World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Nephroprotective Effects of *Emilia sonchifolia*, *Bridelia ferruginea* and *Rhizophora racemosa* Aqueous Extracts against Petrol Fume-Induced Renal Toxicity in Wistar Rats

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World Journal of Advanced Research and Reviews, 2026, 29(02), 529-537

Publication history: Received on 27 December 2025; revised on 03 February 2026; accepted on 06 February 2026

Article DOI: https://doi.org/10.30574/wjarr.2026.29.2.0294

Abstract

Prolonged exposure to petrol fumes has been implicated in oxidative stress-related renal dysfunction. This study evaluated the nephrotoxic effects of petrol fume inhalation and assessed the protective potential of aqueous extracts from *Bridelia ferruginea* and *Emilia sonchifolia* leaves as well as *Rhizophora racemosa* stem bark. Wistar rats(adult-male) were exposed to petrol fumes for 28 days, during which serum urea and creatinine levels, as well as renal antioxidant enzyme activities, glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD), as well malondialdehyde (MDA) levels, were monitored. Petrol fume exposure significantly elevated serum urea and creatinine levels, suppressed CAT, SOD, and GPx activities, and increased MDA, indicating oxidative renal injury. However, treatment with the plant extracts at 200 mg/kg and 400 mg/kg significantly ameliorated these changes in a dose-dependent manner. Notably, *E. sonchifolia* at 400 mg/kg showed the most pronounced reno-protective effect, comparable to that of the standard antioxidant, vitamin E (200 mg/kg). The findings suggest that the protective effects of the extracts may stem from their antioxidant-rich phytochemical composition, which mitigates oxidative damage, enhances endogenous antioxidant defenses, and improves renal function. Histological analysis revealed renal architectural distortions in the petrol-exposed group. This was restored by the extracts. This study highlights the therapeutic potential of these medicinal plants against petrol fume-induced nephrotoxicity and supports further exploration of their bioactive compounds in clinical contexts.

Keywords: Urea; Creatinine; Antioxidants; Petrol; Nephro-Toxicity

1. Introduction

Petrol is a complex mixture of over 150 distinct hydrocarbons, including alkanes, 25-40% isoalkanes, 2-5% alkenes, cycloalkenes, cycloalkanes and aromatic compounds like benzene (typically 0.5–2.5%), toluene, ethylbenzene and xylene (collectively known as BTEX). Furthermore, it contains numerous blending agents and additives, such as anti-knock substances, antioxidants, metal deactivators, lead-clearing agents, anti-corrosion, anti-icing chemicals, upper-cylinder lubricants, detergents and dyes.

These additives are engineered to improve performance and fuel economy. According to the International Agency for Research on Cancer (WHO, 2012), petrol has been categorized as a Group 2B carcinogen, denoting that it is possibly carcinogenic to humans (WHO, 2012).

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Among its constituents, BTEX compounds are considered the most hazardous, with benzene posing the greatest health risk. Benzene has been designated as a Group 1 carcinogen, while chronic exposure to BTEX is strongly associated with toxic effects on the central nervous, respiratory, cardiovascular, hepatic, and renal systems (Adami et al., 2006; Soleimani, 2020; Abduljalel and Al-Saadi, 2022). These toxicities are primarily mediated through oxidative imbalance, lipid peroxidation, and the impairment of cellular antioxidant defense systems, with pronounced impact in highly metabolic tissues like the liver and kidneys (Kim et al., 2024).

Upon inhalation of petrol vapors, hydrocarbons like benzene are rapidly absorbed in the lungs and metabolized in the liver through oxidative pathways involving cytochrome P450 enzymes, especially CYP2E1. This biotransformation produces reactive oxygen species (ROS) and toxic intermediates such as phenol, hydroquinone, benzoquinone, and 1,2,4-benzenetriol (Zhang et al., 2024). Because the kidneys are responsible for excreting these metabolites, they become particularly susceptible to injury. Accumulation of such toxic derivatives promotes excessive free radical generation, which triggers oxidative stress, lipid peroxidation, and structural damage to renal cell membranes (Kishi et al., 2024).

Oxidative stress is a central mechanism in petrol-induced toxicity, making medicinal plants rich in bioactive compounds (flavonoids, alkaloids) a promising therapeutic approach (Tchamgoue et al., 2024). These phytochemicals exhibit potent antioxidant, anti-inflammatory and cytoprotective effects. They operate by scavenging ROS and upregulating the endogenous antioxidant system, particularly through the elevation of SOD, CAT and GPx enzyme activities. Additionally, they contribute to the stabilization of cellular membranes (Said and Ibrahim, 2024), thus alleviating the biochemical injury induced by petrol fume exposure.

Emilia sonchifolia, commonly referred to as lilac tassel flower or cupid's shaving brush—is a tropical herb noted for its vibrant flowers and extensive use in traditional medicine across Ayurveda, Vietnam and Nigeria. It is abundant in secondary metabolites such as terpenoids, alkaloids and flavonoids (Neethu and Gangaprasad, 2018), which underpin its reported antioxidant and anti-inflammatory activities. Traditionally, the plant has been employed in the treatment of wounds, ulcers, tumors, and hypertension (Saratale et al., 2018).

Bridelia ferruginea, a small African tree, is widely utilized in indigenous medicine for the management of dysentery, arthritis, diabetes and febrile illnesses (Afolayan et al., 2019; Olajide et al., 2012). Its leaves and bark are rich in diverse phytochemicals that exhibit antimicrobial, anti-inflammatory, hypoglycemic, and gastroprotective effects, including ulcer protection (Yeboah et al., 2022).

Similarly, *Rhizophora racemosa*, a mangrove species prevalent along the West African coast (de Lacerda et al., 2025), has long been valued in folk medicine for treating gastrointestinal disorders such as ulcers and stomach cramps (Ngeve et al., 2016). The bark and leaves, which contain high levels of flavonoids and tannins (Chiavaroli et al., 2020), not only provide ulcer-healing benefits but also possess antimicrobial activity. This dual functionality supports its continued use in both traditional healing and food preservation practices, particularly within Nigeria's Niger Delta region.

2. Materials And Methods

2.1. Collection of Plant Material and Extract Preparation

Fresh *Emilia sonchifolia* and *Bridelia ferruginea* leaves were obtained from the Niger Delta University campus in Bayelsa State, Nigeria. The plant material was taxonomically identified by Prof. Kola Ajibesin of the Department of Pharmacognosy. Following collection, the leaves were Rinsed using clean water and subsequently dried in the shade for a period of two weeks. The desiccated leaves were milled into a powdered form using an electric blender. For extraction, 100 g of each powdered sample was macerated in 1 L of distilled water for 72 hours with periodic agitation. The aqueous solution was filtered through a Whatman filter paper (110 mm), and the resulting filtrate was concentrated by evaporation in a water bath maintained at 60 °C. The final crude extracts were stored in labeled containers under refrigeration for future use.

The stem bark of *Rhizophora racemosa* was obtained from Edema, Ogbia Local Government Area, Bayelsa State, Nigeria, and also authenticated by Prof. Ajibesin. It was processed similarly washed, shade-dried, pulverized and extracted in distilled water over 72 hours. After filtration and evaporation, the aqueous extract was stored under refrigeration for later use.

2.2. Petrol and Reagents

Petrol (PMS) was sourced from the Nigerian National Petroleum Corporation (NNPC) station in Edepie, Yenagoa, Bayelsa State. Biochemical assay kits were obtained from Randox™ Laboratories Ltd, UK, while other reagents used for this study were obtained from Loba Chemie PVT LTD, India.

2.3. Experimental Animals

Fifty-four (54) healthy male Wistar rats, weighing 211–255g, were obtained from the Department of Pharmacology's animal facility at Niger Delta University. Prior to the experiment, the rats underwent a two-week acclimatization period under standard laboratory conditions, which included a 12-hour light/dark cycle, a controlled temperature of 20–25 °C, adequate ventilation, and ad libitum access to food and water.

Ethical approval for all experimental procedures was obtained from the Research Ethical Committee of the College of Health Sciences, Niger Delta University, located on Wilberforce Island in Bayelsa State. The study was conducted in full accordance with the institution's regulatory guidelines.

2.4. Experimental Design

The 54 rats were randomly assigned to nine groups (n=6 per group) and treated orally for 28 days as follows:

2.4.1. Normal control -distilled water only.

Positive Control -Petrol fumes + distilled water.

- Petrol fumes + *E. sonchifolin* (200 mg/kg).
- Petrol fumes + *E. sonchifolin* (400 mg/kg).
- Petrol fumes + *B. ferrugineol* (200 mg/kg).
- Petrol fumes + *B. ferrugineol* (400 mg/kg).
- Petrol fumes + *R. racemosa* (200 mg/kg).
- Petrol fumes + *R. racemosa* (400 mg/kg).
- Petrol fumes + Vitamin E (200 mg/kg).

All treatments were administered orally using gavage tubes.

2.5. Petrol Fume Exposure Protocol

In accordance with the methodology established by Uboh et al. (2005), and Owagboriaye et al., 2016, rats were exposed to petrol fumes via inhalation. Exposure occurred in a wooden fume chamber (150 × 90 × 210cm) housing two open 1000 ml beakers, each containing 500 ml of petrol. These were placed one hour before exposure to saturate the chamber. Rats were exposed to fumes for four hours daily over 28 days before being returned to a fume-free environment.

2.6. Sample Collection and Biochemical Analysis

Blood samples were collected from the submandibular vein on days 0, 1, 7, 14, 21, and 28 after a light chloroform anesthesia for biochemical analyses Samples were obtained within the time frame of 10:00 a.m. to 12:00 p.m., following a 24-hour post-exposure period. On day 28, the animals were euthanized under chloroform anesthesia, and kidneys were harvested for antioxidant and histopathological evaluation.

Urea and creatinine levels were measured following the manufacturer’s instructions provided in the biochemical assay kits.

Superoxide dismutase (SOD) activity was measured using the method developed by Marklund and Marklund (1974). Catalase (CAT) activity was assessed following the protocol of Aebi et al. (1974). Glutathione peroxidase (GPx) activity was analyzed with a glutathione reductase-coupled system, as described by Lawrence and Burk (1976). Lipid peroxidation was determined by quantifying malondialdehyde (MDA) levels, applying a minor modification to the technique of Armstrong and Al-Awadi (1991).

2.7. Histological Examination

Small tissue fragments (approximately 5 mm^3) were preserved in 10% neutral buffered formalin. Following fixation, the tissues were prepared using conventional histological methods, which included paraffin embedding, sectioning into thin slices, and staining with hematoxylin and eosin (HandE). Examination was conducted using an Olympus CH microscope (Olympus, Tokyo, Japan) at 400× magnification in three randomly selected fields per sample. Histopathological changes and tissue lesions were observed and scored accordingly.

2.8. Statistical Analysis

One-way analysis of variance (ANOVA), with Tukey's post-hoc test applied for comparisons among multiple groups (IBM SPSS Statistics 20, USA). Data are presented as mean ± standard error of the mean (SEM), with statistical significance established at $p < 0.05$.

3. Results

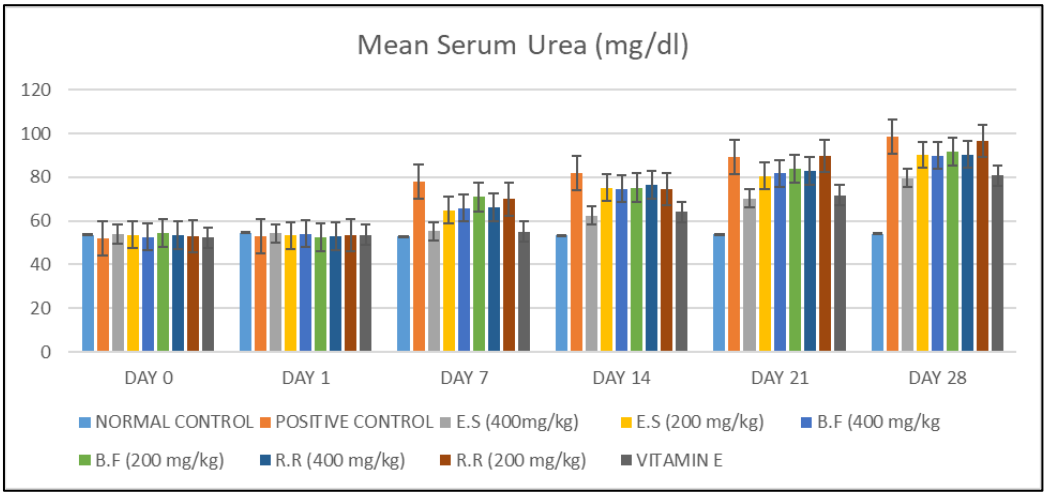


Figure 1 Serum urea levels in rats following 28 days of petrol fume inhalation and subsequent administration of aqueous extracts from *Emilia sonchifolia* (E.S), *Bridelia ferruginea* (B.F) and *Rhizophora racemosa* (R.R). Values are expressed as mean ± SEM. Statistical significance compared to the control group is indicated at $p < 0.05$

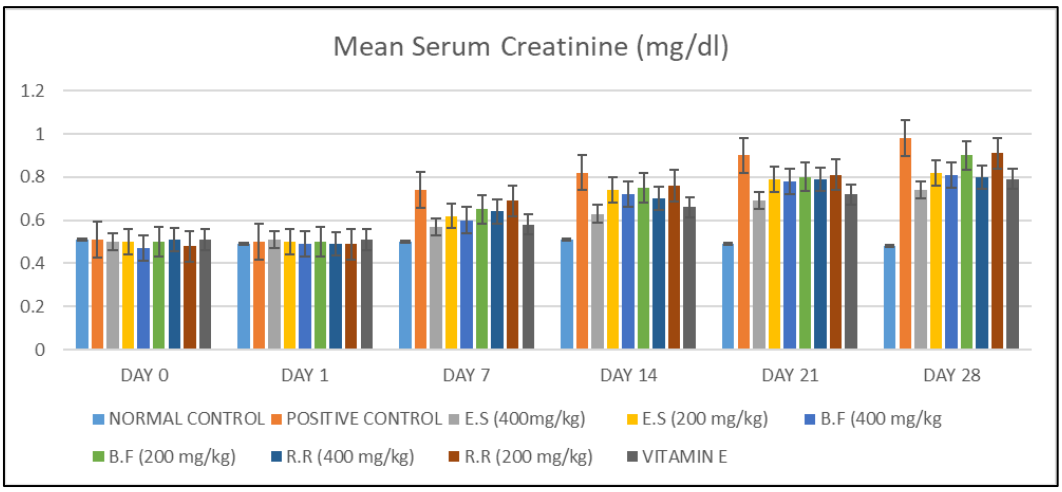


Figure 2 Serum creatinine concentrations in rats after a 28-day period of petrol fume inhalation and therapeutic intervention with aqueous extracts from *Emilia sonchifolia* (E.S), *Bridelia ferruginea* (B.F) and *Rhizophora racemosa* (R.R). Data are expressed as mean ± standard error of the mean (SEM). A statistically significant difference from the control group is indicated at $p < 0.05$

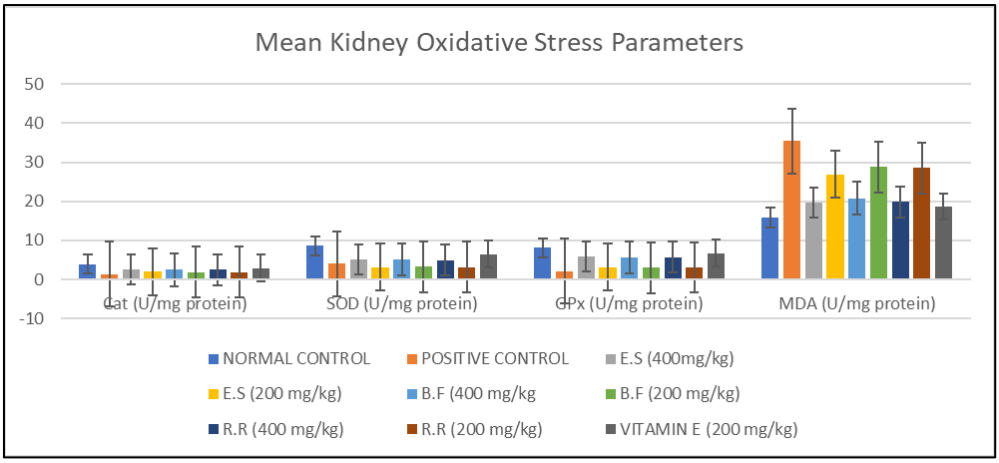
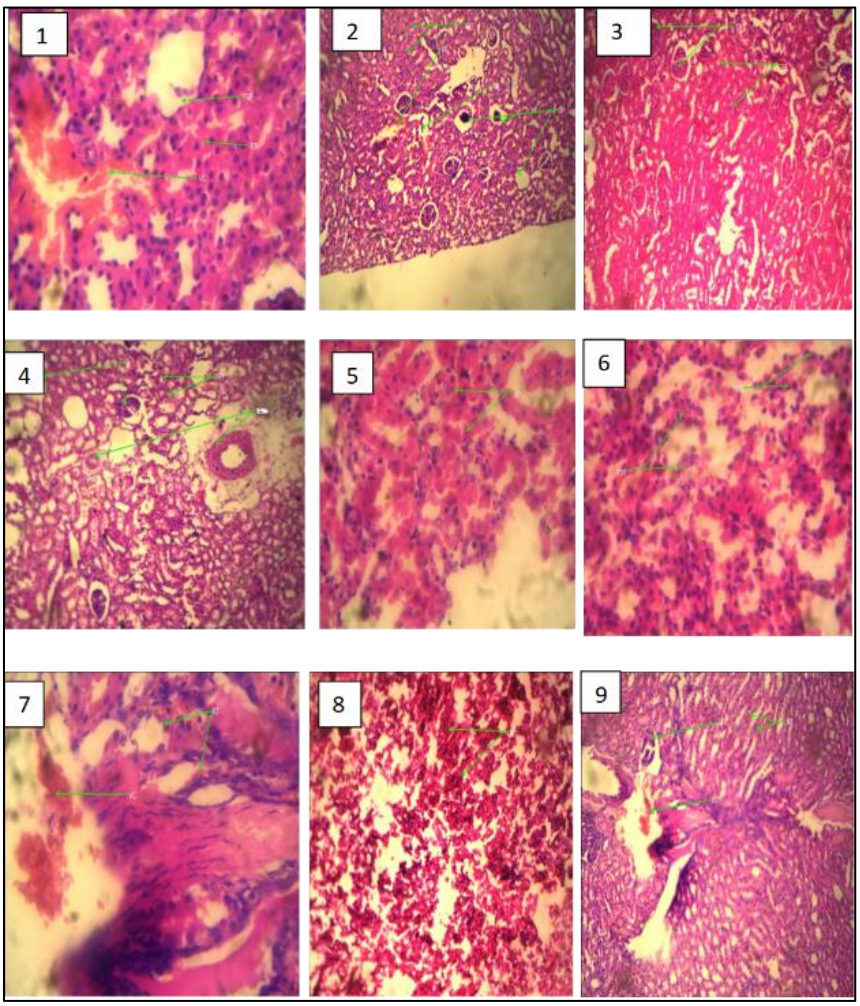


Figure 3 Effects of plant extract administration on markers of oxidative stress in the kidneys of rats following petrol fume exposure. The evaluated parameters comprised the enzymatic activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx), as well as the level of malondialdehyde (MDA). Results are presented as mean ± SEM (n=6). Statistical significance was determined at $p < 0.05$. Extract abbreviations: E.S. (*Emilia sonchifolia*), B.F. (*Bridelia ferruginea*), R.R. (*Rhizophora racemosa*)



Figures 1 through 9 present kidney histology from control and experimental rats, stained with hematoxylin and eosin (HandE). The study assessed the effects of a 28-day exposure to petrol fumes and the potential protective role of aqueous extracts from *Emilia sonchifolia*, *Bridellia ferruginea*, and *Rhizophora racemosa*.

Plate 1 (Normal Control): Displays standard kidney morphology, including intact glomerular parenchyma (GP), vascular congestion (VC), and renal tubules (RT) (HandE, 400x magnification). Plate 2 (Petrol Fumes Only): Reveals significant tissue damage, including Congestion of blood vessels (VC), glomerular herniation (GP), and disruption of normal architecture (AD), and altered renal tubules (RT) (HandE, 400x). Plate 3 (Petrol + 200mg/kg *E. sonchifolia*): Shows a slightly disorganized tissue structure with hypertrophied renal tubules (HRT) and visible glomeruli (G) (HandE, 400x). Plate 4 (Petrol + 400mg/kg *E. sonchifolia*): Appears nearly normal, with preserved kidney architecture, glomeruli (G), and renal tubules (RT) (HandE, 400x). Plate 5 (Petrol + 200mg/kg B. ferruginea): Indicates a mild disruption in the arrangement of the proximal and distal convoluted tubules (HandE, 400x). Plate 6 (Petrol + 400mg/kg B. ferruginea): Demonstrates a moderately disorganized kidney section with visible renal tubules (RT) (HandE, 400x). Plate 7 (Petrol + 200mg/kg R. racemosa): Exhibits vascular congestion (VC) and renal tubules (HandE, 400x). Plate 8 (Petrol + 400mg/kg R. racemosa): Presents a moderate undifferentiation of renal morphorlogy with renal tubules (RT) (HandE, 400x). Plate 9 (Petrol + 200mg/kg Vitamin E): Shows a protective effect, with Intact renal tubules with mild vascular congestion (VC), and glomeruli (G) (HandE, 400x).

4. Discussion

This research work investigated the nephrotoxic effects of prolonged exposure to petrol fumes and the potential protective role of aqueous extracts from *Emilia sonchifolia*, *Bridellia ferruginea*, and *Rhizophora racemosa* in mitigating renal damage. Findings demonstrated that petrol fumes induced significant renal dysfunction, as evidenced by elevated serum urea and creatinine levels, alongside impaired antioxidant defense mechanisms and increased oxidative stress. However, treatment with the plant extracts, particularly at higher doses (400 mg/kg), effectively ameliorated these adverse effects in a dose-dependent manner, comparable to the standard antioxidant, vitamin E.

Exposure to petrol fumes led to a progressive and significant increase in serum urea and creatinine levels from day 7 to day 28 compared to the normal control group. These biomarkers are critical indicators of renal impairment, as urea is a byproduct of protein metabolism excreted by the kidneys, while creatinine is formed from breakdown of creatine and phosphocreatine from skeletal muscle and reflects glomerular filtration rate (GFR) (Bai et al., 2021; Wang et al., 2024). The observed elevation suggests that petrol fumes may impair kidney filtration capacity, possibly due to oxidative damage or direct nephrotoxicity from hydrocarbon constituents such as benzene, toluene, and xylene (Abu-Bakr, 2025).

The study further revealed that petrol fumes significantly suppressed kidney antioxidant enzymes,catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), while increasing malondialdehyde (MDA), a marker of lipid peroxidation. This aligns with previous findings that hydrocarbon exposure generates reactive oxygen species (ROS), overwhelming endogenous antioxidant defenses and leading to cellular damage (D'Souza et al., 2024). The decline in CAT, SOD, and GPx activities indicates compromised ROS scavenging capacity, exacerbating renal oxidative injury.

Treatment with *Emilia sonchifolia*, *Bridellia ferruginea*, and *Rhizophora racemosa* extracts (400 mg/kg and 200 mg/kg) significantly reversed petrol-induced renal damage. The higher dose (400 mg/kg) exhibited superior efficacy, nearly normalizing urea and creatinine levels and restoring antioxidant enzyme activities while reducing MDA. This dose-dependent effect suggests that the extracts contain bioactive compounds (e.g., flavonoids, polyphenols, and alkaloids) with potent antioxidant and nephroprotective properties (Rauf et al., 2024; de Lima et al.,2025).

Interestingly, *Emilia sonchifolia* at 400 mg/kg showed the strongest protective effect, closely followed by *Bridellia ferruginea* and *Rhizophora racemosa*. This may be attributed to differences in phytochemical composition, with *E. sonchifolia* potentially containing high concentrations of radical-scavenging compounds such as flavonoids, alkaloids, and terpenoids (Neethu and Gangaprasad, 2018). Vitamin E (200 mg/kg), a known antioxidant, also demonstrated significant protection, reinforcing the role of oxidative stress in petrol-induced nephrotoxicity.

The findings suggest that petrol fumes induce nephrotoxicity primarily through oxidative stress, leading to renal functional decline. The plant extracts likely mitigate this damage by scavenging ROS, thereby reducing lipid peroxidation (MDA) and preserving membrane integrity, enhancing antioxidant enzymes by pregulating CAT, SOD, and GPx to neutralize free radicals, and chelating toxic metabolites as some phytochemicals may bind hydrocarbon derivatives, preventing renal accumulation.

Histological analysis of kidney tissues after 28 days of petrol fume exposure confirmed significant nephrotoxicity, marked by vascular congestion, glomerular prolapse, and structural distortion (Plate 2), compared to the normal kidney architecture in the control group (Plate 1). Treatment with plant extracts or vitamin E showed varying degrees of reno-protection. *Emilia sonchifolia* offered the most notable protection. At 400 mg/kg (Plate 4), it nearly restored normal

kidney structure, while the 200 mg/kg dose (Plate 3) showed mild improvement. *Bridelia ferruginea* (Plates 5 and 6) and *Rhizophora racemosa* (Plates 7 and 8) showed moderate, dose-dependent improvement, though less effective than *E. sonchifolia*. Vitamin E (Plate 9) also improved kidney histology, with only slight vascular congestion remaining. These findings support the antioxidant-mediated reno-protective potential of the extracts, particularly at higher doses.

The histopathological findings correlate strongly with the biochemical data, affirming that petrol fumes induce notable renal damage characterized by glomerular distortion, tubular degeneration, and vascular abnormalities. Treatment with these plant extracts significantly caused the reduction of the extent of these lesions, especially *Emilia sonchifolia* at 400 mg/kg, which restored near-normal kidney architecture. These effects support the hypothesis that the nephroprotective actions of the extracts are mediated by their antioxidant constituents, which likely reduce ROS-induced cellular damage, stabilize membranes, and preserve renal structure and function.

5. Conclusion

This study confirms that prolonged petrol fume exposure induces significant nephrotoxicity via oxidative stress. However, aqueous extracts of *E. sonchifolia*, *B. ferruginea, and R. racemosa* exhibit dose-dependent nephro-protective effects, comparable to vitamin E. Further research should isolate the active constituents of these plants and explore their mechanisms in clinical settings.

Compliance with ethical standards

Acknowledgments

We are grateful to Victor Okonkwo and Stephen Ofurum for their support during this study.

Disclosure of conflict of interest

We declare that there is no conflict of interest.

Statement of ethical approval

Ethical approval was obtained for this study from the Research Ethics committee of Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria..

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