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Ameliorative Potential of Alcohol Extract of *Ficus exasperata* Leaf on Ethanol-Induced Ovarian Toxicity in Female Wistar Rats

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Abstract:

- ***Background:***

Chronic ethanol consumption is a known risk factor for female reproductive toxicity, primarily mediated through oxidative stress. *Ficus exasperata* (Fe), a medicinal plant, possesses documented antioxidant properties, but its protective role against ovarian damage is underexplored.

- ***Objective:***

This study evaluated the protective effects of an alcohol extract of *Ficus exasperata* leaves (AEFE) against ethanol-induced ovarian damage.

- ***Methods:***

Twenty adult female Wistar rats were randomly divided into four groups (n=5): Control (distilled water), Fe-only (500 mg/kg AEFE), Ethanol-only (10.14 ml/kg of 40% ethanol), and Fe+Ethanol (co-administration). Treatments were administered orally for two weeks. Body and ovarian weights were recorded. Oxidative stress was assessed via serum malondialdehyde (MDA) levels. Ovarian histoarchitecture was evaluated using hematoxylin and eosin staining.

- ***Results:***

The Ethanol-only group exhibited significant histological damage, including severe follicular atresia and distorted ovarian architecture, alongside a marked elevation in serum MDA levels (25.58 ± 0.27 nmol/ml) compared to control (17.23 ± 0.67 nmol/ml, $p<0.05$). Co-administration with AEFE (Fe+Ethanol group) resulted in notable ovarian recovery, evidenced by active follicular growth and restored histoarchitecture. This group also showed a significant reduction in MDA levels (19.45 ± 1.82 nmol/ml) compared to the Ethanol-only group. The Fe-only group displayed normal ovarian histology and the lowest MDA levels. No statistically significant changes were observed in body or absolute ovarian weights across groups.

- ***Conclusion:***

The alcohol extract of *Ficus exasperata* leaves demonstrated significant protective and restorative effects against ethanol-induced ovarian toxicity in rats, potentially mediated through the attenuation of oxidative stress. These findings suggest AEFE as a promising candidate for mitigating alcohol-related ovarian damage.

Keywords: *Alcohol, Ficus exasperata, Malondialdehyde, Ovarian Toxicity, Oxidative Stress, Wistar Rats.*

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I. INTRODUCTION

The global burden of alcohol consumption extends to reproductive health, with chronic intake linked to hormonal imbalance, ovarian dysfunction, and subfertility [1,2]. The primary pathophysiological mechanism is oxidative stress [2], where an imbalance between reactive oxygen species (ROS) and antioxidant defenses leads to lipid peroxidation, protein damage, and cellular apoptosis in ovarian tissues [3]. Biomarkers like malondialdehyde (MDA) are reliable indicators of such oxidative lipid damage [4].

In many cultures, including Nigeria, the use of medicinal plants to manage various ailments is prevalent. *Ficus exasperata* (sandpaper tree), a member of the *Moraceae* family, is widely used in traditional African medicine for its anti-inflammatory, antimicrobial, and antioxidant properties [5, 6]. Phytochemical studies reveal the presence of flavonoids, tannins, and phenolics, which are potent free radical scavengers [7]. This is similar to beniseed (*Sesame* seed) that has been previously investigated [8]. While its systemic antioxidant effects are documented, its specific role in protecting the ovary from ethanol-induced oxidative injury remains largely unexplored. This study, therefore, investigated the potential of an alcohol extract of *Ficus exasperata* leaves (AEFE) to mitigate ethanol-induced ovarian damage in female Wistar rats, using histological and biochemical (MDA) parameters as key endpoints.

II. MATERIALS AND METHODS

➢ *Plant Material and Extraction*

Fresh leaves of *Ficus exasperata* were collected in Ogbomoso, Nigeria, and authenticated at the Department of Biology, LAUTECH (Voucher No: LHO 883). Leaves were air-dried, pulverized, and macerated in 40% ethanol for 72 hours. The filtrate was concentrated using evaporation at room temperature to obtain the crude alcohol extract (AEFE).

➢ *Experimental Animals and Design*

Twenty (20) healthy female Wistar rats (100-120g) were obtained and acclimatized for two weeks under standard conditions (12h light/dark cycle, *ad libitum* access to feed and water). All procedures followed institutional guidelines for animal care and use. The rats were randomly assigned into four groups (n=5):

- Group A (Control): Received distilled water.
- Group B (Fe-only): Received 500 mg/kg b.w. of AEFE.
- Group C (Ethanol-only): Received 10.14 ml/kg b.w. of 40% ethanol.
- Group D (Fe+Ethanol): Received both AEFE (500 mg/kg) and ethanol (10.14 ml/kg).

All treatments were administered orally via cannula once daily for 14 consecutive days.

➢ *Sample Collection and Processing*

On day 15, animals were sacrificed via cervical dislocation under mild anesthesia. Blood was collected via cardiac puncture, allowed to clot, and centrifuged to obtain serum for MDA analysis. Both ovaries were carefully dissected out, cleared of fat, and weighed. The right ovary from each rat was fixed in Bouin's fluid for histology.

➢ *Biochemical Assay*

Serum malondialdehyde (MDA) concentration, a marker of lipid peroxidation, was determined using the thiobarbituric acid reactive substances (TBARS) assay as described by Ohkawa *et al.*, [9]. Absorbance was read at 532 nm.

➢ *Histological Examination*

Fixed ovarian tissues were processed routinely, embedded in paraffin, sectioned at 5µm, and stained with Hematoxylin and Eosin (H&E). Slides were examined under a light microscope by an observer blinded to the treatment groups to assess follicular development, atresia, and general histoarchitecture.

➢ *Statistical Analysis*

Data are presented as Mean ± Standard Error of Mean (SEM). Statistical analysis was performed using GraphPad Prism software (Version 7.0). One-way Analysis of Variance (ANOVA) followed by an appropriate post-hoc test was used for inter-group comparisons. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

III. RESULTS

➢ *Body and Ovarian Weights*

There was a non-significant ($p>0.05$) increase in final body weights across all groups compared to their initial weights. Absolute ovarian weights and ovarian somatic indices showed no statistically significant differences among the groups (Table 1).

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Table 1: Effects of AEFE and Ethanol on Body and Ovarian Weights

Group	Initial BW (g)	Final BW (g)	Ovarian Weight (g)	Ovarian Somatic Index (%)
Control	64.80 ± 2.38	80.20 ± 3.43	0.084 ± 0.019	0.105 ± 0.024
Fe-only	92.40 ± 1.63	106.2 ± 3.53	0.174 ± 0.054	0.164 ± 0.051
Ethanol-only	82.60 ± 3.50	98.80 ± 7.07	0.164 ± 0.034	0.141 ± 0.046
Fe+Ethanol	104.0 ± 3.15	115.8 ± 6.15	0.130 ± 0.017	0.112 ± 0.015

Data: Mean ± SEM; n=5. No significant differences ($p>0.05$) were found between groups for any parameter.

➢ *Oxidative Stress Marker (Serum MDA)*

The Ethanol-only group (C) showed a significant increase ($p<0.05$) in serum MDA levels (25.58 ± 0.27 nmol/ml) compared to the Control group (17.23 ± 0.67 nmol/ml). Co-treatment with AEFE (Group D) significantly reduced MDA levels (19.45 ± 1.82 nmol/ml) compared to the Ethanol-only group. The Fe-only group (B) had the lowest MDA level (15.35 ± 0.13 nmol/ml) (Figure 1).

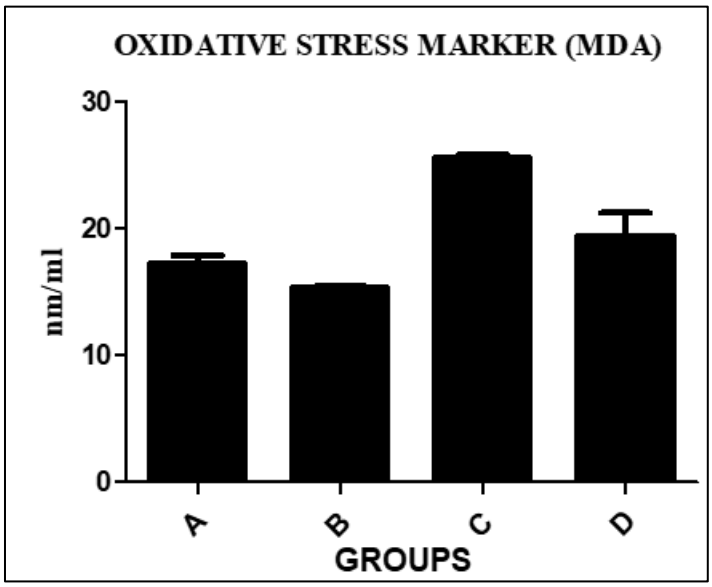

Fig 1: Serum Malondialdehyde (MDA) Levels. Data: Mean ± SEM; n=5. ($p < 0.05$ vs. Control; $p < 0.05$ vs. Ethanol-only).

➢ *Histological Findings*

- Group A (Control): Exhibited normal ovarian architecture with developing follicles at various stages and occasional atretic follicles (Plate 1A).
- Group B (Fe-only): Showed apparently normal histology with visible Graafian follicles, indicating that AEFE alone was not detrimental (Plate 1B).
- Group C (Ethanol-only): Revealed severe histological damage characterized by numerous atretic follicles, stromal distortion, and a marked absence of mature or developing follicles, indicating ethanol-induced ovarian degeneration (Plate 1C).
- Group D (Fe+Ethanol): Demonstrated significant histological recovery. The ovaries showed active follicular growth, developing antral follicles, and restored stromal organization, indicating the protective effect of AEFE (Plate 1D).

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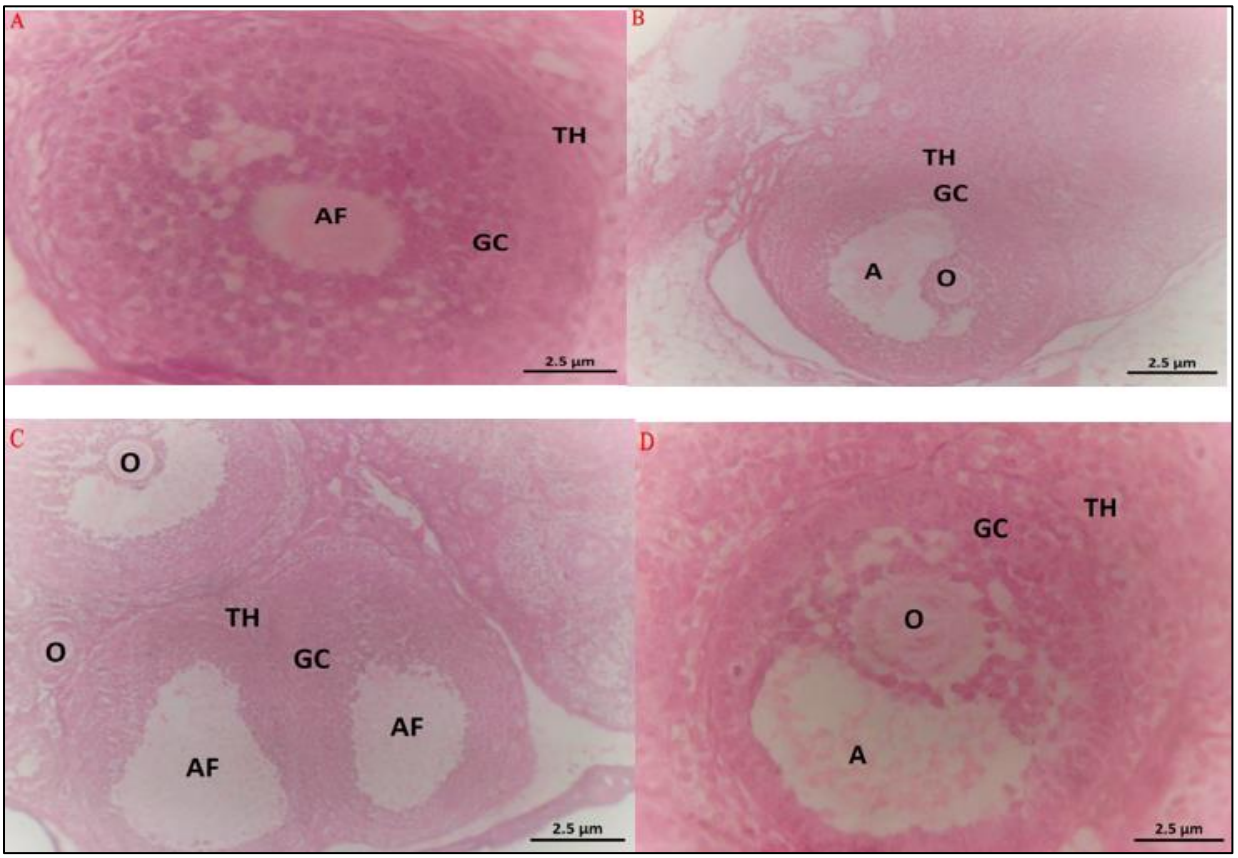

Plate 1: Photomicrographs of ovarian tissue (H&E, 100x). (A) Control (B) Fe-only (C) Ethanol-only (D) Fe+Ethanol. Where oocytes = O, granulosa cells = GC, atretic follicles = AF and theca cells = TH.

IV. DISCUSSION

This study demonstrates that co-administration of *Ficus exasperata* leaf extract (AEFE) ameliorates the deleterious effects of chronic ethanol exposure on the ovary in Wistar rats. The key findings—increased oxidative stress and severe histological damage in the ethanol group, which were significantly reversed by AEFE—strongly support the plant's protective role.

The significant elevation of serum MDA in the Ethanol-only group aligns with established literature that ethanol metabolism exacerbates ROS production, leading to lipid peroxidation and cellular damage in reproductive organs [2, 10]. The reduction in MDA levels in the co-treated group underscores the potent antioxidant capacity of AEFE, likely attributable to its rich content of flavonoids and phenolic compounds [7]. These phytochemicals can donate electrons to neutralize free radicals, thereby preserving membrane integrity and cellular function.

The histological observations provide direct morphological evidence of protection. The follicular atresia and architectural distortion caused by ethanol are hallmarks of oxidative stress-induced apoptosis in granulosa cells [11]. The restoration of follicular development and stromal organization in the Fe+Ethanol group suggests that AEFE not only prevented oxidative damage but also supported ovarian recovery processes. This is consistent with studies on other antioxidant-rich plant extracts showing similar protective effects on ovarian tissue [12].

The lack of significant change in body and ovarian weights, despite clear histological and biochemical changes, suggests that the 14-day exposure period might have been insufficient to manifest in gross morphometric alterations or that the primary insult of ethanol at this dose is functional and micro-architectural rather than grossly trophic.

V. CONCLUSION AND RECOMMENDATIONS

In conclusion, the alcohol extract of *Ficus exasperata* leaves exhibits significant protective effects against ethanol-induced ovarian toxicity in rats, potentially mediated through the attenuation of oxidative stress. These findings validate the ethnomedicinal use of F. exasperata and highlight its potential as a natural adjuvant for mitigating alcohol-related reproductive harm.

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Based on these findings, we recommend:

- Further studies to isolate and characterize the specific bioactive compounds in AEFE responsible for the observed effect.
- Investigations into the precise molecular mechanisms, including effects on antioxidant enzymes (SOD, CAT, GSH) and apoptosis pathways (e.g., Bcl-2, Bax).
- Long-term studies to evaluate the impact on fertility outcomes, such as hormonal profiles and litter size.
- Toxicity profiling to establish a safety margin for potential therapeutic applications.

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➢ *Conflicts of Interest*

The authors declare no conflicts of interest.

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