

**AMELIORATIVE EFFECTS OF AQUEOUS LEAF EXTRACTS
OF *EMILIA SONCHIFOLIA* AND *BRIDELIA FERRUGINEA* AND
AQUEOUS STEM BARK EXTRACT OF *RHIZOPHORA
RACEMOSA* ON SPERM QUALITY IN PETROL FUME-EXPOSED
MALE WISTAR RATS**

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Introduction

Male infertility is a significant global health concern, with a substantial number of cases being attributed to various factors, many of which are linked to environmental and

occupational exposures (Ali *et al.*, 2025). Among these, volatile organic compounds (VOCs) present in petroleum products pose a considerable threat to reproductive health (Wami-Amadi, 2025). Petrol fumes, a complex

mixture of hydrocarbons and additives, are a ubiquitous environmental pollutant, especially in regions with high vehicular traffic and industrial activity. Exposure to these fumes has been demonstrated to induce oxidative stress, a key mechanism in the pathogenesis of sperm damage (Owagboriaye et al., 2022). This oxidative imbalance can lead to impaired spermatogenesis, resulting in reduced sperm count, diminished motility, increased morphological abnormalities, and ultimately, compromised male fertility (Shi et al., 2021).

In the search for interventions to counter such chemically-induced reproductive toxicity, natural products have gained prominence due to their historical use in traditional medicine and their potential for multi-target therapeutic actions with fewer side effects (Vicidomini et al., 2024). *Emilia sonchifolia* and *Bridelia ferruginea* leaves, as well as the stem bark of *Rhizophora racemosa*, are of particular interest. These plants are utilized in ethnobotanical practices in the Niger Delta region of Nigeria for various ailments and their phytochemical profiles are rich in flavonoids, alkaloids, tannins, and phenolic compounds (Neethu and Gangaprasad, 2018; Chiavaroli et al., 2020; Celestine et al., 2025). These constituents are known for their potent antioxidant, anti-inflammatory, and androgenic properties, which could potentially mitigate oxidative damage in the testes and epididymis (Agu and Aja, 2025).

While the individual folk use of these plants suggests a basis for their application in treating

reproductive issues, there is a scarcity of rigorous scientific evidence validating their efficacy against petrol fume-induced sperm damage. Furthermore, a comparative assessment of their restorative potential has not been established. Therefore, this study was designed to investigate the ameliorative effects of the aqueous leaf extracts of *Emilia sonchifolia* and *Bridelia ferruginea*, and the stem bark extract of *Rhizophora racemosa* on sperm quality parameters in male Wistar rats with petrol fume-induced sperm impairment. The study utilized Vitamin E, a known potent antioxidant, as a standard reference control to benchmark the efficacy of the plant extracts.

MATERIALS AND METHODS

Experimental Animals

Male Wistar rats (n=54, 200–250g) were obtained from the animal breeding facility of the Department of Pharmacology at Niger Delta University. All animals were acclimatized for two weeks before the experiment under standard laboratory conditions, which included a 12h light/dark cycle, a temperature of 20–25°C, and free access to food and water.

Ethical Approval

All procedures were approved by the institutional Ethics and Research Committee (Ref No: 03/2025/001/FBMS).

Plant materials

Fresh *Emilia sonchifolia* and *Bridelia ferruginea* leaves were collected from the Niger Delta University College of Health Sciences campus. *Rhizophora racemosa* stem bark was obtained from Edema in the Ogbia Local

Government Area of Bayelsa State. The botanical identity of the first two species was confirmed by Prof. Kola Ajibesin from the Department of Pharmacognosy at Niger Delta University. *Rhizophora racemosa* was identified using the standard reference text "Plants from the Niger Delta" by Prof. B.L. Nyananyo.

Preparation of extracts

The aqueous extracts were obtained using cold maceration. The plant materials were first washed, air-dried in shade, and ground into a powder. For each sample, 100 g of powder was steeped in 1 liter of distilled water for 72 hours, with occasional stirring. Following maceration, the mixture was filtered using Whatman No. 1 paper. The resulting filtrate was then concentrated via evaporation on a water bath maintained at 60°C. The obtained crude extracts were subsequently refrigerated at 4°C until required for use.

Experimental Design

The rats (N=54) were randomly assigned to one of nine groups (n=6) for a 28-day intervention. The groups included a normal control, a petrol fume-exposed negative control, six treatment groups that received petrol fumes plus one of three plant extracts at two doses (200 or 400 mg/kg), and a standard control group that received petrol fumes and Vitamin E (200 mg/kg). All treatments were constituted with distilled water and administered orally by gavage. Food and water were provided *ad libitum*.

Exposure of Animals to Petrol Fumes

Petrol fume exposure was achieved via inhalation in a custom-built chamber (150x90x210 cm) for 4 hours daily over 28 days, adapting the protocol from Uboh et al. (2005) and Owagboriaye et al. (2016). Vapor saturation was ensured by placing 1 liter of petrol (in two 500 ml aliquots) inside the chamber 60 minutes before the rats were introduced. After daily exposure, all animals were returned to a fresh-air holding area.

Collection of Samples

At the end of the 28-day treatment period, rats were anesthetized with urethane and euthanized. Sperm samples were then collected from the epididymis for analysis. The testes were collected for oxidative stress markers assay and histology.

Sperm Analysis

Sperm count and motility were determined following the method previously outlined by Bearden and Fuquay (2000) while sperm viability and morphology were assayed following the method by Kvist and Bjorndahl (2002).

Determination of oxidative stress Parameters

A 10% homogenate of the testes was prepared and used to determine oxidative stress parameters. The activities of the antioxidant enzymes superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) were determined according to Marklund and Marklund (1974), Cohen et al. (1970), and Lawrence and Burk (1976), respectively. GPx activity was measured using a coupled assay system with glutathione reductase (GR). Lipid

peroxidation was assessed using the method of Armstrong and Al-Awadi (1991).

Statistical Analysis

Data was subjected to one-way analysis of variance (ANOVA) followed by Tukey’s test (SPSS Version 25.0, IBM, USA). Differences

were considered statistically significant at a confidence level of $p < 0.05$.

The data were analyzed using a one-way ANOVA with Tukey's post-hoc test in SPSS software (Version 25.0, IBM, USA). A result was deemed statistically significant when the p -value was less than 0.05.

Results

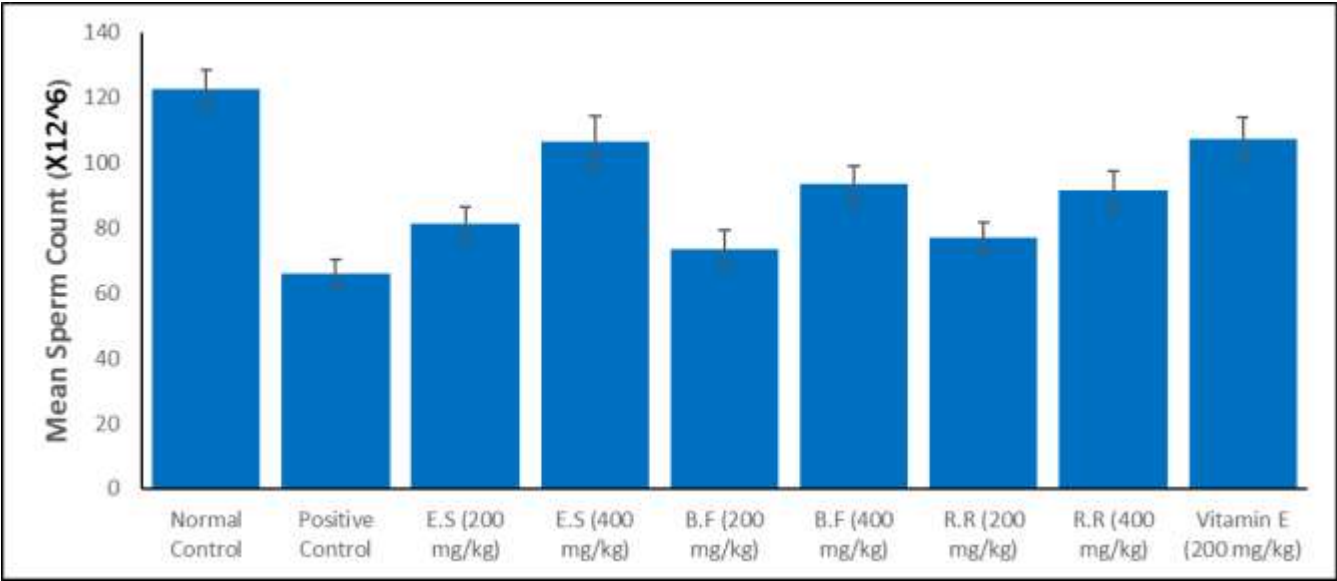


Figure 1. Sperm count in male Wistar rats exposed to petrol fumes and treated with aqueous extracts of *Emilia sonchifolia* (E.S), *Bridelia ferruginea* (B.F), or *Rhizophora racemosa* (R.R).

Values represent the mean $\pm$ standard deviation (n=6). A p -value less than 0.05 was considered statistically significant.

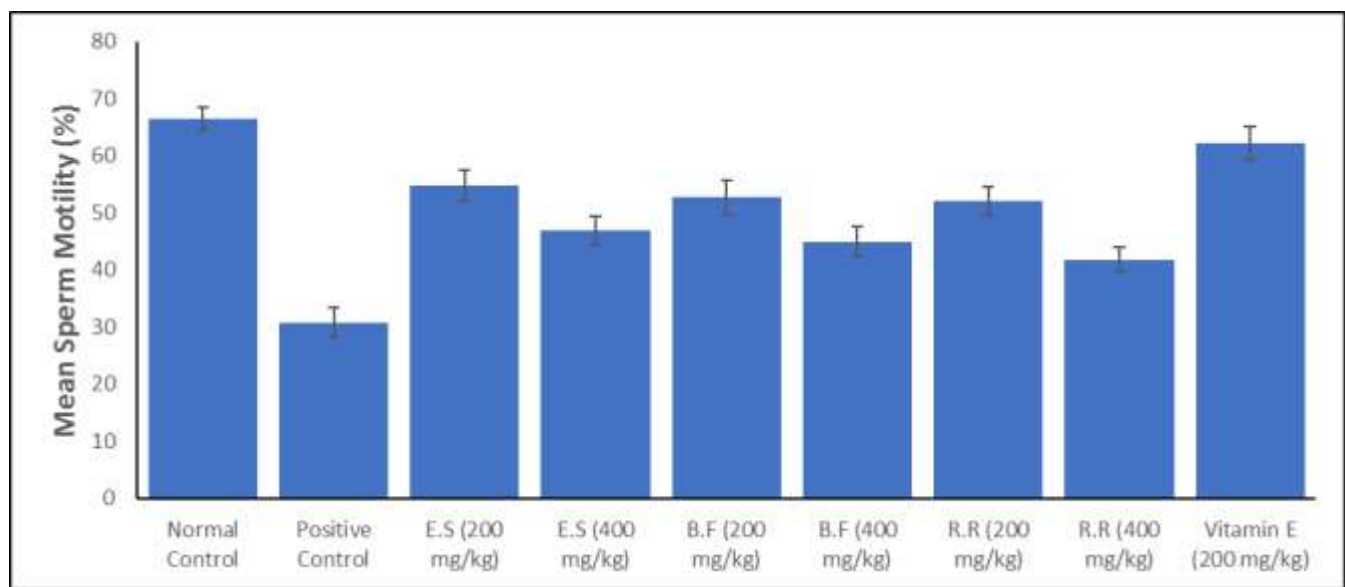


Figure 2. Sperm motility in male Wistar rats exposed to petrol fumes and treated with aqueous extracts of *Emilia sonchifolia* (E.S), *Bridelia ferruginea* (B.F), or *Rhizophora racemosa* (R.R).

Values represent the mean $\pm$ standard deviation (n=6). A *p*-value less than 0.05 was considered statistically significant.

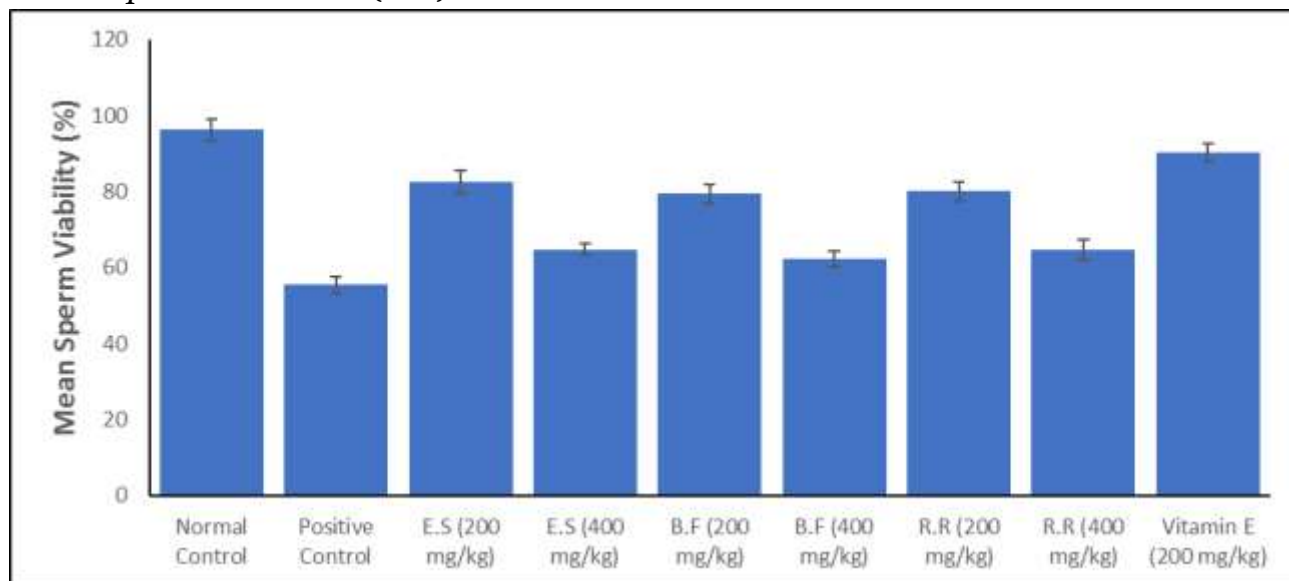


Figure 3. Sperm viability in male Wistar rats exposed to petrol fumes and treated with

aqueous extracts of *Emilia sonchifolia* (E.S), *Bridelia ferruginea* (B.F), or *Rhizophora racemosa* (R.R).

Values represent the mean $\pm$ standard deviation (n=6). A *p*-value less than 0.05 was considered statistically significant.

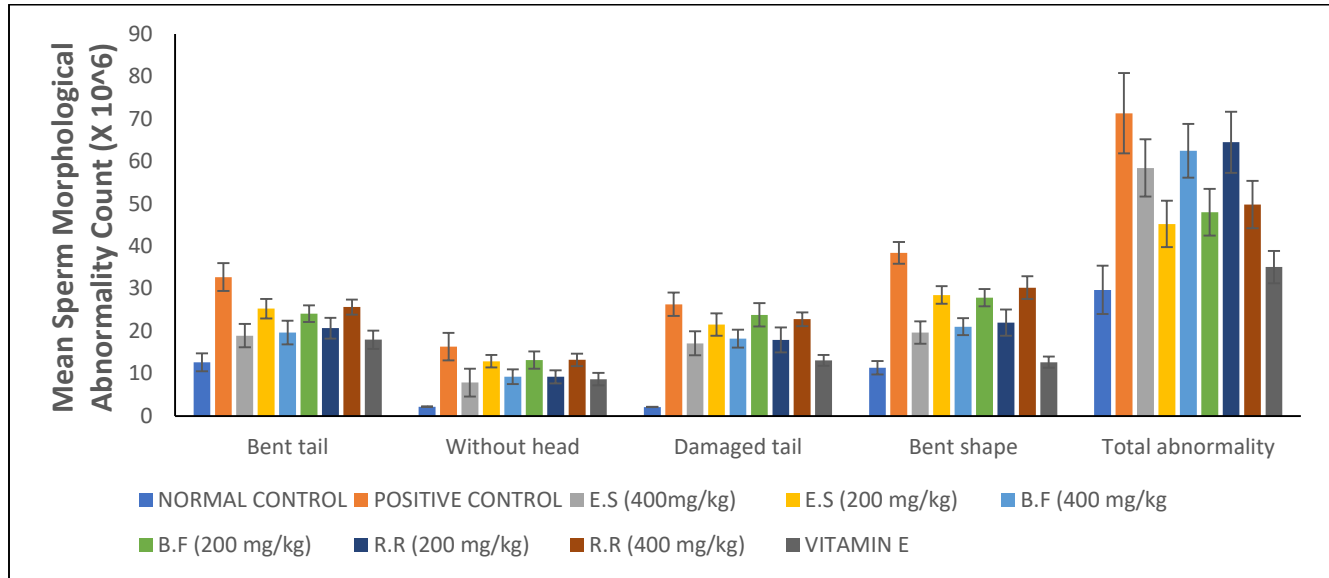


Figure 4. Sperm morphology in male Wistar rats exposed to petrol fumes and treated with aqueous extracts of *Emilia sonchifolia* (E.S), *Bridelia ferruginea* (B.F), or *Rhizophora racemosa* (R.R).

Values represent the mean $\pm$ standard deviation (n=6). A *p*-value less than 0.05 was considered statistically significant.

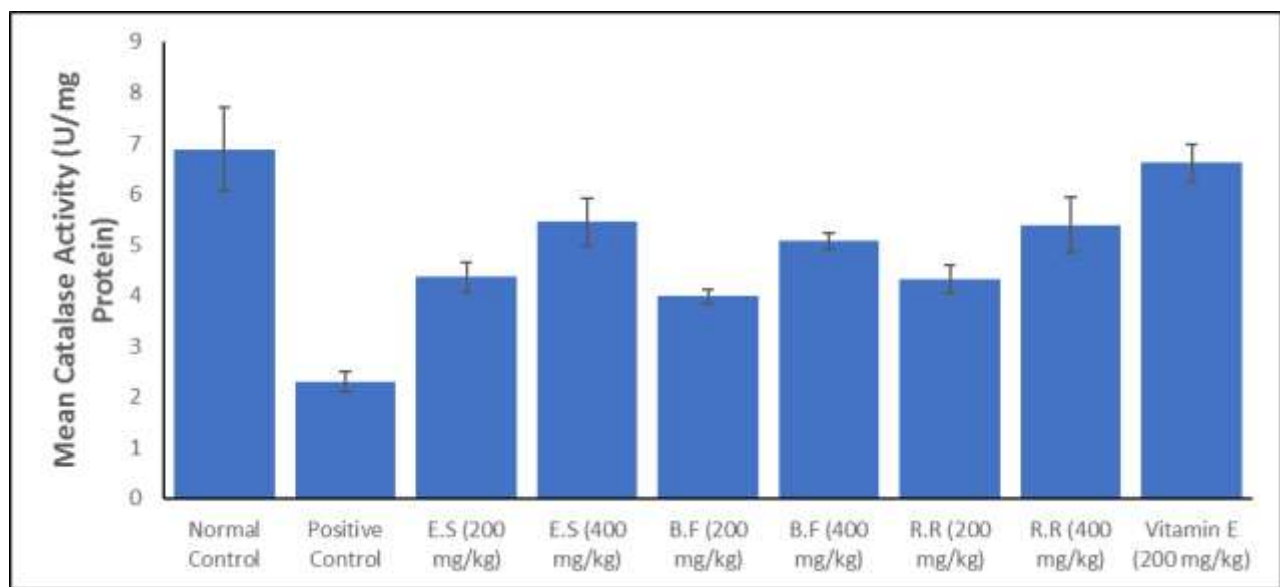


Figure 5. Catalase activity in male Wistar rats exposed to petrol fumes and treated with aqueous extracts of *Emilia sonchifolia* (E.S), *Bridelia ferruginea* (B.F), or *Rhizophora racemosa* (R.R). Values represent the mean $\pm$ standard deviation (n=6). A *p*-value less than 0.05 was considered statistically significant.

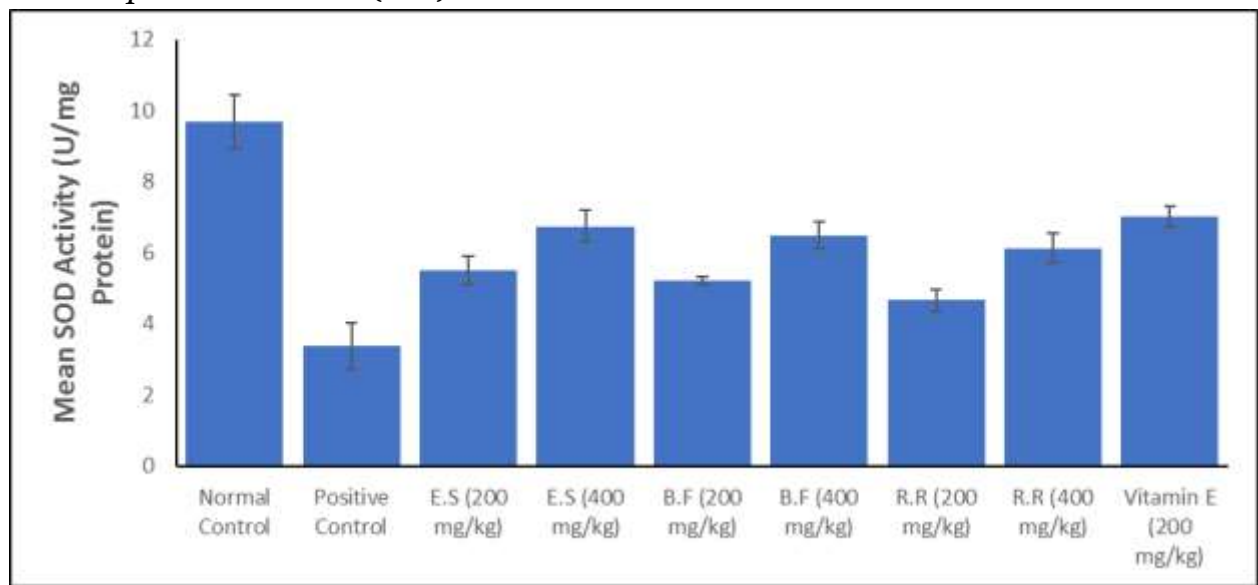


Figure 6. SOD activity in male Wistar rats exposed to petrol fumes and treated with

aqueous extracts of *Emilia sonchifolia* (E.S), *Bridelia ferruginea* (B.F), or *Rhizophora racemosa* (R.R).

Values represent the mean $\pm$ standard deviation (n=6). A *p*-value less than 0.05 was considered statistically significant.

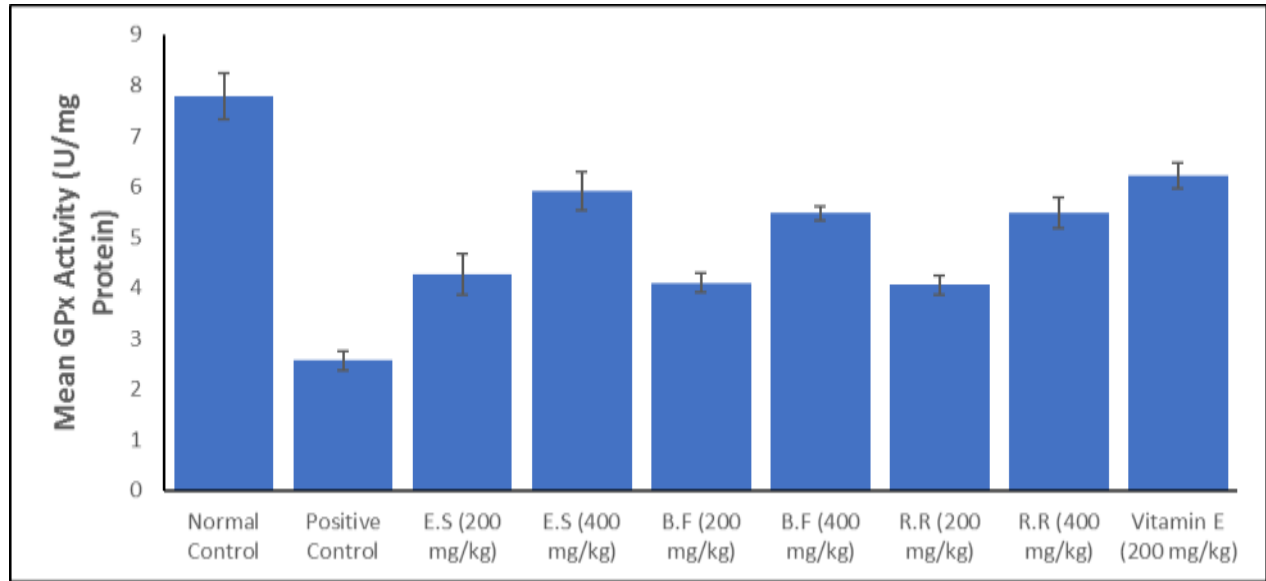


Figure 7. GPx activity in male Wistar rats exposed to petrol fumes and treated with aqueous extracts of *Emilia sonchifolia* (E.S), *Bridelia ferruginea* (B.F), or *Rhizophora racemosa* (R.R).

Values represent the mean $\pm$ standard deviation (n=6). A *p*-value less than 0.05 was considered statistically significant.

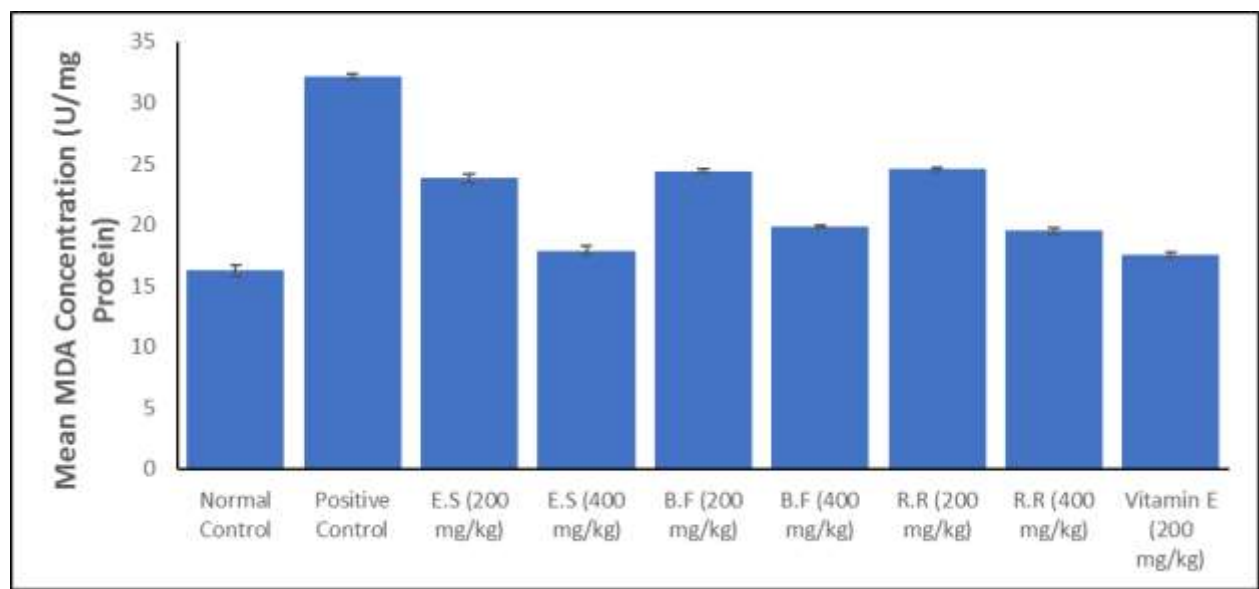


Figure 8. MDA concentration in male Wistar rats exposed to petrol fumes and treated with aqueous extracts of *Emilia sonchifolia* (E.S), *Bridelia ferruginea* (B.F), or *Rhizophora racemosa* (R.R).

Histology

Values represent the mean $\pm$ standard deviation (n=6). A *p*-value less than 0.05 was considered statistically significant.

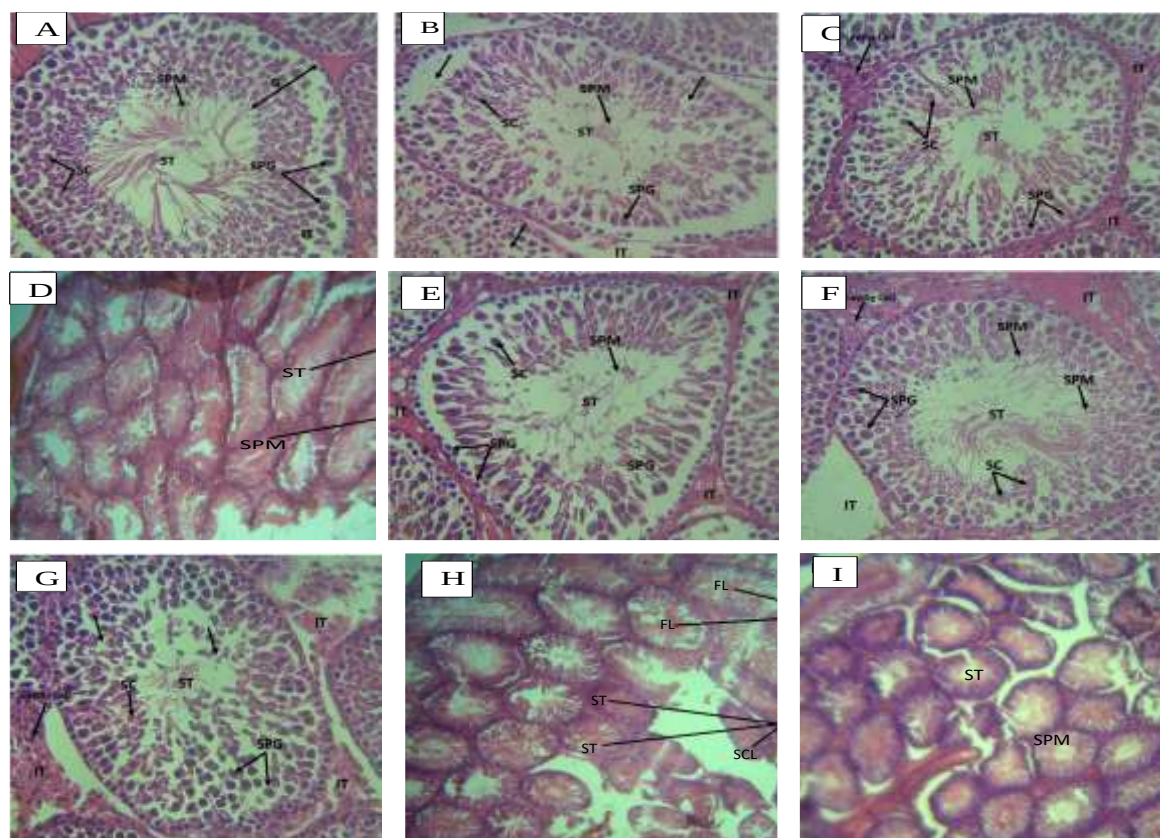


Figure 9 shows the Photomicrographs of hematoxylin and eosin-stained histological sections of testes of controls and normal rats administered aqueous leaf extracts of *Emilia sonchifolia* and *Bridelia ferruginea*, and aqueous stem-bark extract of *Rhizophora racemosa*, following exposure to petrol fumes for 28 days.

A (Normal Control): Photomicrograph (H&E X400) of testis showing moderate thickness of the germinal epithelia (G) associated with Sertoli cells (SC) and Spermatids (SPM) (thread like strands) (arrows). Normal features of the testicular tissue indicated. **B** (Positive Control): Photomicrograph (H&E X400) of the testis with reduced thickness associated with diffused

vacuolation of the germinal epithelia and cell loss (arrows). Severe distortion and hypospermatogenesis of the testicular tissue indicated. **C** (Petrol + 200 mg/kg *Emilia Sonchifolia*): Photomicrograph (H&E X 400) of the testes showing the (ST=seminiferous tubules, SC=Sertoli cells, SPM=Spermatids, SPG=Spermatogonia): with reduced thickness and vacuolation of germinal epithelial associated with germ cells loss and hypospermatogenesis (arrows). Moderate distortion of the testicular tissue indicated. **D** (Petrol + 400 mg/kg *Emilia sonchifolia*): Photomicrograph (H&E X 400) of testes tissue showing distinct spermatids cell layers in seminiferous tubules. Normal tissue

microstructure indicated. **E** (Petrol + 200 mg/kg *Bridelia ferruginea*): Photomicrograph (H&E X 400) of the testes showing the interstitial tissue (IT) with mild vacuolation of the germinal epithelia lining the seminiferous tubules associated with reduced spermatids (arrows). Mild distortion of the germinal epithelia of the testis indicated. **F** (Petrol + 400 mg/kg *Bridelia ferruginea*): Photomicrograph (H&E X 400) of the testes showing focal germinal epithelia distortion with mild enlarged Sertoli cells (arrows). Mild germinal epithelia distortion of the testicular tissue indicated. **G** (Petrol +200 mg/kg *Rhizophora racemosa*): Photomicrograph (H&E X400) of the testes showing mild vacuolation of the germinal epithelia and cell loss (arrows). Mild germinal epithelia distortion of the testicular tissue indicated. **H** (Petrol + 400 mg/kg *Rhizophora racemosa*): Photomicrograph (H&E X 400) of testes tissue showing pockets of normal spermatid cell layers and well flagellated lumens of seminiferous tubules. The interstitial cells of Leydig appear normal. **I** (Petrol + 200 mg/kg Vitamin E): Photomicrograph (H&E X 400) of testes tissue showing seminiferous tubules, spermiogenic cell layers (spermatogonia and spermatids) and flagellated lumen. Tissue microstructure appears normal.

Discussion

Findings from this study demonstrates that sub-chronic exposure (28 days) to petrol fumes significantly impairs sperm quality and testicular antioxidant status in male Wistar rats. The observed decline in sperm count, motility, and

viability, coupled with an increase in morphological abnormalities, confirms the reproductive toxicity of petrol volatile organic compounds (VOCs) like benzene, toluene, and xylene. These findings align with established literature linking petroleum hydrocarbon exposure to male infertility through mechanisms of oxidative stress (Peña-García et al., 2024; Madhu et al., 2022). The toxicity was further evidenced by a significant depletion in the activities of key testicular antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and a concurrent rise in malondialdehyde (MDA), a marker of lipid peroxidation (Kirsch et al., 2022; Ghahri et al., 2024). This compromised antioxidant defense likely leads to reactive oxygen species (ROS)-mediated damage to sperm membranes, DNA, and mitochondrial function, ultimately disrupting spermatogenesis (Wang et al., 2025; Muñoz et al., 2024).

The core finding of this research is the significant, dose-dependent amelioration of this petrol-induced damage by the aqueous extracts of *Emilia sonchifolia*, *Bridelia ferruginea*, and *Rhizophora racemosa*. Administration of these extracts at 200 and 400 mg/kg effectively restored sperm parameters towards normal levels and rebalanced the testicular oxidative state. The 400 mg/kg dose consistently produced a more pronounced restorative effect than the 200 mg/kg dose across all measured parameters, indicating a clear dose-response relationship. This superior efficacy at higher concentrations suggests that the therapeutic impact is directly

related to the bioavailability of bioactive phytoconstituents.

The protective mechanisms are likely multifactorial, centering on the potent antioxidant and anti-inflammatory properties of the phytochemicals abundant in these plants, such as flavonoids, tannins, saponins, and alkaloids (Ramgir et al., 2022; Zohora et al., 2024). These compounds can directly scavenge ROS, inhibit lipid peroxidation, as evidenced by the reduction in MDA levels, and enhance the endogenous antioxidant defense system by upregulating the activities of SOD, CAT, and GPx (Lopes-Ferreira et al., 2025; Rodríguez-Negrete et al., 2024, Ashraf et al., 2024). By preserving mitochondrial integrity and sperm membrane fluidity, these actions directly support sperm motility and viability (Ozoani et al., 2024). Furthermore, the restoration of normal spermatogenesis and sperm morphology implies these extracts may also protect or repair spermatogenic DNA and support the testicular microarchitecture.

The positive control, vitamin E (200 mg/kg), a standard lipid-soluble antioxidant, provided a similar spectrum of protection, significantly reversing petrol-induced damage to sperm quality and oxidative markers (Sengupta et al., 2024; Hajibabaei, 2016). The comparable efficacy between the high-dose plant extracts (400 mg/kg) and vitamin E strongly suggests that the primary mode of action for these botanicals involves robust antioxidant pathways, potentially coupled with anti-inflammatory and hormone-modulating effects (Simón & Mariotti-

Celis, 2024). This positions the extracts of *E. sonchifolia*, *B. ferruginea*, and *R. racemosa* as promising natural therapeutic agents or adjuncts for mitigating chemical-induced oxidative stress in the male reproductive system.

Petrol fumes caused significant testicular damage in rats, characterized by thinned germinal epithelium, vacuolation, and germ cell loss. Treatment with plant extracts and Vitamin E showed varying degrees of protective effect. *Emilia sonchifolia* (400 mg/kg) and *Rhizophora racemosa* (400 mg/kg) promoted strong histological recovery, with organized spermatogenic layers. *Bridelia ferruginea* provided only a mild protective effect at both doses. Normal and Vitamin E groups showed healthy, intact testicular architecture. The results correlate with the known antioxidant properties of the treatments, which counteract the oxidative stress induced by petrol fumes.

Conclusion

The aqueous leaf extracts of *Emilia sonchifolia*, *Bridelia ferruginea*, and *Rhizophora racemosa* stem --bark possess significant protective effects against petrol fume-induced sperm quality impairment and testicular oxidative stress in rats. Their efficacy, which is dose-dependent and comparable to vitamin E, is largely attributed to their rich phytochemical composition and resultant antioxidant activity. These findings provide a scientific basis for the ethnobotanical use of these plants in supporting male reproductive health and warrant further

investigation into their specific active compounds and clinical potential.

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Conflict of Interest

We declare that there is no conflict of interest.

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