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

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 showed that groups treated with 200mg/kg,400mg/kg and 600kg/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.

BACKGROUND OF STUDY: Infertility is a multifaceted health issue affecting men and women globally [1]. Recent research has increasingly focused on the negative impact of alcohol, particularly ethanol, on reproductive health [2]. Ethanol, a key ingredient in alcoholic drinks, has been linked to hormonal imbalances, reduced sperm production, and decreased fertility in men [3]. 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 [4]. 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 [5]. This is particularly relevant in the context of ethanol-related infertility, as oxidative stress is a significant factor in reproductive dysfunction [6]. 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.

2.1.1 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 (BDCP) 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.

Methods

2.2 Extraction of Plant Materials

Extraction of plant material followed the protocol described by [7], 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 45^0C 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 4^0C for further analysis. The fraction with highest percentage yield of the extract was calculated.

2.2.1 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:

$$\text{Percentage Yield} = \frac{\text{Weight of extract}}{X} \times 100$$

Weight of dried pulverized plant

2.2.3 Acute Toxicity Study on Ethyl Acetate Fraction *Terminalia ivorensis* leaves

The acute toxicity study or median lethal dose (LD_{50}) of the EAFTI was determined using the method of [8] 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**

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 [9].

Determination of the effects of EAFTI on Sperm Analysis: The sperm analysis was done using the methods outlined by [10]. 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.

Results

3.2. 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.

Table1: 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

3.3. Result of the Acute Toxicity Test of the Ethyl acetate Fraction of *Terminalia ivorensis*The result in Table2 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

3.4. 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 comparism 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.

Table3: 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,
GROUP2- POSITIVE CONTROL
GROUP3 – STANDARD CONTROL
GROUP4 – 200mg/kg EFRTI
GROUP5 – 400mg/kg EFRTI
GROUP6 – 600mg/kg EFRTI

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.

Table4: 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	$48.70\pm.60^{e}$	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.

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 [11]. 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 [12]. These findings align with those of [13]. 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 [14].

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 [15]. [16] found a correlation between lower testosterone levels and reductions in sperm count, motility, and semen quality, along with an increase in sperm abnormalities. [17] 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 metabolization**.**

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

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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