably, group 3 rats, treated with 3500 mg/kg body weight of crude oil-contaminated *M. indica* stem bark (C) extracts, exhibited the most significant progesterone alterations compared to other treated groups and the control, likely due to the dosage and presence of crude oil metabolites.

Lipids are vital for cell membrane development, serving as precursors for vitamin D, bile salts, and steroid hormones. Total cholesterol (TC) is linked to increased atherosclerosis, while LDL-C and VLDL-C transport TC to tissues. To manage and prevent related diseases, drugs, food supplements, and nutrition are recommended to lower TC, VLDL-C, and LDL-C while raising HDL-C levels (NCEP, 2002; Brunzel *et al*., 2008; Drexel, 2006) [13, 14, 15]. Table 2.0 shows a significant increase (p<0.05) in serum TC, LDL-C, VLDL-C, and triglyceride levels in rats treated with various doses of C extracts compared to the control, indicating crude oil's impact on lipid metabolism. This finding is consistent with Uboh *et al*. (2005) [16], who noted that exposure to kerosene and petrol fumes (crude oil fractions) via inhalation disrupts lipid metabolism. This disruption may stem from liver cell (hepatocyte) damage during crude oil metabolism, reducing cholesterol clearance and elevating LDL-C. Free radicals produced during crude oil metabolism likely compromise liver cell membrane integrity, releasing membrane lipids like cholesterol into circulation. The significant increase (p<0.05) in triglycerides may result from hepatocytes' inability to store triglycerides due to liver cell destruction, aligning with Uboh *et al*. (2005) [16].

Table 2.0 also indicates a significant increase (p<0.05) in TC, LDL-C, VLDL-C, and triglyceride levels in rats treated with F extracts compared to the control. Saponins, tannins, and quercetin in these extracts, known for diverse biological activities, may disrupt biological metabolites, enzymes, and free radicals (Francis *et al*., 2002) [17]. This loss of membrane integrity, driven by free radicals, can oxidize LDL, triggering platelet aggregation and monocyte adhesion to the endothelium, damaging it, as supported by Manach (2005) [18]. Oxidative stress alters blood vessel biology, increasing unhealthy fats like LDL-C and promoting cholesterol plaque buildup in arteries, raising heart attack

and stroke risks (Rice-Evans *et al*., 1996) [19]. The notable increase ($p<0.05$) in triglycerides in rats treated with F extracts may be due to liver dysfunction caused by free radicals, consistent with Owu *et al*. (1998) [19], and could be dose dependent. Additionally, phytochemicals with pro-inflammatory and hormone-like effects may indirectly elevate lipid levels by promoting inflammation in lipid metabolism (Calder, 2015) [21] and interacting with hormonal pathways (Ravichandran, 2012) [22].

High-density lipoprotein (HDL), often called "good" cholesterol, scavenges excess cholesterol from the blood, returning it to the liver for breakdown. However, rats treated with various doses of C extracts showed a significant decrease ($p<0.05$) in HDL-C levels compared to the control (Table 2.0). This reduction may be due to crude oil metabolites (e.g., PAHs) causing oxidative stress, severely damaging hepatocytes and impairing HDL-C production and secretion by the liver, as noted by Uboh *et al*. (2007) [23]. Consequently, reverse cholesterol transport, where HDL removes excess cholesterol from cells for liver excretion, is hindered by crude oil metabolites' harmful effects on the liver. Similarly, a significant decrease ($p<0.05$) in HDL-C levels was observed in rats treated with F extracts compared to the control (Table 2.0). This may result from polyphenols like catechins in the extract, which, despite their antioxidant properties, can cause pro-oxidative effects at high doses, leading to oxidative stress, membrane disruption, and free radical release (Yang *et al*., 2001) [24]. This cascade damages hepatocytes, reducing HDL-C production, as supported by Khoo *et al*. (2017) [25]. The significant decrease in HDL-C is dose-dependent. Notably, group 3 rats, treated with 3500 mg/kg body weight of crude oil-contaminated *M. indica* stem bark (C) extracts, displayed the most significant changes in lipid profiles, including increases in TC, LDL-C, and triglycerides, and decreases in HDL-C, compared to other treated groups and the control. These alterations are likely due to both the dosage and the presence of crude oil and its metabolites in the extract.

Conclusion

The study revealed that aqueous extracts from crude oil-contaminated (C) and uncontaminated (F) *Mangifera indica* stem bark had a significant impact on female Wistar albino rats. It was found that progesterone levels notably decreased ($p<0.05$) in the treated groups, particularly in group 3 (C, 3500 mg/kg), which was attributed to crude oil's endocrine-disrupting effects and the presence of phytochemicals such as phytoestrogens and flavonoids in the F extracts. The study also indicated that lipid profiles showed an increase in total cholesterol, LDL-C, VLDL-C, and triglycerides, alongside a reduction in HDL-C ($p<0.05$), due to liver damage and oxidative stress caused by crude oil and F extract compounds like saponins and quercetin. It was concluded that these dose-dependent effects highlighted the toxicological risks posed by crude oil and phytochemicals, suggesting the need for further research.

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Reference

1. Uzoije AP, Onunkwo A, Egwuonwu N. Crude oil sorption onto groundnut shell activated carbon: kinetic and isotherm studies. Res J Environ Earth Sci. 2011;3(5):555-563.
2. Guo F, Huang C, Liao X, Wang Y. Beneficial effects of mangiferin on hyperlipidemia in high-fat-fed hamsters. Mol Nutr Food Res. 2011;55(12):1809-1818.
3. Imran M, Arshad MS, Butt MS, Kwon JH, Arshad MU, Sultan MT. Mangiferin: A natural miracle bioactive compound against lifestyle related disorders. Lipids Health Dis. 2017;16(1):84.
4. Niu Y, Li S, Na L, Feng R, Liu L, Li Y, *et al*. Mangiferin decreases plasma free fatty acids through promoting its catabolism in liver by activation of AMP. PLoS One. 2012;7(1):e30782.
5. Matkowski A, Kuś P, Góralska E, Woźniak D. Mangiferin - a bioactive xanthonoid, not only from mango and not just antioxidant. Mini Rev Med Chem. 2013;13(3):439-455.
6. Na L, Zhang Q, Jiang S, Du S, Zhang W, Li Y, *et al*. Mangiferin supplementation improves serum lipid profiles in overweight patients with hyperlipidemia: a double-blind randomized controlled trial. Sci Rep. 2015;5(1):10344.
7. Ite A, Udo I, Ite M, Petters S. Petroleum exploration and production: past and present environmental issues in the Nigeria's Niger Delta. Am J Environ Protect. 2013;1(4):78-90.
8. Smiths JE. Industrial pollution and its effects on birds: from individual birds to populations and communities. In: Silva EM, Lu JJ, editors. Environmental contamination. 2011. p. 207-28.
9. Achuba FI, Osakwe SA. Petroleum induced free radical toxicity in African catfish (Clarias gariepinus). Fish Physiol Biochem. 2003;29(2):97-103.
10. Cassidy A. Dietary phytoestrogens and bone health. J Br Menopause Soc. 2003;9(1):17.
11. Marques-Lopes J, Van Kempen T, Waters EM, Pickel VM, Iadecola C, Milner TA. Slow-pressor angiotensin II hypertension and concomitant dendritic NMDA receptor trafficking in estrogen receptor beta-containing neurons of the mouse hypothalamic paraventricular nucleus are sex and age dependent. J Comp Neurol. 2015;69(2):148-165.
12. Wilson K, Hofmann A, Walker JM, Clokie S. Editors. Wilson and Walker's principles and techniques of biochemistry and molecular biology. Cambridge University Press; c2018.
13. National Cholesterol Education Program (NCEP). Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults–adult treatment panel III, final report. NCEP, National Heart, Lung, and Blood Institute, National Institutes of Health, USA; c2002.
14. Brunzell JD, Davidson M, Furberg CD. Lipoprotein management in patients with cardiometabolic risk–consensus statement from the American Diabetes

Association and the American College of Cardiology Foundation. Diabetes Care. 2008;31(4):811-822.
15. Drexel H. Reducing risk by raising HDL-cholesterol: the evidence. Eur Heart J Suppl. 2006;8(1):23-29.
16. Uboh FE, Akpanabiatu MI, Eyong UE, Ebong PE, Eka OO. Evaluation of toxicological implications of inhalation exposure to kerosene fumes and petrol fumes in rats. Acta Biol Szegediensis. 2005;49(1):19-22.
17. Francis G, Kerem Z, Makkar H, Becker K. The biological action of saponins in animal systems: a review. Br J Nutr. 2002;88(6):587-605.
18. Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans: I. Review of 97 bioavailability studies. Am J Clin Nutr. 2005;81(1):230S-42S.
19. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 1996;20(7):933-956.
20. Owu D, Osime E, Ebong P. Serum liver enzymes profile of Wistar rats following chronic consumption of fresh or oxidized palm oil diets. Acta Trop. 1998;69(1):65-73.
21. Calder PC. Functional roles of fatty acids and their effects on human health. J Parenter Enteral Nutr. 2015;1(1):18S-32S.
22. Ravichandran R, Venugopal JR, Sundarrajan S, Mukherjee S, Ramakrishna S. Precipitation of nanohydroxyapatite on PLLA/PBLG/collagen nanofibrous structures for the differentiation of adipose derived stem cells to osteogenic lineage. Biomaterials. 2012;33(3):846-855.
23. Uboh FE, Akpanabiatu MI, Atangwho IJ, Ebong PE, Umoh IB. Effect of gasoline vapours on serum lipid profile and oxidative stress in hepatocyte of male and female rats. Acta Toxicology. 2007;15(1):13-18.
24. Yang CS, Landau JM, Huang MT, Newmark HL. Inhibition of carcinogenesis by dietary polyphenolic compounds. Annu Rev Nutr. 2001;21(1):381-406.
25. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res. 2017;61(1):1361779.