



Effect of Ethyl Acetate Fraction of *Terminalia ivorensis* on Ethanol Induced Infertility in Male Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors UOC and ACA developed the design of the study. Authors ACC and OOR performed the statistical analysis. Author UOC developed the first draft of the manuscript. Authors FKA, ILO, NCK, UCD and OCG performed the analysis of the study. Author UCC and UOP contributed to the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: This research investigates the Effect of Ethyl Acetate Fraction of *Terminalia ivorensis* on Ethanol Induced Infertility in Male Rats. Infertility is the inability to conceive after one year of unprotected intercourse for couples under 35, or after six months for those over 35. It affects both men and women, with various factors contributing to the condition. *Terminalia ivorensis* has been claimed from traditional healers for the treatment of infertility hence the study.

Method: Quantitative phytochemical analysis, toxicity test and other biochemical analysis was carried out using standard method.

Results: Seven hundred grammes (700 g) of dried crude sample of Ethyl acetate Fraction of *Terminalia ivorensis* leaves powder gave a percentage yield of 6.28%. The study revealed that flavonoids, tannins, and phenols were present in high concentration, alkaloids, terpenoids and glycosides were present in moderate concentration, whereas saponins, steroids and hydrogen cyanide were present in low concentration. The toxicity result showed that the extract was not toxic even at the highest dosage (5000 mg/kg body weight) administered, indicating that the extract is safe for consumption. The results shown in the Table 3 indicates that groups treated with 200mg/kg, 400mg/kg and 600mg/kg body weight of extract significantly ($P < 0.05$) increased in testosterone level when compared to positive control; whereas groups treated with 200mg/kg, 400mg/kg and 600mg/kg body weight of extract showed significantly ($P < 0.05$) higher in LH and FSH when compared with positive control. The results in the Table 3 showed that groups treated with 200mg/kg, 400mg/kg and 600mg/kg body weight of extract significantly ($P < 0.05$) decreases in sluggish, and dead sperm level when compared with positive control whereas groups treated with 200mg/kg, 400mg/kg and 600mg/kg body weight significantly ($P < 0.05$) increased active, sperm count and normal sperm when compared with positive control.

Conclusion: The plant extract has ant infertility properties and can be recommend as an alternative therapy for infertility in male.

Keywords: Toxicity; infertility; alkaloids; sperm production.

1. INTRODUCTION

Infertility is a multifaceted health issue affecting men and women globally (Chaudhuri et al., 2022). Recent research has increasingly focused on the negative impact of alcohol, particularly ethanol, on reproductive health (De Angelis et al., 2020). Ethanol, a key ingredient in alcoholic drinks, has been linked to hormonal imbalances, reduced sperm production, and decreased fertility in men (Finelli et al., 2021). *Terminalia ivorensis* or Tasmanian blue gum, has a long history of use in traditional indigenous Australian medicine. It is known for its various pharmacological benefits, including anti-inflammatory, antioxidant, and antimicrobial properties (Thoms et al., 2024). These characteristics make *Terminalia ivorensis* a

promising candidate for exploring therapies that may help counteract the reproductive effects of ethanol. Research has indicated that extracts from *Terminalia ivorensis* may help reduce oxidative stress and inflammation (Moomin et al., 2020). This is particularly relevant in the context of ethanol-related infertility, as oxidative stress is a significant factor in reproductive dysfunction (Soni et al., 2022). However, there is still a lack of comprehensive understanding regarding the therapeutic potential of *Terminalia ivorensis* specifically for ethanol-induced infertility in male rats. This study aims to fill this gap by systematically examining the phytochemical components of *Terminalia ivorensis* leaf extracts and their effects on other parameters in albino rats exposed to ethanol. By rigorously investigating these factors, the research aspires

to establish a scientific foundation for considering *Terminalia ivorensis* as a potential treatment for reproductive issues linked to alcohol consumption.

2. METHODOLOGY

2.1 Materials

2.1.1 Plant materials

Fresh leaves of *Terminalia ivorensis* were collected from a location within the Enugu State University of Science and Technology community. Mr. Ozioko, A. E., a staff member of the Bioresources Development Conservation Program (BDPC) at the Research Centre in Nsukka, Enugu State, identified the plant. The leaves were then shade-dried and pulverized prior to analysis.

2.1.2 Animals

Thirty male rats were acquired and kept in the Animal House of the Faculty of Pharmaceutical Sciences Enugu State University of Science and Technology. The rats were acclimated for one week before the experiment began, living under a 12-hour light and dark cycle and having access to standard food and water ad libitum.

2.1.3 Equipment, chemicals and reagents

Analytical grades chemicals and reagents were used for the study and were purchased from reputable companies.

2.2 Methods

2.2.1 Extraction of plant materials

Extraction of plant material followed the protocol described by (Kumar et al., 2016), with slight modifications. Briefly, a weight of the pulverized leaves of *Terminalia ivorensis* (700g) was macerated in 4 liters of 70% ethanol using a maceration flask. The mixture was left for 72 hours with occasional stirring, after which it was filtered into a flat-bottomed flask using a muslin cloth. Further filtration was achieved with the help of Whatman No. 1 filter paper so as to remove fine residues. The filtrates were concentrated using a rotary evaporator at 45°C to obtain the crude ethanol extract. The crude extract was fractionated with n-hexane, ethyl acetate and ethanol. The fractions were

completely dried under high vacuum on a rotary evaporator and stored in the refrigerator at 4°C for further analysis. The fraction with highest percentage yield of the extract was calculated.

2.2.2 Determination of the percentage yield of the ethyl acetate Fraction *Terminalia ivorensis* leaves

The percentage yield of the of Ethyl Acetate Fraction *Terminalia ivorensis* leaves was determined from the weight of the dried pulverized pulp before maceration and the weight of the extract after concentration following the formula below:

$$\text{Percentage Yield} = \frac{\text{Weight of extract}}{\text{Weight of dried pulverized plant}} \times 100$$

2.2.3 Acute toxicity study on ethyl acetate fraction *Terminalia ivorensis* leaves

The acute toxicity study or median lethal dose (LD₅₀) of the EAFTI was determined using the method of (Lorke, 1983) with little modification

2.2.4 Experimental design

Thirty adult male albino rats were distributed into six groups of four rats each, and treatment was done as follows: Group 1: Normal control (Not induced, not treated, normal saline only), Group2:Positive control 30% Ethanol induced rats at 7ml/kg body weight, Group 3: 30% Ethanol induced rats + 1.04 mg/kg body weight of the standard drug, Group 4: 30% Ethanol induced rats + 200 mg/kg body weight of the EAFTI, Group 5: 30% Ethanol induced rats + 400 mg/kg body weight of the EAFTI and Group 6: 30% Ethanol induced rats + 600 mg/kg body weight of the EAFTI

2.2.5 Determination of the effects of EAFTI on Hormonal assay

The levels of serum sex hormones: Testosterone, Follicle-stimulating hormone (FSH), and Luteinizing hormone (LH), was assayed using Accubind Kits based on the enzyme-linked immunosorbent assay (ELISA) technique (Behmanesh et al., 2019).

2.2.6 Determination of the effects of EAFTI on sperm analysis

The sperm analysis was done using the methods outlined by (Eliasson, 2010). The cauda

epididymis was squeezed to release the semen, then 1.5 ml of the semen from the epididymis was then diluted with warm 0.1 M phosphate buffered saline.

3. RESULTS

3.1 Quantitative Phytochemical Compositions of Ethyl Acetate Fraction of *Terminalia ivorensis*

Quantitative phytochemical analyses of EAFTI as depicted in Table 1 revealed that flavonoids, tannins, and phenols were present in high concentration, alkaloids, terpenoids and glycosides were present in moderate concentration, whereas saponins, steroids and hydrogen cyanide were present in low concentration.

Table 1. Quantitative Phytochemical Compositions of EFRTI

Phytochemical	Quantitative Remarks
Alkaloids	891.34±4.90
Glycosides	222.33±2.61
Terpenoids	592.71±5.58
Saponins	61±0.53
Steroids	48±0.05
Tannins	1124.95±24.28
Flavonoids	1378.89±47.13
Phenols	1069.62±5.72

3.2 Result of the Acute Toxicity Test of the Ethyl acetate Fraction of *Terminalia ivorensis*

The result in Table 2 showed that the extract was not toxic even at the highest dosage (5000 mg/kg body weight) administered, indicating that the extract is safe for consumption.

Table 2. Result of the Acute Toxicity Test of the Ethyl acetate Fraction of *Terminalia ivorensis*

	Doses (mg/kg)	Mortality
Phase 1	10mg/kg	0/3
	100mg/kg	0/3
	1000mg/kg	0/3
Phase 2	1600mg/kg	0/3
	2900mg/kg	0/3
	5000mg/kg	0/3

3.3 Effect Ethyl Acetate Fraction of *Terminalia ivorensis* on Hormonal Analysis

The results shown in the Table 3 indicates that groups treated with 200mg/kg, 400mg/kg and 600mg/kg body weight of extract significantly ($P < 0.05$) increased in testosterone level when compared to positive control; whereas groups treated with 200mg/kg, 400mg/kg and 600mg/kg body weight of extract showed significantly ($P < 0.05$) higher in LH and FSH when compared with positive control. However, in terms of comparison with standard drug, 200mg/kg, 400mg/kg and 600mg/kg body weight of extract showed significantly ($P < 0.05$) reduction when compared with standard drug.

The results depicted in Table 4 showed that group treated with group 200, 400 and 600 mg/kg b.w significantly ($P < 0.05$) increase activeness, normal sperm and sperm count when compared with positive control. However, group 200, 400 and 600 mg/kg b.w significantly ($P < 0.05$) decrease the percentage of dead sperm and sperm sluggish when compared with positive.

Table 3. Effect Ethyl Acetate Fraction of *Terminalia ivorensis* on Hormonal Analysis

GROUPS	TESTESTERONE (mol/l)	LH(Iu/l)	FSH(Iu/l)
GROUP 1	10.49 ±0.36 ^F	9.52 ±0.31 ^e	11.14 ±0.17 ^e
GROUP2	4.55 ±0.46 ^a	5.27 ±0.23 ^a	4.30 ± 0.24 ^a
GROUP3	8.85 ± 0.47 ^e	7.17 ±0.26 ^c	5.15 ± 0.87 ^b
GROUP4	7.04 ±0.35 ^c	8.17 ±0.26 ^d	10.17 ±0.12 ^e
GROUP5	6.44 ± 0.15 ^b	6.32 ±0.35 ^b	7.16 ±0.89 ^c
GROUP6	8.26±0.18 ^d	7.50 ±0.29 ^c	8.26 ±0.21 ^d

GROUP 1-NORMAL CONTROL, GROUP 2- POSITIVE CONTROL, GROUP 3 – STANDARD CONTROL, GROUP 4 – 200mg/kg EFRTI, GROUP 5 – 400mg/kg EFRTI, GROUP 6 – 600mg/kg EFRTI

Table 4. Effect ethyl acetate fraction of *Terminalia ivorensis* on sperm analysis

Groups	Active %	Sluggish %	Dead %	Normal %	Count%
GROUP1	55.26±0.57 ^f	20.36±0.21 ^c	35.12±0.19 ^e	67.30±0.28 ^f	93.12±0.08 ^f
GROUP2	15.58±0.33 ^a	45.51±0.29 ^f	55.26±0.27 ^f	20.30±0.18 ^a	50.94±0.64 ^a
GROUP3	45.76±0.79 ^d	17.32±0.09 ^a	30.13±0.08 ^d	55.20±0.07 ^e	74.94±0.77 ^d
GROUP4	25.10±0.70 ^b	19.14±0.13 ^b	22.38±0.12 ^c	26.00±0.51 ^b	55.44±0.60 ^b
GROUP5	35.38±0.46 ^c	22.73±0.17 ^d	19.32±0.28 ^b	34.77±0.67 ^c	68.34±0.25 ^c
GROUP6	48.70±.60 ^e	26.17±0.75 ^e	17.42±0.26 ^a	46.62±0.69 ^d	82.62±0.36 ^e

GROUP 1-NORMAL CONTROL, GROUP2- POSITIVE CONTROL, GROUP3 – STANDARD CONTROL, GROUP4 – 200mg/kg EFRTI, GROUP5 – 400mg/kg EFRTI, GROUP6 – 600mg/kg EFRTI

4. DISCUSSION

The ethyl acetate fraction contains active components including flavonoids, tannins, phenols, alkaloids, terpenoids, glycosides, saponins, steroids, and hydrogen cyanide. Animal studies indicate that moderate tannin intake can enhance testicular strength, increase semen volume, boost sperm concentration, and lower the percentage of abnormal or poorly motile sperm (Ahmed et al., 2021). Plant steroids, which vary due to their side chains, methyl groups, and carbon oxidation states in the tetracyclic core, have been linked to various health benefits, such as lowering cholesterol, and exhibiting antiviral, anticancer, immunomodulatory, and anti-inflammatory properties (Vikasari et al., 2024). These findings align with those of (Tali et al., 2022). Additionally, the acute toxicity (LD50) test on the ethyl acetate fraction of *Terminalia ivorensis* showed it was non-toxic at doses up to 5000 mg/kg body weight, indicating the leaf extract is safe for consumption in humans and animals, supporting earlier research (Zaza et al., 2018).

Testosterone is crucial for spermatogenesis, and results indicated a significant decrease in testosterone levels in both treated and untreated groups compared to the normal control group. Similar results were noted by (Rahayu et al., 2019; Almeida et al., 2017) found a correlation between lower testosterone levels and reductions in sperm count, motility, and semen quality, along with an increase in sperm abnormalities (Anderson et al., 2022) suggested that decreased testosterone levels might result from heightened activity of testosterone reductase, which accelerates testosterone breakdown. He noted that the testes contain enzymes essential for testosterone production, which play a role for alcohol metabolism.

5. CONCLUSION

The ethyl acetate fraction of *Terminalia ivorensis* shows promising potential to improve infertility when given orally in moderate doses to experimental rats. Thus, this plant could be considered a therapeutic option for individuals experiencing infertility, in addition to its other benefits.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image

generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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