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Combined administration of *Carpalobia lutea* and *Sabicea calycina* mitigates crude oil-induced hepatorenal and haematological toxicity in Wistar rats

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Abstract

Environmental petroleum insult is associated with oxidative stress and systemic toxicity, often manifesting as haematological, hepatic, and renal impairments. This study evaluated the protective efficacy of a composite extract from *Sabicea calycina* and *Carpalobia lutea* on male Wistar rats exposed to Nigeria Light Crude Oil (BLCO). Rats were randomized into five groups (N=5), including a positive control, negative control (BLCO exposure only), and treatment groups receiving 50, 100, or 200 mg/kg of the extract. Following 4 weeks of BLCO exposure via bedding contamination, and concomitant extract administration. Crude oil exposure significantly reduced RBC count, Hb, PCV, and platelet levels, while altering red cell indices, and elevated WBC counts and renal biomarkers (urea, creatinine, uric acid). This exposure also caused hepatic and renal histoarchitectural damage. Treatment with 100 and 200 mg/kg extract doses significantly mitigated these abnormalities in a dose-dependent manner, restoring haematological parameters, renal function, and tissue integrity to near-normal levels. Histological analyses confirmed marked improvement in hepatic and renal architecture at higher doses. These findings suggest that the composite extract possesses strong antioxidant, anti-inflammatory, and organ-protective properties, highlighting its potential as a phytotherapeutic agent against environmental toxin-induced haematotoxicity, hepatotoxicity, and nephrotoxicity.

Keywords: Haematotoxicity, hepatotoxicity, phytotherapeutic, nephrotoxicity

Introduction

Crude oil contamination remains a significant environmental and public health concern, particularly in petroleum-producing regions, where frequent spills, inadequate cleanup, and poor waste management contribute to ecosystem degradation [1-2]. It contains several toxic constituents, notably polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs), alkanes, heavy metals and radioactive materials such as uranium and thorium which are known to induce oxidative stress, inflammation, and multi-organ toxicity in mammals [3]. Visceral organs such as the liver and kidneys, which are primarily responsible for biotransformation and excretion of xenobiotics, are especially vulnerable to such toxic insults, resulting in hepatocellular damage, nephrotoxicity, and hematological disturbances [4-5].

Recent studies have underscored the role of botanical therapeutics in mitigating chemically induced toxicities due to their rich reservoir of antioxidant and anti-inflammatory phytochemicals [6-7]. *Carpalobia lutea* and *Sabicea calycina* are traditionally used in various cultures to manage ailments related to oxidative stress and organ dysfunction [8]. *C. lutea* is a small tree native to the tropical rainforests of Africa. Traditionally, its phytotherapeutic applications span a wide spectrum of ailments. The leaves are employed as antipyretics and in the treatment of ulcers, malaria, dermatological infections, venereal diseases, infertility, gastrointestinal disturbances, and helminthiasis. They are also applied in obstetric care and wound healing. The root bark is used for rheumatism, analgesia, and neuropsychiatric conditions, while dried stem bark is inhaled for migraine relief. In Southern Nigeria, root decoctions are reputed to possess aphrodisiac properties [8]. *S. calycina* is traditionally used as a laxative.

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Its mashed leaves are applied to children's limbs to support bone development and ambulation, and also to treat wounds. Infusions are reputed to enhance memory and alleviate senile dementia [9]. Phytochemical analyses have revealed the presence of flavonoids, alkaloids, and polyphenols in these plants, suggesting their therapeutic potential against xenobiotic-induced organ injury [10].

Although previous studies have demonstrated the individual hepatoprotective and nephroprotective activities of *C. lutea* and *S. calycina* [11-12], there is limited information on their combined effects. Polyherbal formulations may provide enhanced protective efficacy due to additive or synergistic interactions among their bioactive compounds. Consequently, this study examines the efficacy of the co-administration of *C. lutea* and *S. calycina* in attenuating crude oil-induced hepatorenal and hematological toxicity in Wistar rats. The findings are expected to provide a scientific basis for the therapeutic potential of polyherbal strategies in environmental toxicology.

Materials and Methods

Plant source and authentication

The stems of *S. calycina* and *C. lutea* were collected from Odi Town, Kolokuma/Opokuma LGA, Bayelsa State, Nigeria. Botanical identification and authentication were conducted at the Herbarium of Ekiti State University, Ado-Ekiti, with voucher specimens UHAE2019-808 and UHAE2019-809 deposited in the institutional herbarium for future reference.

Preparation of composite extract of *C. lutea* and *S. calycina*

Freshly collected samples of *C. lutea* and *S. calycina* were rinsed with distilled water to eliminate dirt and contaminants. The plant materials were air-dried in a shaded but well-ventilated area until a constant weight was achieved, then pulverised using an electric blender. Equal quantities (100 g each) of the powdered plant materials were combined, yielding 200 g of composite powder. This was extracted with 600 mL of absolute ethanol at room temperature for 24 hours under continuous agitation using a Denly A-500 flask shaker. The resulting crude extract was subjected to vacuum filtration using Whatman No. 1 filter paper. The clarified filtrate was subsequently concentrated to dryness under reduced pressure at 40 °C using a rotary evaporator, yielding 6.39 g of dry residue. The dried extract was then reconstituted in distilled water to obtain a homogenous solution for subsequent experimental administration.

Experimental Animals

Thirty healthy male Wistar rats (average weight: 162.22±5.63g) were obtained from the Animal House Unit, Department of Biochemistry, Federal University Otuoke, Bayelsa State, Nigeria. The animals were kept in clean, well-ventilated pens at 28-30 °C under a natural light/dark cycle. They were fed standard rat pellets and provided water *ad libitum* throughout the study. A 2-week acclimatisation period preceded the 28-day exposure to the crude oil-contaminated environment and the treatment phase. All procedures involving animals were conducted in full compliance with the protocols stipulated by the Directorate of Research and Quality Assurance regarding the use and care of experimental animals. A comprehensive ethical review and approval were also granted by the Directorate of Research & Quality Assurance at Federal University,

Otuoke (Approval No DRQA/FUO/0100/13/12/23), ensuring that the work met recognised principles of humane care to minimise animal distress.

Experimental Design and Crude Oil Exposure

The twenty-five male Wistar rats were randomly assigned to five groups (N=5 per group) and subjected to the following treatment protocols. Group A (positive control) received 50 mL of distilled water without crude oil exposure. Group B (negative control) was exposed to Nigerian Light Crude Oil and administered 50 mL of distilled water. Groups C, D, and E were exposed to the BLCO and treated orally with 50, 100 and 200 mg/kg of the composite extract, respectively. To simulate a crude oil-contaminated environment, 20 mL of BLCO was evenly applied to the bedding material of metabolic cages designated for the exposed groups, mimicking environmental exposure. This exposure and treatment protocol was designed to assess the protective efficacy of the composite extract against BLCO-induced toxicity in a controlled setting.

Extract Administration

Oral oropharyngeal drug delivery using a dosing cannula was used for the crude extract to ensure accurate and consistent dosing. Doses of 50, 100, and 200 mg/kg body weight were given once daily in the morning between 07:30 and 08:00 hours for 28 days. The extracts were reconstituted in distilled water, which served as the vehicle. All administrations were conducted under controlled conditions to ensure precision and to minimize animal stress throughout the treatment period.

Blood and Tissue Harvesting

On the 29th day of the experiment, the animals were anaesthetized in a closed chamber containing chloroform-soaked cotton wool to induce deep anaesthesia. Following complete anaesthesia, blood samples were obtained via cardiac puncture using sterile syringes and transferred into EDTA sample bottles. Immediately after blood collection, the abdominal cavity was carefully opened using sterile dissection instruments to expose the liver and kidneys. These were excised promptly and rinsed in cold normal saline to remove residual blood and debris.

Assay kits/ Reagents

Commercial assay kits for the determination of renal function parameters were procured from Randox Laboratories Ltd. (United Kingdom). All other reagents and chemicals used in the study were of analytical grade and obtained from reputable suppliers. All procedures were performed following the manufacturer's protocols and quality control standards.

Haematological and biochemical assessments of renal function

Blood urea nitrogen (BUN) levels were determined using the modified Berthelot method as described by Tobacco *et al.* [13]. Creatinine (CRT) concentrations were measured via the colorimetric kinetic method developed by Bartels *et al.* [14], while uric acid (UA) levels were quantified using the enzymatic colorimetric method outlined by Duncan *et al.* [15]. Complete blood count (CBC) analyses were performed on samples from the respective experimental groups using the Erba Mannheim Automatic Hematology Analyzer (Elite 580), following the procedure established by Coulter [16].

Histopathological evaluation of liver and kidney tissues

Liver and kidney tissues were fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin. Tissue sections of 5 μm thickness were cut using a rotary microtome, mounted on glass slides, and stained with hematoxylin and eosin (H&E). The stained sections were examined under a light microscope Olympus CX43 (Olympus Corporation, Tokyo, Japan) at magnifications ranging from $\times 100$ to $\times 400$ for the assessment of structural alterations and pathological changes [17].

Statistical Analysis

Data are presented as mean \pm standard deviation (SD), with five replicates per experimental group. Statistical comparisons among groups were conducted using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to identify significant differences between group means. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS software, version 17.0 (IBM Corp., Armonk, NY, USA).

Results

Effects of 50, 100 and 200 mg/kg body weight of composite mixture of *S. calycina* and *C. lutea* on haematological indices of male Wistar rats after 28 days challenge in crude oil environment

The haematological profiles of male Wistar rats following 28 days of oral administration of a composite extract of *S. calycina* and *C. lutea* at doses of 50, 100, and 200 mg/kg body weight are summarized in Table 1.0. Parameters assessed included packed cell volume (PCV), red blood cell count (RBC), haemoglobin concentration (Hb), white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and platelet count. Statistical analysis indicated that the haematological markers in the group treated with 50 mg/kg of the extract and the negative control group differed significantly ($p < 0.05$) from those observed in the positive control and the group administered 200 mg/kg body weight of the extract.

Table 1: Effects of 50, 100 and 200 mg/kg body weight of composite mixture of *S. calycina* and *C. lutea* on haematological parameters of male Wistar rats after 28 days challenge in crude oil environment

| Parameter | Dose in mg/kg body weight of composite mixture of <i>S. calycina</i> and <i>C. lutea</i> | | | | |
|--|--|-------------------------------|----------------------------------|----------------------------------|----------------------------------|
| | Positive control | Negative control | 50 | 100 | 200 |
| PCV (%) | 43.32 \pm 3.89 ^a | 31.12 \pm 3.54 ^b | 36.29 \pm 4.18 ^c | 40.82 \pm 3.11 ^a | 41.97 \pm 3.23 ^a |
| RBC ($\times 10^6 \mu\text{L}^{-1}$) | 7.41 \pm 1.17 ^a | 5.11 \pm 0.81 ^b | 6.46 \pm 1.76 ^c | 6.97 \pm 1.20 ^a | 7.11 \pm 1.22 ^a |
| Hb (g dL ⁻¹) | 13.45 \pm 1.43 ^a | 9.33 \pm 1.62 ^b | 10.63 \pm 1.85 ^c | 11.65 \pm 1.43 ^d | 12.12 \pm 1.55 ^a |
| WBC ($\times 10^3 \mu\text{L}^{-1}$) | 9.91 \pm 3.05 ^a | 13.70 \pm 2.15 ^b | 12.99 \pm 3.00 ^c | 10.05 \pm 3.03 ^a | 10.01 \pm 2.87 ^a |
| MCV (fL) | 69.43 \pm 4.37 ^a | 54.47 \pm 5.13 ^b | 58.89 \pm 4.62 ^c | 64.33 \pm 4.23 ^d | 68.39 \pm 4.45 ^a |
| MCH (pg) | 35.54 \pm 3.18 ^a | 24.73 \pm 2.25 ^b | 27.12 \pm 3.10 ^c | 32.72 \pm 3.28 ^d | 35.44 \pm 3.36 ^a |
| MCHC (g dL ⁻¹) | 46.14 \pm 4.10 ^a | 33.41 \pm 4.16 ^b | 36.10 \pm 3.86 ^c | 42.94 \pm 3.66 ^d | 45.98 \pm 4.52 ^a |
| Platelet (10^9L^{-1}) | 9159.98 \pm 25.99 ^a | 6861 \pm 21.54 ^b | 7153.12 \pm 20.88 ^c | 8158.44 \pm 21.73 ^d | 8364.33 \pm 23.07 ^c |

Data for haematological parameters are mean \pm standard deviation (N=5), Mean values on the same row with different superscript letters as that of the positive control are significantly different ($p < 0.05$) from the control one-way analysis of variance (ANOVA).

Effects of 50, 100 and 200 mg/kg Body Weight of Composite Mixture of *S. calycina* and *C. lutea* on renal function indices of male Wistar rats after 28 days challenge in crude oil environment: As shown in Figure 1, oral administration of graded doses of the composite mixture of *S. calycina* and *C. lutea* for 28 days produced notable effects on renal function indices. The negative

control group demonstrated values that were significantly different from those of the positive control group ($p < 0.05$). Notably, administration of the mixture at 100 and 200 mg/kg resulted in a significant mitigation of renal impairment, with values approaching those of the positive control group ($p < 0.05$).

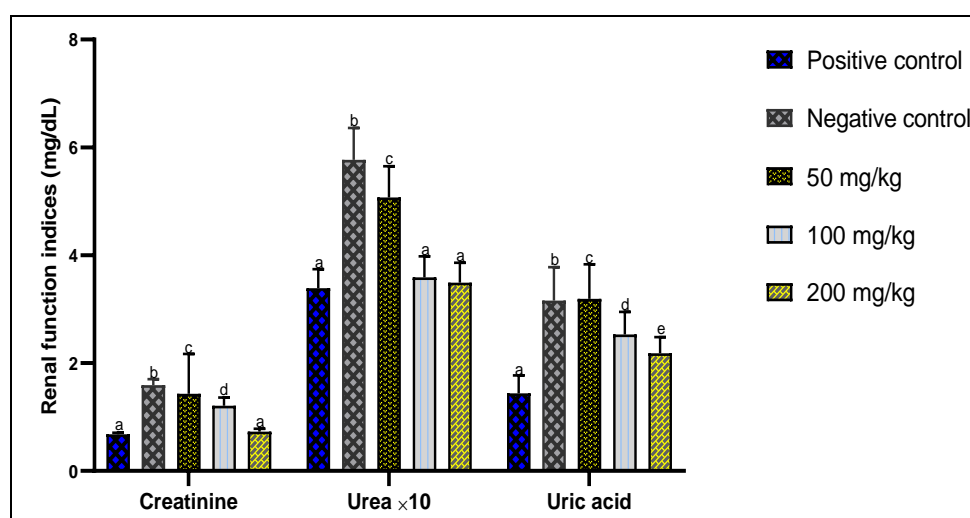

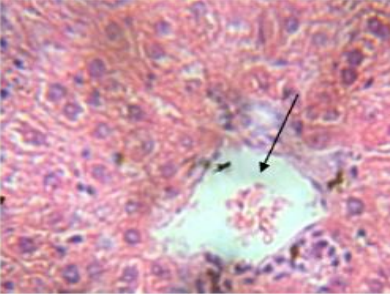
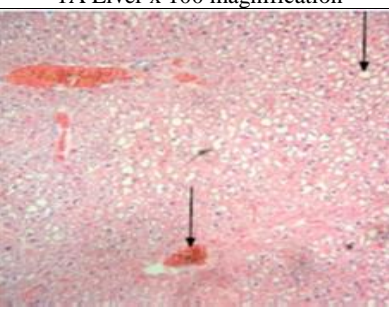
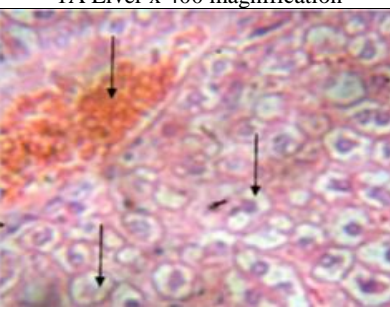
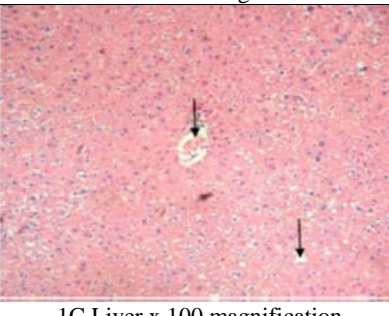
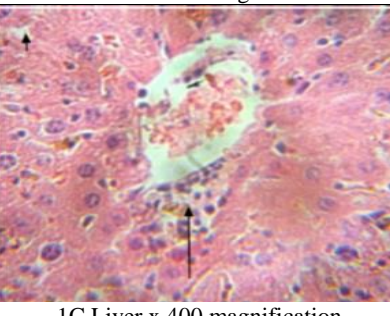
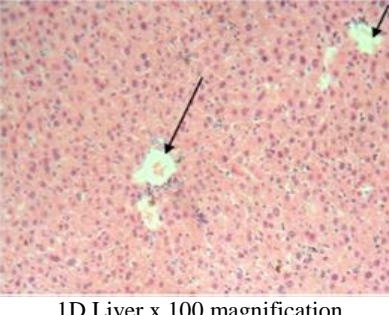
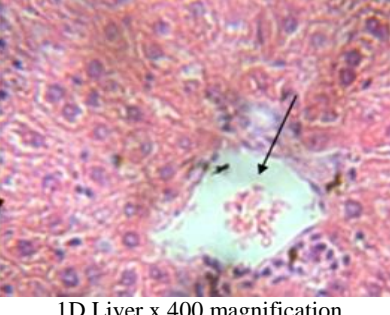
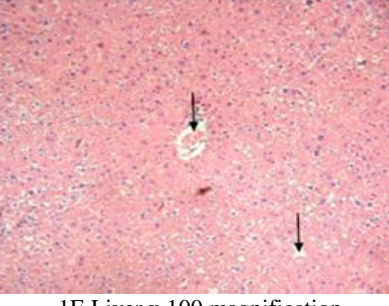
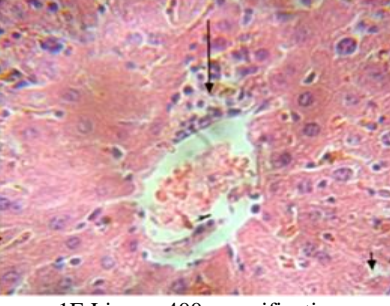


Fig 1: The effects of daily 50, 100 and 200 mg/kg body weight of composite mixture of *S. calycina* and *C. lutea* on renal function parameters of male Wistar rats after 21 days challenge in crude oil contaminated environment, Values are means of five replicate determinations \pm standard deviation, Values on the same column with same superscript letters a are not significantly different ($p > 0.05$) from the control, while those with different superscript letters b, c, d, e are significantly different ($p < 0.05$) from the control group, One-way analysis of variance (ANOVA).

Effects of 50, 100 and 200 mg/kg Body Weight of Composite Mixture of *S. calycina* and *C. lutea* on Liver Histology of Male Wistar Rats after 28 Days Challenge in Crude Oil Environment: The effects of 50, 100 and 200

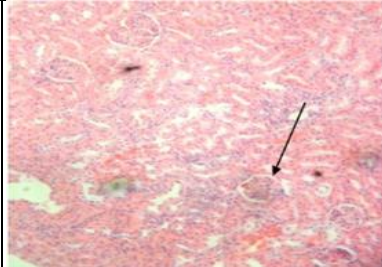
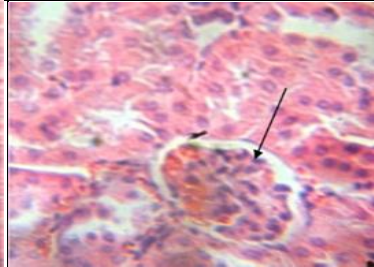
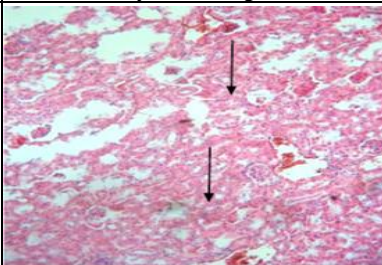
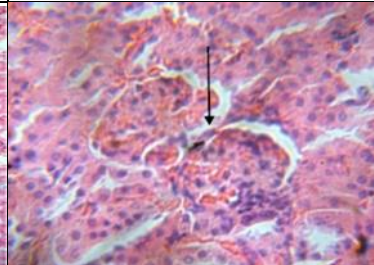
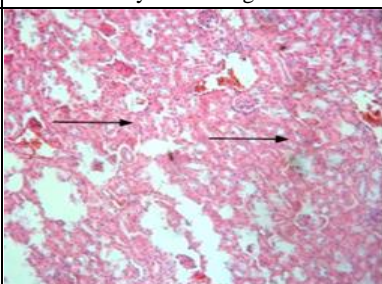
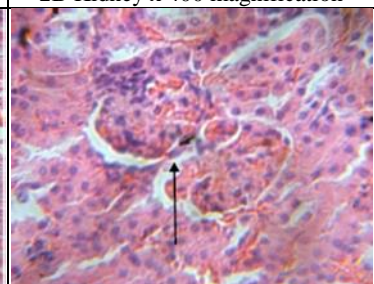
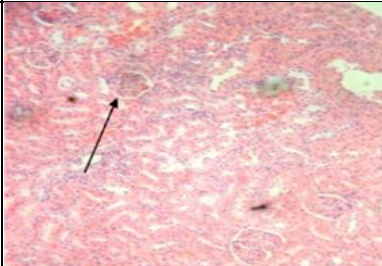

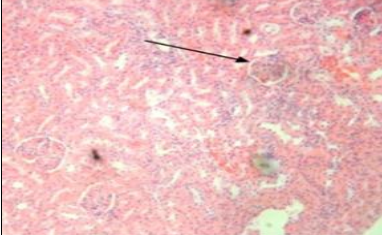
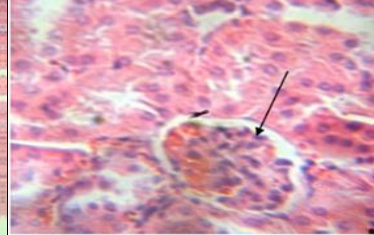
mg/kg body weight of composite mixture of *s. calycina* and *c. lutea* on liver histological architecture of male Wistar rats after 28 days' challenge in crude oil environment is presented in Table 2.

Table 2: Histological architecture of liver of male Wistar rats challenged in crude oil environment and diagnosis

| | | |
|---|--|---|
| <p>Slide 1A: Representative photomicrographs of liver sections from positive control (H&E, $\times 100$ and $\times 400$). Histological examination of liver tissue revealed well-preserved hepatic architecture. The hepatic lobules displayed clearly demarcated hepatocytes with distinct, centrally located nuclei and prominent nucleoli. Sinusoidal spaces were uniformly distributed and fenestrated, with no evidence of congestion or dilation. Central veins and surrounding hepatocytes appeared structurally intact. No histopathological abnormalities were observed (see arrow).</p> |  |  |
| <p>Slide 1B: Representative photomicrographs of liver sections from negative control (H&E, $\times 100$ and $\times 400$). Histological evaluation of liver sections revealed prominent fatty change (steatosis) and hydropic degeneration of hepatocytes, observable at both low and high magnifications (long arrow). Additionally, multifocal areas of hepatocellular coagulative necrosis were evident, accompanied by portal biliary hyperplasia (arrow), indicative of ongoing hepatic injury and regenerative activity.</p> |  |  |
| <p>Slide 1C: Representative photomicrographs of liver sections from 50 mg/kg of composite extract (H&E, $\times 100$ and $\times 400$). Low- and high-power microscopic examination revealed prominent macrovesicular steatosis and hydropic degeneration of hepatocytes (long arrow), accompanied by multifocal areas of hepatocellular coagulative necrosis and portal biliary hyperplasia (arrow).</p> |  |  |
| <p>Slide 1D: Representative photomicrographs of liver sections from 100 mg/kg of composite extract (H&E, $\times 100$ and $\times 400$). Low- and high-power microscopic examination demonstrated reduced macrovesicular steatosis and diminished hydropic degeneration of hepatocytes (long arrow). Additionally, there was a noticeable decrease in multifocal centrilobular congestion (arrow), with occasional neutrophilic infiltration observed in proximity to the central vein (arrow).</p> |  |  |
| <p>Slide 1E: Representative photomicrographs of liver sections from 200 mg/kg of composite extract (H&E, $\times 100$ and $\times 400$). Well-defined central veins and prominently fenestrated sinusoidal architecture were observed. Hepatocytes appeared distinct, with well-differentiated nuclei evident at both low and high magnification. Overall, the hepatic parenchyma exhibited normal histological architecture (arrow).</p> |  |  |

Effects of 50, 100 and 200 mg/kg body weight of composite mixture of *S. calycina* and *C. lutea* on kidney histology of Male Wistar rats after 28 days challenge in crude oil environment:

The effects of 50, 100 and 200 mg/kg body weight of composite mixture of *s. calycina* and *c. lutea* on kidney histological architecture of male Wistar rats after 28 days challenge in crude oil environment is presented in Table 3.

| | | |
|---|---|--|
| <p>Slide 2A: Representative photomicrographs of kidney sections from positive control (H&E, $\times 100$ and $\times 400$)</p> <p>Histological examination of kidney sections revealed normal renal architecture. The cortical parenchyma was well-preserved, and the renal corpuscles were observed as distinct, densely stained, spherical structures (arrow).</p> |  <p>2A Kidney x 100 magnification</p> |  <p>2A Kidney x 400 magnification</p> |
| <p>Slide 2B: Representative photomicrographs of kidney sections from negative control (H&E, $\times 100$ and $\times 400$). Histological evaluation of the kidney revealed varying degrees of distortion and disruption in the microarchitecture of the renal cortex, with features suggestive of interstitial edema, when compared to the positive control group</p> |  <p>2B Kidney x 100 magnification</p> |  <p>2B Kidney x 400 magnification</p> |
| <p>Slide 2C: Representative photomicrographs of kidney sections from 50 mg/kg of composite extract (H&E, $\times 100$ and $\times 400$).</p> <p>Microscopic assessment of renal tissue demonstrated variable alterations and disorganization in the structural integrity of the cortical region, with indications of possible interstitial fluid accumulation, relative to the positive control group.</p> |  <p>2C Kidney x 100 magnification</p> |  <p>2C Kidney x 400 magnification</p> |
| <p>Slide 2D: Representative photomicrographs of kidney sections from 100 mg/kg of composite extract (H&E, $\times 100$ and $\times 400$).</p> <p>Microscopic analysis of the renal tissue showed moderate structural alterations and disarray within the cortical architecture, along with evidence suggestive of interstitial edema, in comparison to the positive control group.</p> |  <p>2D Kidney x 100 magnification</p> |  <p>2D Kidney x 400 magnification</p> |
| <p>Slide 2E: Representative photomicrographs of kidney sections from 200 mg/kg of composite extract (H&E, $\times 100$ and $\times 400$).</p> <p>The kidney section exhibited preserved histological architecture. The cortical parenchyma appeared well-defined, and the renal corpuscles were visualized as compact, spheroidal structures (arrow)</p> |  <p>2E Kidney x 100 magnification</p> |  <p>2E Kidney x 400 magnification</p> |

Discussion

Effects of 50, 100 and 200 mg/kg body weight of composite mixture of *S. calycina* and *C. lutea* on haematological parameters of male Wistar rats after 28 days challenge in crude oil environment

Crude oil exposure induces oxidative stress and systemic toxicity, commonly reflected in altered haematological indices and immunological dysregulation [18]. The present study demonstrates that co-administration of *S. calycina* and *C. lutea* extracts exerted dose-dependent haematoprotective effects. The negative control group exhibited significant reductions in Packed Cell Volume (PCV), Red Blood Cell (RBC) count, and Haemoglobin (Hb) concentration ($31.12 \pm 3.54\%$, $5.11 \pm 0.81 \times 10^6/\mu\text{L}$, and $9.33 \pm 1.62 \text{ g/dL}$, respectively), suggestive of crude oil-induced anaemia via

oxidative haemolysis and impaired erythropoiesis [19]. Treatment with 100 and 200 mg/kg of the extract significantly restored these parameters toward control levels, indicating antioxidant and erythropoietic enhancement by phytoconstituents [20]. White Blood Cell (WBC) count was markedly elevated in the negative control ($13.70 \pm 2.15 \times 10^3/\mu\text{L}$), reflecting a systemic inflammatory response, which was attenuated in treated groups, implying immunomodulatory activity. Microcytic hypochromic anaemia, evidenced by reduced MCV and MCH ($54.47 \pm 5.13 \text{ fL}$; $24.73 \pm 2.25 \text{ pg}$), was reversed post-treatment, suggesting restored erythrocyte morphology. Platelet suppression in untreated rats ($6861 \pm 21.54 \times 10^9/\text{L}$) was significantly ameliorated by treatment, reflecting improved thrombopoiesis [21-22]. Overall, these findings

indicate that *S. calycina* and *C. lutea* synergistically counteract crude oil-induced haematotoxicity through antioxidant, anti-inflammatory, and haematopoietic mechanisms.

Effects of 50, 100 and 200 mg/kg body weight of composite mixture of *S. calycina* and *C. lutea* on renal function indices of male Wistar rats after 28 days challenge in crude oil environment

The nephroprotective efficacy of a composite extract derived from *S. calycina* and *C. lutea* was investigated in male Wistar rats exposed to crude oil over a 28-day period. As illustrated in Figure 1, crude oil exposure significantly elevated renal biomarkers of serum creatinine, urea, and uric acid relative to the positive control group ($p < 0.05$), indicating marked nephrotoxicity. This is consistent with prior studies demonstrating that crude oil and its constituents induce renal oxidative stress via the generation of reactive oxygen species (ROS) and nephrotoxic [23]. Notably, administration of the composite extract at 100 mg/kg and 200 mg/kg resulted in statistically significant reductions in these biomarkers ($p < 0.05$), suggesting dose-dependent renal protection. The observed renoprotective effect may be attributed to phytoconstituents such as flavonoids, tannins, and saponins, which are known for their antioxidant, anti-inflammatory, and free radical-scavenging activities [24]. Rats treated with 50 mg/kg showed only minimal improvements, implying that this dose is likely sub-therapeutic under the present experimental conditions. Importantly, the 100 mg/kg and 200 mg/kg treatments restored renal indices to levels comparable with the positive control, reinforcing the therapeutic potential of this herbal formulation [25]. These findings corroborate previous reports on the individual nephroprotective effects of *S. calycina* and *C. lutea*, attributed to their capacity to inhibit lipid peroxidation and preserve renal histoarchitecture [26]. The observed synergistic interaction in the composite may enhance bioefficacy, highlighting its promise as a phytotherapeutic candidate for managing crude oil-induced nephrotoxicity.

Effects of 50, 100 and 200 mg/kg body weight of composite mixture of *S. calycina* and *C. lutea* on liver histology of male Wistar rats after 28 days challenge in crude oil environment

Histological examination of liver tissues from the positive control group revealed preserved hepatic architecture, characterized by polygonal hepatocytes with centrally located nuclei, prominent nucleoli, and well-organized hepatic cords. The sinusoids were intact with no signs of congestion, cellular degeneration, or inflammatory infiltration, indicating normal liver physiology (Slide 1A, $\times 100$ and $\times 400$). These findings align with the classical hepatic microanatomy described in healthy mammalian livers [27]. In contrast, liver sections from the negative control group (crude oil-exposed, untreated) demonstrated severe pathological alterations, including micro vesicular steatosis, hydropic degeneration, and multifocal areas of coagulative necrosis. Portal triads showed marked biliary hyperplasia and lymphocytic infiltration, indicative of hepatocellular injury and regenerative attempts (Slide 1B). These changes are consistent with hydrocarbon-induced hepatotoxicity mediated via oxidative stress and lipid peroxidation [28]. Treatment with 50 mg/kg of the composite

extract resulted in partial histological improvement, evidenced by reduced necrotic foci and less extensive steatosis, although hydropic degeneration and portal hyperplasia persisted (Slide 1C), reflecting limited hepatoprotection at lower doses [29]. At 100 mg/kg, liver sections showed substantial architectural restoration, with reduced steatosis, minimal centrilobular congestion, and decreased inflammatory cell presence (Slide 1D), suggesting enhanced free radical scavenging and anti-inflammatory activity [30]. Notably, at 200 mg/kg, hepatic tissue appeared near-normal, with clear lobular organization, intact sinusoidal spaces, and no detectable lesions (Slide 1E), indicating complete hepatoprotection, likely due to synergistic antioxidant and membrane-stabilizing actions of the phytochemicals present [31]. These results confirm a dose-dependent hepatoprotective efficacy of the extract against crude oil-induced liver toxicity.

Effects of 50, 100 and 200 mg/kg Body weight of composite mixture of *S. calycina* and *C. lutea* on kidney histology of male Wistar rats after 28 days challenge in crude oil environment

Histopathological analysis of renal tissues from male Wistar rats exposed to crude oil-contaminated environments and subsequently treated with graded doses (50, 100, and 200 mg/kg) of a composite extract from *S. calycina* and *C. lutea* revealed a dose-dependent attenuation of nephrotoxicity. In the untreated control group (Slide 2A), kidney sections exhibited normal histoarchitecture with intact cortical parenchyma and prominent renal corpuscles, confirming baseline renal integrity [32]. In contrast, the negative control group (Slide 2B), subjected to Nigerian Light Crude Oil (BLCO) without treatment, showed extensive cortical disruption, interstitial edema, and glomerular atrophy, indicative of significant crude oil-induced nephrotoxicity. These alterations are consistent with hydrocarbon-mediated renal injury via oxidative stress, lipid peroxidation, and pro-inflammatory cytokine induction [4]. Rats treated with 50 mg/kg of the composite extract (Slide 2C) exhibited mild histological improvement. While interstitial edema and tubular disarray persisted, partial preservation of cortical architecture was evident, suggesting limited ameliorative effects likely due to suboptimal phytochemical concentration [33-34]. At 100 mg/kg (Slide 2D), renal sections demonstrated moderate structural restoration, with reduced tubular distortion and attenuated interstitial changes, signifying enhanced renoprotection suggesting a dose-dependent therapeutic response [35]. The most profound histological recovery was observed at 200 mg/kg (Slide 2E), where renal morphology closely resembled the positive control, with well-defined glomeruli and minimal pathological lesions. The renoprotective effects observed are plausibly attributed to the antioxidant and membrane-stabilizing activities of flavonoids, saponins, and tannins inherent in the composite extract [36-38]. These findings provide compelling evidence for the dose-responsive nephroprotective potential of *S. calycina* and *C. lutea* in ameliorating crude oil-induced renal injury, with implications for phytotherapeutic interventions in environmental toxicology.

Conclusion

This study demonstrates that environmental exposure to Nigerian Light Crude Oil induces significant

haematological, hepatic, and renal toxicity in male Wistar rats, characterized by oxidative stress and histopathological damage. Administration of a composite extract from *S. calycina* and *C. lutea* at 100 and 200 mg/kg effectively ameliorated these toxic effects in a dose-dependent manner, restoring key physiological parameters and tissue architecture. These findings underscore the extract's potent antioxidant and organ-protective properties, supporting its potential application as a phytotherapeutic intervention against crude oil-induced systemic toxicity.

Declaration of generative AI and AI assisted technologies in the manuscript preparation

We disclose that Grammarly was utilised for spelling, language editing and refining the manuscript's structure. All scientific content, data interpretation, and conclusions were developed solely by the authors.

Conflict of Interest

The authors declare no competing interests

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