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Biochemical effects of ingested crude oil contaminated *Mangifera indica* stem bark aqueous and ethanol extracts in wistar albino rats

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Abstract
The impact of crude oil spills on food safety is increasingly concerning due to associated health issues. In Nigeria, rural areas, which are major sites of crude oil exploration, produce most of the food crops. This study aimed to evaluate the biochemical effects of aqueous and ethanolic extracts of crude oil-contaminated *Mangifera indica* stem bark on adult female Wistar albino rats. One hundred and twenty-five rats were divided into three groups: control, aqueous, and ethanol extract groups, with each extract group further divided into crude oil-free and contaminated sub-groups. The sub-groups were administered doses of 85 mg/kg, 170 mg/kg, 255 mg/kg, 340 mg/kg, 425 mg/kg, and 510 mg/kg. After 12 weeks of treatment, the rats were euthanized, and blood samples were analyzed for renal function markers such as creatinine and urea levels. Results indicated that crude oil-contaminated extracts significantly ($p<0.05$) elevated the concentrations of these renal biomarkers compared to both control and crude oil-free extract groups ($p>0.05$), with the ethanolic extract showing the most significant ($p<0.05$) increase. This suggests that the extracts of crude oil-contaminated *Mangifera indica* stem bark may have nephrotoxic effects, as elevated serum creatinine and blood urea nitrogen (BUN) levels are indicative of renal failure.

Keywords: *Mangifera indica*, creatinine, crude oil and wistar albino rats

Introduction
Petroleum, a naturally occurring liquid found beneath the earth's surface, is refined into various fuels through fractional distillation. It comprises hydrocarbons and other organic compounds, with crude oil and its refined products both falling under the term petroleum (Aboribo, 2001) [2]. Crude oil contains toxic and carcinogenic organic compounds, including benzene, which is linked to leukemia and immune system diseases (Aboribo, 2001) [2]. Crude oil spills, whether accidental or deliberate, are significant sources of environmental pollution, contaminating air, water, and soil (Uquetan *et al*., 2018) [15]. These oil spills occur through various means, including vehicle accidents, vessel spills, pipeline vandalism, and drilling rig incidents (Uquetan *et al*., 2018) [15]. Such spills, especially in water bodies, cause long-lasting environmental damage and are difficult to clean up (Aladeitan *et al*., 2019) [4]. In Nigeria's Niger Delta region, agricultural lands have suffered due to oil spills, pipeline vandalization, and crude oil transportation. These activities result in oil spills that adversely affect plant germination, seedling growth, and nutrient availability, leading to reduced crop yields (Ogbo *et al*., 2009) [10]. Crude oil pollution has been shown to decrease the protein, dry matter, and crude fiber content of plants like cassava and reduce growth parameters in crops such as *Arachis hypogea L*. and *Zea mays L*. (Uboh *et al*., 2005) [14]. Given the reliance on plant-based natural products for treating diseases it was crucial to investigate the effects of consuming crude oil-contaminated plants in animals. Hence, this study was undertaken to determine the biochemical effects of administering aqueous and ethanol extracts of crude oil-contaminated *Mangifera indica* on the kidney of normal Wistar albino rats.

Materials and Method

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

Stem Bark of *Mangifera indica* was obtained from an oil polluted farmland in a community in Ibeno, Rivers State, Nigeria while crude oil free *Mangifera indica* Stem Bark was obtained from the University of Benin, a crude oil free environment. They were then sealed, labelled, and identified in the Department of Plant Biology and Biotechnology for identification where a voucher no; UBHM 0129 was given by Dr. Akinnibosun Henry.

Preparation of crude drug powder

The preparation of the crude drug powder from the stem barks of crude oil free and contaminated *M. indica* stem barks involved air-drying the stem bark samples for two weeks, grinding them into a powder, and sieving to obtain a coarse consistency.

Preparation of Aqueous and Ethanolic Extract

Preparation of Aqueous Extract

Using distilled water as solvent, 100 g of powdered sample of the herb was extracted by soaking in 1000 ml of distilled water in a beaker, stirred for 72 hours at room temperature. Thereafter, the solution was filtered using sintered glass to remove cellulose fibers; the filtrate was retained. The filtrate was concentrated using a rotary evaporator. The concentrate was freeze-dried using a freeze dryer. The extract was stored in a refrigerator at 4°C using clean plastic bottles.

Preparation of Ethanolic Extract

Using distilled water as solvent, 100 g of powdered sample of the herb was extracted by soaking in 1000 ml of 90% ethanol in a beaker (Covered), stirred for 72 hours at room temperature. Thereafter, the solution was filtered using sintered glass to remove cellulose fibers; the filtrate was retained. The filtrate was concentrated using a rotary evaporator. The concentrate was freeze-dried using a freeze dryer. The extract was stored in a refrigerator at 4 °C using clean plastic bottles. During the oral administration of both extracts via the means of a Gavage, a known weight was dissolved in physiological saline.

Experimental animals

Female albino rats (120-160 g) were housed in the Animal House of the Biochemistry Department, University of Benin, and acclimatized for two weeks. They were fed commercial grower pellets and given water *ad libitum*. One hundred and twenty-five rats were divided into three groups: control, aqueous, and ethanol extract groups, with each extract (Treated) group further divided into crude oil-free and contaminated sub-groups. The sub-groups were administered doses of 85 mg/kg, 170 mg/kg, 255 mg/kg, 340 mg/kg, 425 mg/kg, and 510 mg/kg body weight of either aqueous or ethanolic extracts of crude oil free and contaminated *Mangifera indica* stem barks.

Biochemical sample preparation

After 35 days of treatment, the rats were euthanized, and blood samples were collected, centrifuged, and the serum obtained was used for biochemical analyses. The serum was separated from the whole blood at 3500 rpm for 10 minutes and stored at -40 °C for further analyses. The animals were then sacrificed by cervical dislocation, causing minimal pain. The liver and kidney tissues were collected separately and stored at -20 °C for further analysis.

Biochemical analysis

Determination of Serum Urea

Serum urea levels were determined using a urea kit from Dialab (Austria), following the method outlined by Veniamin and Vakirtzi (1970) [16].

Evaluation of Serum Creatinine

Serum creatinine concentration was measured using a Dialab diagnostic kit (France), according to the method described by Blass *et al*. (1994) [7].

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

The renal function indices of rats exposed to different doses of both aqueous and ethanol extracts of crude oil-free and contaminated *Mangifera indica* stem bark are shown in Figures 1-4. The results indicates that serum creatinine and urea concentrations significantly ($p<0.05$) increased in rats administered these extracts, compared to the control group. Notably, the rats administered ethanol extracts exhibited the most significant ($p<0.05$) increase in creatinine and urea concentrations

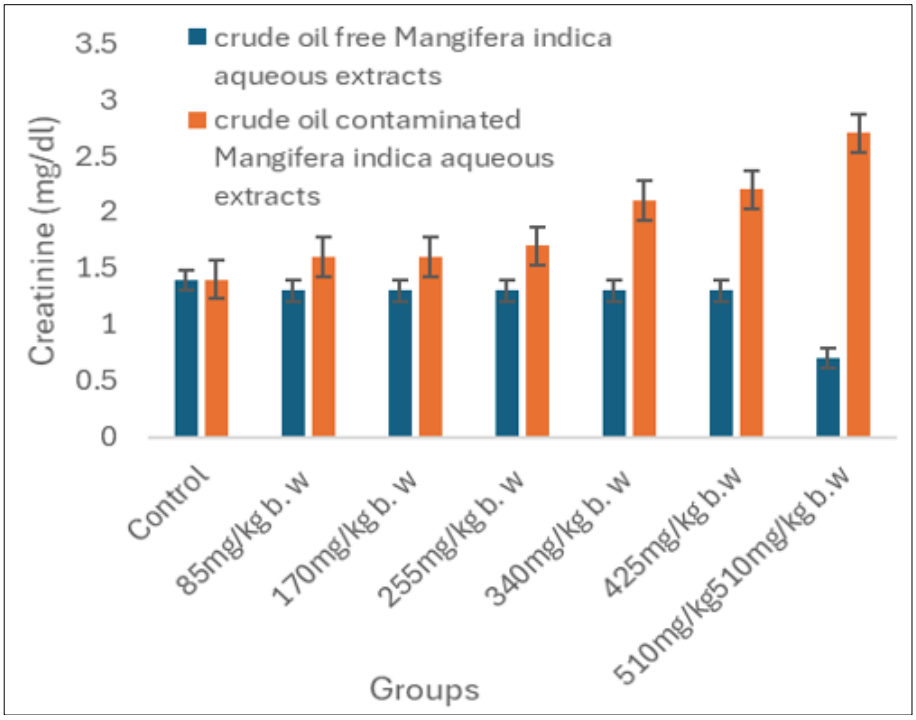

Fig 1: Concentration of creatinine in the serum of female Wistar albino rats treated with various doses of aqueous extracts of crude oil free *Mangifera indica* stem bark. Values are expressed as mean ± SEM (n=5)

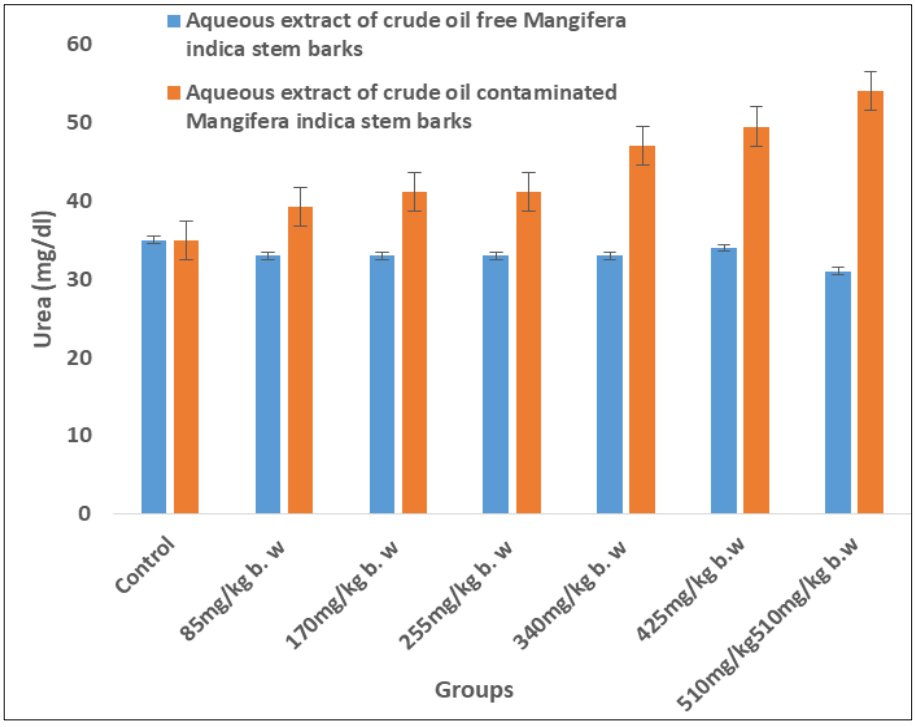

Fig 2: Concentration of urea in the serum of female Wistar albino rats treated with various doses of aqueous extracts of crude oil free *Mangifera indica* stem bark. Values are expressed as mean ± SEM (n=5)

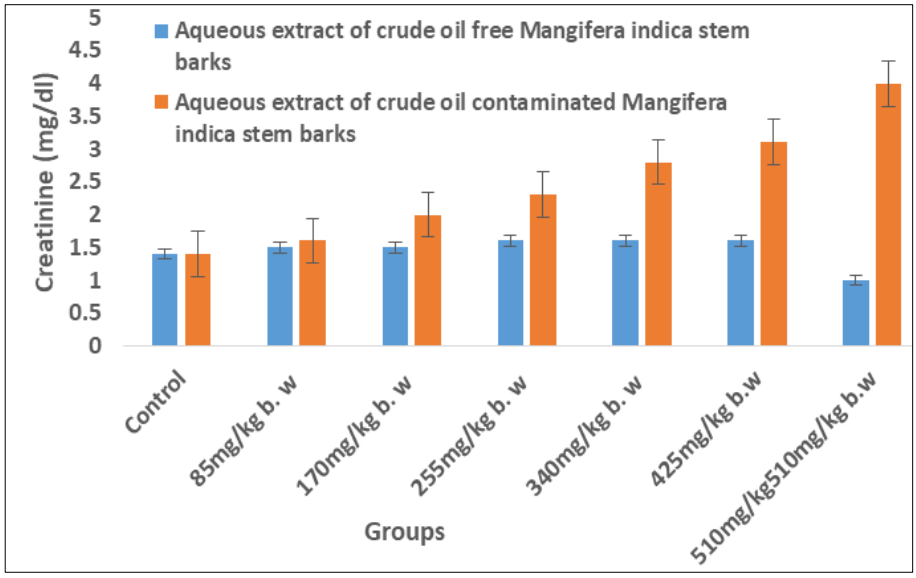

Fig 3: Concentration of creatinine in the serum of female Wistar albino rats treated with various doses of aqueous extracts of crude oil contaminated *Mangifera indica* stem bark. Values are expressed as mean ± SEM (n=5)

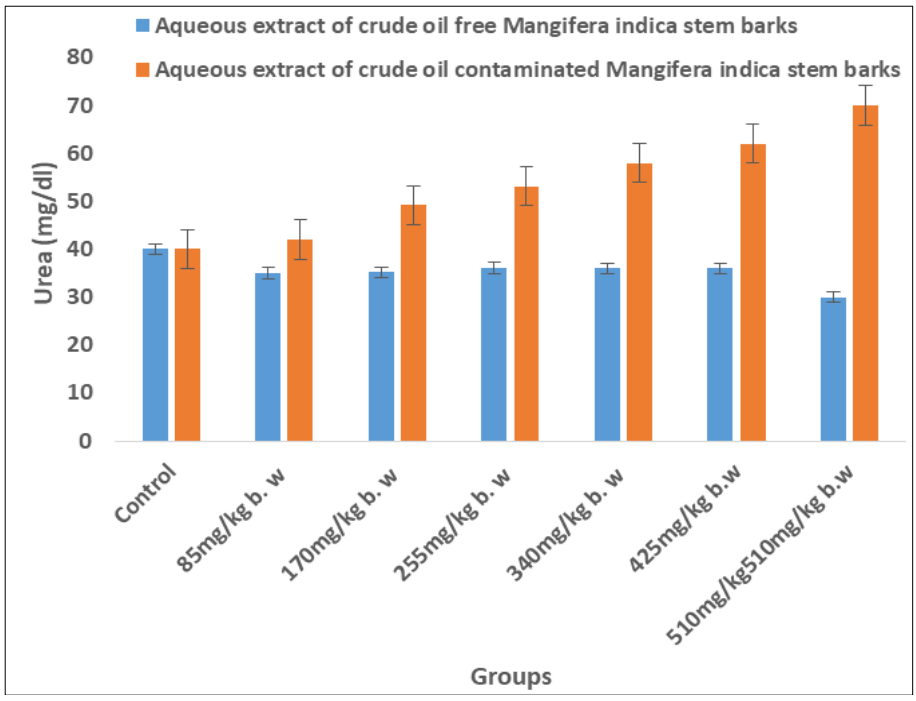

Fig 4: Concentration of urea in the serum of female Wistar albino rats treated with various doses of aqueous extracts of crude oil contaminated *Mangifera indica* stem bark. Values are expressed as mean ± SEM (n=5).

Discussion

Exposure of humans and animals to crude oil, increasing due to environmental contamination and direct application, poses significant health risks (Ovuru *et al*., 2004) [12]. Crude oil exposure can lead to cell damage via oxidative stress in several organs, particularly the liver and kidneys, resulting in elevated levels of cytoplasmic enzymes in the blood, which aids in diagnosing liver and kidney diseases. Urea, a waste product formed in the liver during protein metabolism, is released into the blood and filtered by the kidneys into the urine. Normally, a small, stable amount of urea nitrogen is present in the blood. However, kidney disease or damage impairs this filtration, leading to increased blood urea levels (Aladeitan *et al*., 2020) [5]. Similarly, creatinine is a reliable indicator of kidney function. High levels of creatinine and urea are critical indicators of kidney malfunction or failure. For instance, research conducted by Szaro *et al*. (1980) [13] and Abolagba *et al*. (2017) [1] reported increased levels of creatinine and urea in rats exposed to crude oil. This study demonstrated a significant increase (p<0.05) in serum urea and creatinine levels in rats administered with both aqueous and ethanol extracts of crude oil contaminated *Mangifera indica* stem bark. Clinical and experimental studies by Ovuru *et al*. (2004) [12] showed that crude oil exposure is a risk factor for renal disease. Groups administered aqueous and ethanol extracts of crude oil-contaminated *Mangifera indica* exhibited a steady, significant (p<0.05) increase in serum creatinine and urea concentrations as the dose increased. The highest increase was observed at 510 mg/kg bw. Notably, animals treated with ethanol extracts of crude oil-contaminated *Mangifera indica* showed the most significant

(p<0.05) increase in serum creatinine and urea concentrations.

Which could be due to a possible distortion of the membrane integrity and functions by the toxicants in crude oil. This observation aligns with Aladeitan *et al*. (2020) [5], who found higher concentrations of creatinine and urea in individuals living in oil-polluted regions compared to a control group. In contrast, groups administered crude oil-free aqueous and ethanol extracts showed only a slight increase in these concentrations compared to the control. This suggests that consistent intake of crude oil-contaminated extracts of *Mangifera indica* may indicate nephrotoxicity over time, with ethanol extracts posing a greater threat due to better solvent properties for crude extraction (Madunagu *et al*., 1990) [9]. Certain plants like *Mangifera indica* contain toxic compounds such as alkaloids, glycosides, and lectins, which can adversely affect kidney function if consumed in significant amounts. For example, certain alkaloids found in plants like tobacco or nightshades can be toxic to these organs when ingested by rats. These findings align with Madunagu *et al*. (1990) [9] and Yakubu *et al*. (2008) [17] on *Fadogia agrestis* stem. Increased serum levels of these metabolites in groups administered crude oil-contaminated extracts suggest decreased glomerular filtration rate (GFR) and kidney dysfunction, supported by Counts *et al*. (1995) [8]. The study also noted physiological changes such as hair loss and aggressive behavior in rats treated with these crude oil contaminated *Mangifera indica* stem bark, indicating toxicity, which aligns with Counts *et al*. (1995) [8]. The observed pattern of these increases also suggests a dose-dependent relationship.

Conclusion

According to the findings of this study and its correlation with other research, it is apparent that hydrocarbons present in crude oil may significantly influence the physiology, health, growth, and reproduction of living organisms. The observed physiological changes indicate the toxic effects of crude oil, and the results demonstrate that ethanol is a more effective solvent for extraction. Moreover, the findings suggest that consuming crude plant extracts could potentially lead to organ toxicity.

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