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Impact of crude oil-contaminated plant extracts on renal and hepatic function in rats: A biochemical perspective

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Abstract
Crude oil, derived from ancient marine life through heat and pressure, has shaped Nigeria's oil industry since its 1956 discovery in Bayelsa State. This has positioned Nigeria as a major oil producer but introduced environmental issues, including frequent spills due to negligence or sabotage, with minimal cleanup in areas like Ogoniland. The use of oil-contaminated plants for traditional medicine, spurred by inadequate healthcare, led to this study. We examined how *Mangifera indica* stem bark extracts from both polluted and clean sites affect enzyme activity in female Wistar albino rats. Eighty rats, each around 155 grams, were involved. The contaminated extract was from an oil spill in Umuechem, Ikwere, Rivers State, while the clean extract was from the University of Benin. Rats were divided into five groups: Group I was the control; Groups II and III received 250 mg/kg and 3500 mg/kg of contaminated extract, respectively; Groups IV and V got the same dosages of clean extract. The study duration was 90 days.. This study revealed that renal function parameters in rats exposed to various doses of aqueous extracts C and F showed significant alterations ($p<0.05$). Concentrations of creatinine, urea, sodium, potassium, bicarbonate, and chloride either significantly increased or decreased ($P<0.05$) in the serum of rats from groups II, III, and V when compared to the control group. Notably, animals in group III rats, which received 3500mg/kg body weight of extract C, exhibited markedly higher concentrations ($p<0.05$), indicating substantial organ damage. Conversely, group IV rats displayed a significant increase ($P<0.05$) in creatinine, urea, potassium, and sodium, but only a non-significant decrease ($P>0.05$) in chloride and bicarbonate levels compared to the controls, suggesting a dose-dependent response. Similarly, liver function markers like ALT, ALP, AST, total bilirubin, albumin, and total protein were significantly altered in rats treated with 250 and 3500mg/kg body weight of extracts C and F. The serum concentrations of these markers significantly increased or decreased ($P<0.05$) in groups II, III, and V when compared to the control. Animals in group III, receiving the highest dose of extract C, had the most pronounced changes in ALT, ALP, total bilirubin, and AST levels. Meanwhile, rats in group IV showed a non-significant increase ($P>0.05$) in ALT and ALP, a significant increase in total protein ($P<0.05$), and a significant decrease in albumin ($P<0.05$) compared to the control group, highlighting that the impact on rat physiology is influenced by the dose, the nature of the extract, and exposure duration. These findings suggest that crude aqueous extracts *Mangifera indica* stem barks, especially when contaminated with crude oil, can cause significant kidney and liver toxicity in rats. The extent of damage increases with higher doses, highlighting the need for careful consideration when using plant extracts from polluted areas.

Keywords: *Mangifera indica*, creatinine, urea, electrolytes, hepatocytes

Introduction
In the early 20th century, crude oil was first discovered in Nigeria, with a pivotal commercial find made in 1956 by Shell D'Arcy Petroleum, which later became Shell Petroleum Development Company of Nigeria Limited (SPDC), in Oloibiri, now within Bayelsa State (Amu, 1983) [1]. This event catalyzed the development of Nigeria's oil sector, elevating the nation to one of the foremost oil producers globally. Over time, exploration extended into the Niger Delta, where major multinational corporations like Shell, ExxonMobil, Chevron, Total, and Eni have established significant operations. Oil serves as a principal energy source in Nigeria, with recent months witnessing an uptick in domestic production. The continuous transport of vast oil quantities through pipelines across the country invariably leads to oil spills (Stevens, 2003) [2]. These spills have been a major environmental concern for decades,

with several significant incidents causing long-term ecological and economic damage. The environmental impact of an oil spill varies based on factors like spill volume, release rate, oil type, and spill location. Even smaller spills can have profound effects on individual organisms and whole ecosystems, with impacts manifesting over various timescales, from immediate to long-lasting effects that might span decades (Nwilo & Badejo, 2005) [3]. In regions where healthcare infrastructure is lacking, local communities have turned to traditional remedies, including the use of plant extracts such as those from the stem bark of *Mangifera indica* (mango) for medicinal purposes. The mango tree, from the Mangifera genus within the *Anacardiaceae* family, has been utilized in traditional medicine for over 4,000 years due to its diverse medicinal properties (Gilberto *et al.*, 2008) [4]. Mangiferin, a key compound in mango, is recognized for its antioxidant, anti-lipid peroxidation, immunomodulatory, cardiotonic, hypotensive, wound healing, anti-degenerative, and antidiabetic effects (Jelena *et al.*, 2023) [5]. In the current research, we administered extracts from both oil-contaminated and uncontaminated *Mangifera indica* to Wistar albino rats, subsequently analyzing the biochemical impacts on their health and overall well-being.

Materials and methods

Chemicals

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

Collection of plant

The stem bark of *Mangifera indica* (C) was collected from an oil-polluted agricultural land in Umuechem, located in the Ikwere Local Government Area of Rivers State. Conversely, the uncontaminated *Mangifera indica* stem bark (F) was gathered from the University of Benin in Edo State, Nigeria, which is free from oil pollution. Both specimens were carefully sealed, labeled, and dispatched to the Department of Plant Biology and Biotechnology for accurate identification. There, Dr. Akinnibosun Henry issued them the voucher number UBHM 0249.

Preparation of crude drug powder

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 and then further sieved to obtain a coarse powder. The pieces were then air-dried indoors in a well-ventilated area for three weeks. After drying, they were ground using a milling machine in the Department of Pharmacy at the University of Benin. The resulting powdered materials were subsequently used for phytochemical and proximate analyses.

Preparation of Aqueous and Ethanolic Extract

Preparation of Aqueous Extract: A 100 g portion of the powdered herb was extracted by soaking it in 1000 ml of distilled water, using the water as the solvent. The mixture was stirred and left at room temperature for 72 hours. Afterward, the solution was filtered through sintered glass to remove cellulose fibers, with the filtrate being collected. This filtrate was then concentrated using a rotary evaporator and subsequently freeze-dried with a freeze dryer. The resulting extract was stored in clean plastic bottles and refrigerated at 4°C.

Experimental Design and Administration of Extract

The research was carried out in the animal facility of the Biochemistry Department, within the Faculty of Life Sciences at the University of Benin, Edo State, Nigeria. The study, encompassing acclimatization, experimental protocols, and data analysis, extended over four weeks; however, the administration of the plant extracts in water form continued for three months. The rats were randomly assigned to five groups: four for treatment and one as a control, with each group comprising 16 rats. The treatment groups were given oral doses of 250 mg/kg and 3500 mg/kg based on body weight of the extract daily for 90 days using gavage. The control group received normal saline. During these 90 days, the animals were observed for signs of death and behavioral alterations. The dosages were chosen based on prior research. The treatment process was as follows: (specific details to follow)

Group 1 (Control): Female Wistar Albino rats were administered distilled water orally.

Group 2 (C- 250 mg/kg bwt): Female Wistar Albino rats were orally given 250 mg/kg body weight of crude oil-contaminated aqueous extract of *Mangifera indica* Linn stem bark.

Group 3 (C- 3500 mg/kg bwt): Female Wistar Albino rats were orally administered 3500 mg/kg body weight of crude oil-contaminated aqueous extract of *Mangifera indica* Linn stem bark.

Group 4 (F- 250 mg/kg bwt): Female Wistar Albino rats were orally administered 250 mg/kg body weight of crude oil-free aqueous extract of *Mangifera indica* Linn stem bark.

Group 5 (F- 3500 mg/kg bwt): Female Wistar Albino rats were orally given 3500 mg/kg body weight of crude oil-free aqueous extract of *Mangifera indica* Linn stem bark.

Biochemical Sample Collection and Preparation

On the 90th day, after an overnight fast, the rats were sacrificed by cervical dislocation. Blood samples were drawn from the heart using a syringe and collected into sterile bottles for serum analysis.

Assessment of serum biochemical parameters

Estimation of liver function parameters

Liver function was assessed by measuring serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) with UV spectrophotometry at wavelengths of 505/670 nm using commercial kits. Results for these enzymes were reported in International Units per Liter (IU/L). Serum alkaline phosphatase (ALP) levels were quantified at an absorbance of 405 nm. Albumin concentration in the rats' serum was determined using Bromocresol Green (BCG) according to the method of Doumas *et al.*, 1971[6]. Total bilirubin was analyzed using the Cobas C111 automated chemistry analyzer, adhering to the manufacturer's protocol for the diazo bilirubin assay.

Estimation of renal serum function parameters

Determination of serum creatinine according to colorimetric method was described by Tietz and Andresen (1986) [8]. Urea was determined in serum by modified urease-Berthlot

method according to Tietz *et al.* (1990) [7]. Na+, K+, HCO_3- and Cl− contents in serum were determined using the automatic biochemistry analyzer.

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 displays the renal function parameters of rats exposed to various doses of aqueous extracts C and F. It shows that levels of creatinine, urea, sodium, potassium, bicarbonate, and chloride significantly increased or decreased ($P<0.05$) in the serum of rats from groups 2, 3, and 5, when compared to the control group. Specifically, group 3 rats, which received 3500mg/kg body weight of extract C, showed markedly higher concentrations, suggesting significant organ damage. In contrast, group 4 rats experienced a significant rise ($P<0.05$) in creatinine, urea, potassium, and sodium, alongside a non-significant drop ($P>0.05$) in chloride and bicarbonate levels compared to controls. The data from groups 4 and 5 imply a dose-dependent effect. Table 4.7 outlines significant alterations in liver function markers like ALT, ALP, AST, total bilirubin, albumin, and total protein in rats treated with 250 and 3500mg/kg body weight of extracts C and F. The serum levels of ALT, ALP, AST, total bilirubin, and total protein significantly increased or decreased ($P<0.05$) in groups 2, 3, and 5 compared to the control group. Group 3, dosed with 3500mg/kg of extract C, showed the highest concentrations of ALT, ALP, total bilirubin, and AST among the treated groups. Group 4 exhibited a non-significant increase ($P>0.05$) in ALT and ALP, a significant rise in total protein ($P<0.05$), and a significant decrease in albumin ($P<0.05$) relative to controls. These outcomes indicate that the effects on the rats are modulated by the dose, the specific extract used, and the length of exposure

Table 1.0 Renal function indices of female Wistar albino rats treated with various doses of *Mangifera indica* aqueous extracts.

Treatment	**Renal function indices**						
Control	Creatinine	Urea (mg/dl)	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	$HCO3^-$ (mmol/l)	
	(mg/dl)						
	1.754±0.019[a]	46.850±0.548[a]	142.000±0.577[a]	4.900±0.058[a]	110.000±0.333[a]	21.000±0.577[a]	
250mg/kg body weight	2.820±0.012[b]	52.134±0.164[b]	146.000±1.155[b]	9.300±0.115[b]	108.000±0.882[b]	19.000	±1.000[b]
(C)							
3500mg/kg body	2.974±0.012[c]	54.123±0.506[c]	148.000±0.333[c]	11.000±0.882[c]	106.000±0.334[c]	17.000	±1.155[c]
weight(C)							
250mg/kg body weight	2.488±0.094[d]	48.950±0.462[d]	143.000±0.578[a]	8.900±0.115[d]	110.000±0.577[a]	20.000	±1.154[ab]
(F)							
3500mg/kg body	2.820±0.035[b]	51.734±0.453[b]	145.000±0.578[b]	11.100±0.231[c]	108.000±0.333[b]	18.000	±1.155[bc]
weight(F)							

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

Table 2.0 Serum liver function indices of female Wistar albino rats treated with various doses of *Mangifera indica* aqueous extracts.

Treatment	**Liver Function Indices**					
Control	ALT (U/L)	AST (U/L)	ALP (U/L)	Total bilirubin	Albumin (g/dl)	Total protein(g/dl)
	(mg/dl)					
	19.000±1.732[a]	75.000±2.887[a]	30.000±0.577[a]	0.116±0.004[a]	2.852±0.042[a]	4.378±0.085[a]
250mg/kg body	24.000±1.732[b]	90.000±4.041[b]	44.000±3.464[b]	0.356±0.032[b]	2.466±0.077[b]	4.838±0.182[b]
weight (c)						
3500mg/kg	28.000±0.882[c]	112.000±4.041[c]	52.000±3.464[c]	0.367±0.010[b]	2.440±0.069[b]	5.414±0.168[c]
body weight (c)						
250mg/kg body	20.000±1.155[a]	81.000±6.350[d]	30.000±1.154[a]	0.248±0.001[c]	2.624±0.165[c]	4.423±0.005[a]
weight (f)						
3500mg/kg body weight (f)	23.000±1.732[b]	89.000±1.732 [b]	42.000±1.732[b]	0.346±0.009[b]	2.472±0.023 [b]	5.3345±0297 [c]

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

Discussion

Renal failure is characterized by a progressive decline in kidney function, often resulting from genetic predispositions or conditions like cancer, diabetes, and hormonal disruptions (Eduardo *et al.*, 2015) [9]. The assessment of kidney health relies on monitoring serum levels of urea and creatinine, which increase as the kidneys' ability to filter waste diminishes, signaling compromised glomerular filtration and overall renal function. In our experimental setup, we observed a significant increase ($p<0.05$) in the serum concentrations of creatinine and urea in rats treated with aqueous extracts of *Mangifera indica* stem barks C and F, notably higher in those administered with 3500mg/kg body weight of extract C, as detailed in Table 1.0. This increase suggests renal toxicity, likely due to the presence of crude oil in the extract. Crude oil is known to harbor toxic compounds that can adversely affect kidney function by disrupting normal filtration processes, leading to the buildup of

metabolic waste in the blood. This observation is consistent with previous research by Szaro *et al.* (1980) [10] and Abolagba *et al.* (2017) [11], who documented similar increases in rats exposed to crude oil, and Aladeitan *et al.* (2020) [13], who noted elevated creatinine and urea in human populations in oil-polluted areas. The degree of renal impairment appears influenced by exposure duration, the concentration of crude oil, and individual variability in response. Moreover, our study revealed a dose-dependent increase in these markers in rats treated with extracts from *Mangifera indica* stem bark (F), hinting at the potential nephrotoxic effects of prolonged use of these extracts. Plants like *Mangifera indica* contain compounds such as alkaloids, glycosides, and lectins, which can be harmful to kidney function when consumed in high amounts (Cheeke, 1989; Panter and James, 1990) [12, 14]. This is corroborated by studies on other plant extracts, like those from *Fadogia agrestis* by Yakubu *et al.* (2008) [15] and Mitchell and Kline (2006) [16], indicating similar nephrotoxic effects. The most pronounced alterations in urea and creatinine were observed in animals in group III, which received the highest dose of C extract, suggesting that both the dosage and the contamination with crude oil play critical roles in these effects. The study also highlighted significant changes in electrolyte balance, with an increase ($p<0.05$) in serum potassium and a decrease in chloride and bicarbonate levels, as shown in Table 1.0. These shifts point to potential renal pathology, aligning with findings by Kluwe (1981) [17]. Crude oil can disturb electrolyte regulation by interfering with ion channels and transporters, and the inflammatory cascade it induces can exacerbate these imbalances. Research by Xie *et al.* (2016) [18] and others, including Wang and Wang (2018) [20] and Gupta and Rico-Medina (2020) [19], have shown how crude oil exposure alters electrolyte levels, leading to potential health issues ranging from metabolic disturbances to cardiovascular and neurological dysfunction. The phytochemicals in the F extract might also contribute to these toxic effects, potentially disrupting electrolyte management by acting on various organs, including the kidneys (Maughan and Griffins, 2003) [21].

Liver function was equally affected, as evidenced by significant increased ($p<0.05$) levels of liver enzymes like AST, ALT, and ALP, and total bilirubin, indicating hepatocyte damage. These significant changes ($p<0.05$) are likely due to the toxic components of crude oil or plant compounds like saponins, tannins, and alkaloids (Mehdi-Araghi and Ahmadi, 2013; Offor *et al.*, 2015) [22, 23]. Such compounds can lead to oxidative stress, causing membrane damage and enzyme leakage from the liver. The significant decrease in serum albumin levels further suggests liver dysfunction, as albumin is predominantly produced by the liver, and its reduction could be linked to increased bilirubin levels due to hepatotoxicity or hemolysis induced by crude oil (Lee, 2003; Orisakwe *et al.*, 2005) [24, 26]. The dose-dependent nature of these changes underscores the potential for chronic liver conditions like fibrosis or cirrhosis with continued exposure, highlighting the hepatotoxic potential of both crude oil and certain plant extracts.

Conclusion

This study illustrates the severe risks associated with the use of plant extracts from environments contaminated with crude oil, emphasizing the need for caution in both medicinal and environmental contexts. The implications for toxicology and public health are profound, calling for further investigation into the interaction between environmental toxins and biological systems, as well as stringent regulations on the sourcing and use of natural remedies in contaminated regions.

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