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Biochemical and Histopathological Evidence of Renal Injury Following Sub-Acute Crude Oil Exposure in Wistar Rats

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ABSTRACT

Crude oil contamination remains a major environmental concern in petroleum-producing regions, where repeated exposure to hydrocarbon mixtures may pose significant risks to human and animal health. The kidney is particularly vulnerable to petroleum-derived toxicants because of its high blood flow and central role in the filtration and excretion of xenobiotics. Despite increasing environmental exposure to crude oil pollutants, limited information exists on the renal effects of sub-acute crude oil exposure through multiple environmental pathways. This study investigated the biochemical and histopathological effects of sub-acute crude oil exposure on renal injury in Wistar rats. Twenty adult Wistar rats were randomly assigned to four groups (n = 5). The control group received normal feed and water, whereas the experimental groups were exposed to crude oil through contaminated feed, contaminated drinking water, or inhalation of crude oil vapour for 21 days. At the end of the exposure period, blood samples were collected for the analysis of renal biochemical parameters, including serum urea, creatinine, and electrolytes, while kidney tissues were processed for histopathological examination using hematoxylin and eosin staining. Crude oil exposure produced significant alterations in renal biochemical parameters compared with the control group. The highest serum urea concentration occurred in the feed-exposed group, whereas the vapour-exposed group showed the highest creatinine level. These biochemical changes were accompanied by electrolyte disturbances and histological abnormalities, including tubular degeneration, glomerular distortion, glomerular shrinkage, and expansion of urinary spaces. In conclusion, sub-acute crude oil exposure induced route-dependent biochemical and histopathological alterations indicative of renal injury in Wistar rats. Continuous environmental monitoring and effective pollution control strategies are recommended to reduce petroleum-related health risks in crude oil-contaminated environments.

Keywords: Crude oil exposure, renal injury, nephrotoxicity, renal biomarkers, petroleum hydrocarbons, Wistar rats, environmental toxicology.

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1. BACKGROUND OF STUDY

Crude oil remains a major global energy resource, but its exploration, production, transport, and storage continue to generate substantial environmental contamination and chronic exposure to petroleum hydrocarbons. Chemically, crude oil is a highly complex mixture of aliphatic and aromatic hydrocarbons, resins, asphaltenes, and trace metals, many of which have recognized toxic potential in biological systems. In polluted settings, these compounds can enter soil, water, air, and ultimately food chains, thereby extending exposure beyond occupational settings to nearby human and animal populations (Speight, 2006; Atlas and Hazen, 2011). In oil-producing regions such as the Niger Delta, repeated spills and poor remediation have sustained long-term concerns about organ toxicity and environmental health consequences, making mechanistic toxicology studies especially relevant to local risk assessment (UNEP, 2011).

Among the organs vulnerable to petroleum-derived toxicants, the kidney is of particular concern because of its high blood flow, filtrative function, and central role in the excretion of xenobiotics and their metabolites. The kidney receives a substantial fraction of cardiac output and is therefore continuously exposed to circulating toxicants; this physiological advantage for filtration simultaneously increases susceptibility to toxicant concentration within glomerular and tubular compartments. For this reason, renal injury from environmental contaminants may manifest biochemically even before overt structural failure becomes evident, and traditional biomarkers such as serum urea and creatinine remain widely used indicators of functional disruption despite their limitations in sensitivity (Ferguson *et al.,* 2008; Vaidya *et al.,* 2008). More broadly, recent nephrology literature has emphasized that environmental pollution is an under-recognized contributor to kidney disease, with oxidative stress and inflammation featuring prominently in the pathophysiological link between exposure and renal injury (Lao *et al.,* 2024; Yadav *et al.,* 2024).

A central mechanism by which crude oil and related petroleum fractions induce nephrotoxicity is oxidative stress. During hydrocarbon biotransformation, Cytochrome P450-dependent metabolism generates reactive intermediates and reactive oxygen species capable of overwhelming endogenous antioxidant defenses. Once redox homeostasis is lost, lipid peroxidation, protein oxidation, mitochondrial dysfunction, DNA injury, and apoptosis may follow, all of which can compromise nephron integrity and renal function (Guengerich, 2008; Hsu and Tain, 2020; Tain and Hsu, 2022). This mechanistic framework is consistent with a broader toxicology literature showing that environmental nephrotoxicants frequently converge on oxidative stress, inflammatory signaling, and mitochondrial injury as shared pathways of kidney damage (Yadav *et al.,* 2024).

Experimental evidence specifically implicating petroleum hydrocarbons in renal injury is already substantial. In albino rats exposed to Bonny Light crude oil, Orisakwe et al. reported significant renal biochemical disturbances alongside histopathological changes such as necrosis and oedema, supporting the nephrotoxic potential of crude oil itself rather than only its refined fractions (Orisakwe *et al.,* 2004). Similarly, Azeez et al. showed that petroleum hydrocarbon exposure significantly increased serum urea, creatinine, and renal malondialdehyde while reducing renal glutathione, superoxide dismutase, and catalase, directly linking hydrocarbon exposure to oxidative-stress-mediated renal injury (Azeez *et al.,* 2013). Additional evidence from Nigerian crude oil studies indicates that Bonny Light crude oil can induce oxidative stress in both liver and kidney tissues, reinforcing the view that renal injury occurs within a wider systemic toxicodynamic response to hydrocarbon exposure (Adedara *et al.,* 2011).

Human data, although often less mechanistically controlled than animal studies, point in the same direction. Occupational exposure to gasoline and petroleum vapors has been associated with altered renal function indices in exposed workers, suggesting that chronic real-world hydrocarbon exposure may have measurable renal consequences outside experimental models. (Asefaw *et al.,* 2020). Beyond occupational cohorts, recent studies of environmental contaminants relevant to petroleum pollution have strengthened the plausibility of hydrocarbon-linked renal injury. For instance, contemporary studies have linked exposure to pollutant mixtures, including polycyclic aromatic hydrocarbons and co-occurring contaminants, with impaired renal function, higher uric acid burden, and oxidative-damage-related mechanisms in exposed populations (Ding *et al.,* 2024; Liao *et al.,* 2024). These findings are important because crude oil is itself a complex mixture, and real exposure scenarios are rarely limited to a single purified compound.

Recent toxicological work also underscores the importance of studying complex mixtures and sub-chronic exposure scenarios rather than relying only on acute, single-agent models. In a 2024 rat study of oral exposure to contaminated groundwater mixtures, Boamah et al. demonstrated nephrotoxicity that was dose-dependent and sex-dependent, emphasizing how persistent low-to-moderate environmental exposure can damage the kidney even when the exposure matrix is chemically heterogeneous rather than singularly defined (Boamah *et al.,* 2024). This is particularly relevant to crude oil pollution, where exposed organisms encounter mixtures of parent hydrocarbons, partially weathered products, combustion by-products, and associated metals. At the same time, the broader kidney-environment literature now recognizes oxidative stress, chronic inflammation, and cumulative mixture effects as key mediators of pollutant-associated renal injury across exposure settings (Lao *et al.,* 2024; Yadav *et al.,* 2024).

Despite this growing body of evidence, important gaps remain. First, many petroleum toxicology studies have focused on acute exposure, single refined products, or broad organ toxicity, leaving fewer studies centered specifically on sub-acute crude oil exposure and its renal biochemical consequences. Second, although oxidative stress is repeatedly implicated, there is still a need for more integrated studies that connect renal function markers with the biological plausibility of oxidative and inflammatory injury under environmentally relevant exposure conditions. Third, because crude oil exposure in affected communities may occur through multiple routes and over sustained periods, sub-acute animal models remain essential for clarifying early renal responses before irreversible kidney damage becomes established (Orisakwe *et al.,* 2004; Azeez *et al.,* 2013; Lao *et al.,* 2024; Yadav *et al.,* 2024).

Accordingly, the present study was designed to investigate renal injury following sub-acute crude oil exposure in Wistar rats by assessing key biochemical indices of kidney function. It was hypothesized that repeated sub-acute exposure to crude oil would significantly disrupt renal biochemical homeostasis, reflected by altered urea and creatinine handling and supported by the established mechanistic framework of hydrocarbon-induced oxidative stress and nephrotoxicity. By focusing specifically on the kidney within a controlled experimental model, this study seeks to strengthen the evidence base for petroleum-associated renal injury and provide data relevant to environmental health risk evaluation in crude-oil-contaminated settings (Ferguson *et al.,* 2008; Vaidya *et al.,* 2008; Asefaw *et al.,* 2020).

2. MATERIALS AND METHODS

2.1. Experimental Animals

Twenty healthy adult Wistar albino rats weighing between 150–200 g were used for this study. The animals were obtained from the Animal House Unit of the Faculty of Basic Medical Sciences, Imo State University, Owerri, Nigeria. The rats were allowed to acclimatize for two weeks under standard laboratory conditions prior to the experiment.

The animals were housed in well-ventilated cages under a 12-hour light–dark cycle, with ambient temperature maintained at 22–25^{0}C and relative humidity between 50–60%. The rats were fed with standard commercial rat pellets and provided with clean drinking water ad libitum throughout the study.

2.2. Ethical Approval

All experimental procedures involving animals were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. Ethical approval for the study was obtained from the Research Ethics Committee of the Department of Medical Biochemistry, Imo State University, Owerri, Nigeria.

2.3. Crude Oil Procurement

Crude oil samples used in this study were obtained from Seplat Petroleum Development Company located in Ohaji-Egbema Local Government Area, Imo State, Nigeria. The crude oil was collected in sterile glass containers and transported to the laboratory, where it was stored under appropriate laboratory conditions prior to use. Crude oil from the Niger Delta region has been widely reported to contain complex mixtures of hydrocarbons capable of inducing biochemical and physiological alterations in experimental animals (Speight, 2014; Atlas and Hazen, 2011).

2.4. Experimental Design

The rats were randomly divided into four groups of five animals each (n = 5) as follows. Animals were assigned to experimental groups using a simple randomization technique to minimize selection bias:

- **Group A (Control):** Rats received normal feed and clean drinking water without crude oil exposure.
- **Group B:** Rats were exposed to crude oil-contaminated feed.
- **Group C:** Rats were exposed to crude oil-contaminated drinking water.
- **Group D:** Rats were exposed to crude oil vapour through inhalation (air exposure).

The exposure period lasted 21 days, representing a sub-acute exposure model commonly used in toxicological investigations (OECD, 1995). The exposure concentration was selected based on previously established sub-acute toxicity models for petroleum hydrocarbons (OECD, 1995; Azeez *et al.,* 2013). During the experimental period, the animals were monitored daily for signs of toxicity and behavioural changes. Body weights were recorded at the beginning and end of the exposure period.

2.5. Sample Collection

At the end of the 21-day exposure period, the animals were sacrificed under mild anaesthesia. Blood samples were collected via cardiac puncture using sterile syringes and transferred into plain sample containers.

The blood samples were allowed to clot and subsequently centrifuged at 3000 rpm for 10 minutes to obtain serum for biochemical analysis.

2.6. Determination of Renal Function Parameters

Renal function was evaluated by measuring serum urea and creatinine concentrations, which are widely used biomarkers of renal impairment (Burtis and Bruns, 2015). These parameters were determined using standard enzymatic colorimetric methods according to the manufacturer's instructions.

Serum electrolytes, including sodium (Na^+), potassium (K^+), chloride (Cl^-), and bicarbonate (HCO_3^-) were determined using an ion-selective electrode analyzer, which provides reliable assessment of electrolyte imbalance associated with renal injury (Tietz, 2012).

2.7. Histological Examination of Kidney Tissue

Following sacrifice, the kidneys were carefully excised, rinsed in normal saline, and fixed in 10% buffered formalin for histological examination. The tissues were processed using standard histological techniques, embedded in paraffin wax, and sectioned at 5 μm thickness using a microtome.

The sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope for pathological alterations in renal structures. Histological examination is a standard approach used to evaluate tissue injury and structural changes associated with toxic exposure (Bancroft and Gamble, 2008).

2.8. Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. Post-hoc comparisons were conducted using the Least Significant Difference (LSD) test, and a p-value less than 0.05 ($p < 0.05$) was considered statistically significant.

3. RESULTS

3.1. Effect of crude oil exposure on Body Weight of Wistar rats

The effect of crude oil exposure on body weight of Wistar rats is presented in Table 1. After the 21-day exposure period, rats exposed to crude oil exhibited significantly reduced weight gain ($p < 0.05$) compared with the control group. The lowest weight gain was observed in rats exposed to crude oil-contaminated feed, while animals exposed through contaminated air showed relatively higher weight gain among the exposed groups.

Table 1. Effect of crude oil exposure on body weight of Wistar rats.

Treatment Groups	Initial body weight (g)	Final body weight (g)	Weight gain (g)	Percentage weight gain (%)
Control (0.5ml NS)	123.33±3.81^{a}	166.70±3.78^{c}	43.37±0.17^{c}	35.23±1.10^{c}
Treated (5ml oil/100g feed)	116.47±2.99^{a}	138.33±5.91^{a}	21.87±3.45^{a}	18.70±2.66^{a}
Treated (5ml oil/100ml water)	121.80±1.67^{a}	148.87±3.08^{ab}	27.07±3.20^{ab}	22.25±2.72^{ab}
Exposed (5ml oil/100g cotton wool)	121.10±4.28^{a}	153.40±3.46^{bc}	32.30±0.90^{b}	26.78±1.62^{b}

(n = 5, mean ± SD; values with different superscripts differ significantly at $p < 0.05$)

3.2. Effects of Crude Oil Exposure on Relative Organ Weights

The relative kidney weights of rats exposed to crude oil are presented in Table 2. Rats exposed to crude oil through contaminated feed, water, and air showed slight variations in kidney weight compared with the control group. However, these differences were not statistically significant ($p > 0.05$), indicating that crude oil exposure did not produce a significant change in the relative kidney weight of the animals within the duration of the study.

Table 2. Effect of crude oil exposure on relative kidney weight of Wistar rats.

Treatment Groups	Final body weight (g)	Kidney weight (g)	ROW, kidney (%)
Control (0.5ml NS)	166.70±3.78^{c}	1.13±0.04^{c}	2.75±0.10^{a}
Treated (5ml oil/100g feed)	138.33±5.91^{a}	0.92±0.01^{a}	2.99±0.16^{a}
Treated (5ml oil/100ml water)	148.87±3.08^{ab}	1.01±0.02^{ab}	2.79±0.08^{a}
Exposed (5ml oil/100g cotton wool)	153.40±3.46^{bc}	1.04±0.04^{bc}	2.73±0.08^{a}

(n = 5, mean ± SD; $p > 0.05$)

3.3. Effect of Crude Oil Exposure on Renal Function Parameters of Rats

The effect of crude oil exposure on renal biochemical parameters is summarized in Table 3. Significant alterations ($p < 0.05$) were observed in several renal indices, including urea, creatinine, and electrolyte concentrations. Rats exposed to crude oil-contaminated feed exhibited the highest urea concentration, whereas rats exposed through inhalation showed the highest creatinine levels among the experimental groups.

Table 3. Effect of crude oil exposure on renal biochemical parameters of Wistar rats.

Treatment Groups	Urea (mg/dL)	Creatinine (mg/dL)	Potassium (mmol/L)	Sodium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)
Control (0.5ml NS)	14.89±4.40^{c}	0.52±0.03^{a}	5.03±0.07^{a}	132.87±0.52^{a}	91.69±1.16^{a}	23.60±0.36^{a}
Treated (5ml oil/100g feed)	24.96±4.20^{d}	0.58±0.03^{ab}	6.91±0.11^{a}	131.52±4.89^{a}	103.25±1.36^{b}	29.40±0.45^{c}
Treated (5ml oil/100ml water)	11.55±1.39^{b}	0.54±0.01^{a}	8.06±0.08^{a}	132.62±1.70^{a}	95.23±1.15^{a}	29.00±0.23^{c}
Exposed (5ml oil/100g cotton wool)	8.04±1.59a	0.63±0.02^{b}	5.90±2.71^{a}	140.57±1.10^{a}	94.70±0.49^{a}	27.90±0.25^{b}

(n = 5, mean ± SD; $p < 0.05$)

3.4. Kidney Histology

Histological examination of kidney tissues revealed structural alterations in rats exposed to crude oil compared with the control group (Plates 1–4).

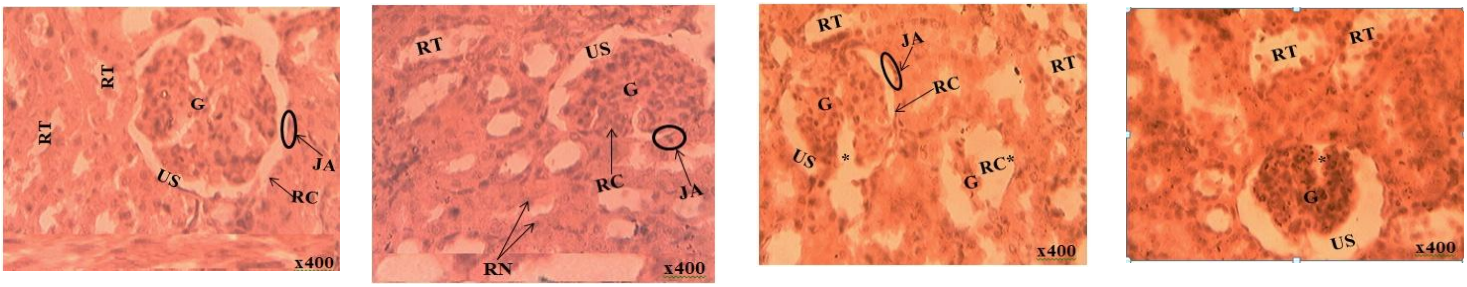
Kidney sections from the control group showed normal renal architecture characterized by well-organized renal tubules, intact renal corpuscles, and normal glomerular structures.

In contrast, kidney sections from rats exposed to crude oil exhibited varying degrees of pathological alterations. Rats exposed to crude oil-contaminated feed showed mild tubular degeneration and fading of tubular nuclei, although the renal corpuscles and glomeruli remained largely intact.

Rats exposed to crude oil-contaminated water displayed glomerular distortion and partial degeneration of renal tubular structures, indicating structural disruption of the renal cortex.

More pronounced alterations were observed in rats exposed through crude oil vapour, including enlarged renal tubules, glomerular shrinkage, and expanded urinary spaces, suggesting more evident renal structural changes.

These histopathological findings corroborate the biochemical evidence of renal injury observed in crude oil–exposed rats.



Group A Control n-Saline Kidney **Group B** Crude Oil Polluted feed **Group C** Crude Oil Polluted water **Group D** Crude Oil Polluted Cotton Wool

Kidney Keys: RT = Renal Tubules, RC= Renal Corpuscle, US= Urinary Space, JA= Juxtar- Glomerula Apparatus, G= Glomerulus, RN= Renal Nuclei, RC*= Distorted Renal Corpuscle, G*= Splitting Glomerulus, *= Affected Parts.

Figure 1. Histopathological changes in kidney tissues of Wistar rats following sub-acute crude oil exposure. Representative photomicrographs of renal cortex sections stained with hematoxylin and eosin (H&E), ×400.

A: Control group showing normal renal architecture with intact glomeruli and well-organized renal tubules.

B: Crude oil-contaminated feed group showing mild tubular degeneration and fading of tubular nuclei.

C: Crude oil-contaminated water group showing glomerular distortion and partial degeneration of renal tubular structures.

D: Crude oil vapour-exposed group showing enlarged renal tubules, glomerular shrinkage, and expanded urinary spaces.

4. DISCUSSION

The present study provides integrated biochemical and histopathological evidence demonstrating that sub-acute crude oil exposure induces measurable renal injury in Wistar rats. The major findings included reduced body weight gain, significant alterations in serum urea, creatinine, and electrolyte concentrations, as well as renal histological lesions ranging from tubular degeneration to glomerular distortion and expansion of urinary spaces. Taken together, these findings indicate that repeated crude oil exposure, even over a relatively short duration, can disrupt renal physiological homeostasis and compromise nephron integrity. These observations are broadly consistent with earlier studies demonstrating that Bonny Light crude oil and related petroleum hydrocarbons possess nephrotoxic potential in laboratory animals (Orisakwe *et al.,* 2004; Adedara *et al.,* 2012; Azeez *et al.,* 2013).The reduction in body weight gain observed among crude-oil-exposed rats suggests that petroleum hydrocarbons adversely affected normal growth and metabolic performance. Similar reductions in body weight have been reported in experimental studies investigating crude oil toxicity. For instance, Orisakwe et al. (2004) reported significant weight reduction in rats exposed to Bonny Light crude oil, while Ikanone et al. (2017) also observed decreased body weight gain following sub-acute crude oil exposure.

Such growth impairment may result from reduced feed palatability, impaired nutrient utilization, and metabolic stress associated with hydrocarbon toxicity. Polycyclic aromatic hydrocarbons (PAHs), which are major toxic constituents of crude oil, are known to interfere with cellular energy metabolism and induce oxidative stress, thereby affecting normal physiological processes and growth performance (Kim *et al.,* 2013). The more pronounced reduction in weight gain observed in the feed-exposed group may therefore reflect higher internal exposure resulting from direct gastrointestinal absorption of crude oil components.

The absence of significant changes in relative kidney weight in the present study is also noteworthy. While Orisakwe et al. (2004) reported reductions in both absolute and relative kidney weights following crude oil exposure, the present findings are more consistent with those of Adedara et al. (2012), who observed that kidney weight remained largely unchanged despite biochemical evidence of renal oxidative stress and tissue injury. Differences between studies may be attributed to variations in exposure duration, crude oil composition, administered dose, and route of exposure. In toxicological investigations, organ weight alterations often represent relatively late manifestations of toxicity, whereas biochemical disturbances may appear earlier due to disruption of metabolic and physiological processes. Thus, the present findings support the concept that biochemical indicators of renal injury may precede detectable morphometric changes in kidney tissues.

The significant alteration in serum urea observed across the exposure groups, particularly the marked increase in the feed-exposed rats, suggests that crude oil exposure may impair renal excretory function in a route-dependent manner. Urea accumulation in the bloodstream is a recognized indicator of reduced glomerular filtration and impaired renal clearance of nitrogenous metabolic waste products. Similar increases in serum urea have been reported in experimental studies of petroleum hydrocarbon toxicity (Orisakwe *et al.,* 2004; Azeez *et al.,* 2013). Adedara et al. (2012) also reported dose-dependent increases in serum urea following Bonny Light crude oil exposure. The particularly high urea level observed in rats exposed through contaminated feed may reflect greater gastrointestinal absorption of crude oil constituents, resulting in higher systemic toxicant burden and greater impairment of renal filtration processes.

The increase in serum creatinine, most pronounced in the vapour-exposed group, further supports the occurrence of renal injury following crude oil exposure. Creatinine is widely used as a biomarker of glomerular filtration rate because it is primarily eliminated from the circulation through renal filtration (Burtis & Bruns, 2015). Elevated serum creatinine therefore reflects reduced glomerular filtration efficiency and impaired kidney function. Similar increases in serum creatinine following crude oil exposure have been reported in earlier experimental studies (Orisakwe *et al.,* 2004; Azeez *et al.,* 2013). The relatively higher creatinine concentration observed in the inhalation group suggests that volatile hydrocarbon fractions present in crude oil vapours may significantly contribute to systemic toxicity affecting renal function. However, previous toxicological studies have shown that creatinine levels may remain relatively stable during early renal injury because of the substantial functional reserve of the kidney, with histological alterations often appearing before marked biochemical changes.

Electrolyte disturbances observed in crude-oil-exposed rats indicate that petroleum hydrocarbons likely affected renal tubular handling of ions in addition to glomerular filtration processes. The observed changes in sodium, potassium, chloride, and bicarbonate concentrations suggest alterations in renal reabsorption and secretion mechanisms. Similar electrolyte disturbances have been reported in petroleum hydrocarbon toxicity studies where toxicant-induced damage to renal tubular cells disrupted ion transport and homeostatic regulation (Azeez *et al.,* 2013).

Differences in electrolyte patterns among exposure groups may be explained by variations in toxicant absorption, metabolic processing, and the nephron segments preferentially affected by the hydrocarbon mixture. For instance, ingestion through contaminated feed may lead to higher systemic exposure, whereas inhalation exposure may preferentially deliver volatile hydrocarbon fractions with distinct toxicokinetic profiles.

Histopathological examination of kidney tissues further strengthened the biochemical evidence of renal injury observed in the present study. Control animals displayed normal renal architecture characterized by intact glomeruli and well-organized renal tubules. In contrast, crude-oil-exposed rats exhibited structural alterations including tubular degeneration, glomerular distortion, glomerular shrinkage, and expansion of urinary spaces. These lesions are consistent with histopathological findings reported in previous petroleum toxicity studies. Orisakwe et al. (2004) described renal necrosis and oedema following crude oil exposure, while Owagboriaye et al. (2022) reported renal tubular degeneration and glomerular damage following exposure to gasoline vapours. The concordance between biochemical markers and histological findings in the present study provides strong evidence that crude oil exposure induced structural as well as functional renal injury.

The mechanistic basis for these renal alterations is most plausibly linked to oxidative stress and inflammatory injury. Hydrocarbon metabolism via Cytochrome P450 enzymes generates reactive oxygen species capable of damaging cellular membranes, proteins, and nucleic acids (Guengerich, 2008). When antioxidant defense mechanisms are overwhelmed, oxidative damage may disrupt mitochondrial function and induce apoptosis in renal cells. Several studies have demonstrated that oxidative stress plays a central role in environmental pollutant-induced kidney injury (Hsu & Tain, 2020; Tain & Hsu, 2022). Indeed, petroleum hydrocarbon exposure has been shown to increase lipid peroxidation and reduce antioxidant enzyme activities in renal tissues, further supporting oxidative stress as a key mechanism underlying hydrocarbon-induced nephrotoxicity (Adedara *et al.,* 2012; Azeez *et al.,* 2013).

Environmental exposure to petroleum-derived pollutants has also been associated with renal injury in human populations. Polycyclic aromatic hydrocarbons originating from petroleum products, industrial emissions, and combustion processes are widely recognized environmental contaminants. Exposure to PAHs has been linked to multiple adverse health outcomes, including kidney disease and systemic inflammation (Kim *et al.,* 2013). Epidemiological investigations have also reported associations between environmental pollutant exposure and impaired renal function in human populations (Afsar *et al.,* 2019; Rahman *et al.,* 2022). These findings further strengthen the biological plausibility of the renal alterations observed in the present experimental study.

An important strength of the present study is the comparison of three environmentally relevant exposure routes: contaminated feed, contaminated water, and inhalation of crude oil vapours. Many previous petroleum toxicity studies relied on a single exposure pathway, whereas environmental petroleum pollution often involves simultaneous contact with multiple contaminated media. By incorporating multiple exposure routes, the present study provides a more realistic representation of environmental exposure scenarios and offers insight into how exposure pathway may influence the severity and pattern of nephrotoxicity. The differences observed among exposure groups suggest that exposure route, dose, and hydrocarbon composition may significantly influence toxicological outcomes.

Overall, the present findings are consistent with previous experimental and epidemiological studies demonstrating that petroleum hydrocarbon exposure can compromise renal structure and function. Where minor differences exist between studies, these discrepancies may be explained by variations in exposure duration, crude oil composition, administered dose, and route of exposure, as well as the temporal lag between structural renal injury and detectable changes in serum biomarkers. Collectively, the results of the present study provide further evidence that sub-acute crude oil exposure is nephrotoxic, and that the combined assessment of biochemical markers and histopathological alterations offers a more comprehensive evaluation of renal injury than either approach alone (Orisakwe *et al.,* 2004; Adedara *et al.,* 2012; Azeez *et al.,* 2013; Asefaw *et al.,* 2020)..

5. CONCLUSION

Sub-acute crude oil exposure produced measurable biochemical and histopathological alterations in the kidneys of Wistar rats. Significant changes were observed in renal biochemical parameters, including serum urea, creatinine, and electrolyte concentrations. These biochemical disturbances were accompanied by structural abnormalities in renal tissues, such as tubular degeneration, glomerular distortion, glomerular shrinkage, and expansion of urinary spaces. Together, these findings indicate that repeated crude oil exposure can disrupt renal physiological homeostasis and compromise nephron integrity.

The severity and pattern of renal injury appeared to be influenced by the route of exposure. Rats exposed to crude oil-contaminated feed exhibited the highest serum urea concentrations, suggesting impaired renal excretory function following gastrointestinal absorption of petroleum hydrocarbons. In contrast, animals exposed to crude oil vapours showed relatively higher creatinine levels and more evident histological alterations, indicating that inhalational exposure to volatile hydrocarbon fractions may also contribute substantially to systemic toxicity affecting renal function.

From an environmental health perspective, these findings highlight the potential risks associated with chronic exposure to petroleum-derived contaminants in polluted environments. Communities residing in oil-producing regions or areas affected by petroleum spills may encounter crude oil pollutants through multiple pathways, including contaminated food, drinking water, and air. Such exposure scenarios may therefore pose significant risks to renal health.

Overall, the combined biochemical and histopathological evidence obtained in this study demonstrates that sub-acute crude oil exposure can induce renal injury in experimental animals. These findings underscore the importance of environmental monitoring, effective pollution control measures, and improved remediation strategies in crude oil-contaminated environments. Further investigations incorporating molecular biomarkers, oxidative stress indicators, and longer exposure durations are recommended to better elucidate the mechanisms underlying petroleum-induced nephrotoxicity and to improve risk assessment for populations exposed to petroleum pollution.

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