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RESEARCH PAPER

EFFECTS OF ETHANOLIC LEAF EXTRACT OF *Ficus mucoso* ON HAEMATOLOGICAL PARAMETERS AND MALE FERTILITY OF THE ALBINO WISTAR RATS

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ABSTRACT

This 21-day study investigated the effects of ethanolic leaf extract of *Ficus mucoso* on haematological parameters and male fertility in albino Wistar rat. The extract was subjected to phytochemical screening tests prior to its administration to three groups of fifteen matured male rats, respectively. Groups 2 and 3 received 500 mg/kg and 200 mg/kg body weight of *Ficus mucoso* leaf extract, respectively, while Group 1 (Control) received 0.9% physiological saline. After the treatment period, the rats were sacrificed for blood/semen sample collection and laboratory analysis. Haematological parameters were assessed using an automated cell counter, while sperm morphology, motility, and count were analyzed following standard protocols. Phytochemical screening results verified the presence of alkaloids, tannins, phenols, saponins and flavonoids in the *Ficus mucoso* extract, while results from laboratory analysis indicated that there was no significant difference in the hematological parameters across all the groups. However, there were adverse effects on sperm morphology, count, and motility across the test groups compared to control, suggesting potential reproductive toxicity; hence the need for further studies to elucidate the underlying mechanisms, long-term effects, and potential reversibility of these reproductive alterations.

Keywords: *Ficus mucoso*, haematological parameters, male fertility, Wistar rats

INTRODUCTION

The medicinal value of any plant lies in some chemical substances that produce a definite physiological action on the human body, generally known as metabolites (Lattanzio *et al.*, 2009). From antiquity until now, medicinal plants have been an integral part of the healthcare system globally (Jamshidi-Kia *et al.*, 2017). Medicinal plants have been used extensively in the food, cosmetics, pharmaceutical, and agricultural sectors (Bolouri *et al.*, 2022). These therapeutic plants are utilized for spiritual purposes, as well as for food, medicine, scent, and flavonoids. Some plants are considered a source of nutrition they are recommended for their therapeutic values (Alamgir, 2017). These plants include ginger, green tea, walnuts, aloe, pepper and turmeric (Khan, 2016). Other plant derivatives are considered a source of active ingredients used in aspirin and toothpaste (Hassan, 2012). The major indices of physiological, pathological, and nutritional status of an individual/animal are blood parameters (Etim *et al.*, 2014). The metabolic state of an individual/animal can be interpreted from the changes in the components of blood compared to normal values (Gao *et al.*, 2022).



A complete blood count (CBC) is a blood test that provides detailed information about the types of cells in the blood, including red blood cells and platelets (George-Gay and Parker, 2003). Each type of blood cell plays an important role in the body's normal function Salvagno *et al.*, 2015). Over the last decade, our understanding of the male reproductive function and the importance of male factors in infertility has advanced significantly (Fainberg and Kashanian 2019). Infertility is defined as the inability to achieve a pregnancy after one year of unprotected intercourse (Jain *et al.*, 2004). Herbal therapy for medical ailments has recently become available despite the lack of experimentation to assess its effectiveness and safety (Calixto 2000). It's been shown that high concentrations of some medicinal plants such as saw-palmetto, Echinacea Purpurea, ginkgo biloba, St. John's wort induced inhibitory effects on sperm motility (Alzahrani *et al.*, 2023). High concentrations of certain medicinal plants can cause male reproductive toxicity through a variety of mechanisms, including direct effects on testicular tissue, altered secretions from accessory sex glands that lower the quality of semen, and indirect endocrine effects that suppress the production of steroids (Sorelle and 2019).

Ficus belongs to the family of Moraceae and is a genus of about 800 species (Akesa *et al.*, 2017). They are collectively known as Figs (Shi *et al.*, 2018). The fruits of most *Ficus* are edible, while some are used as food resources for wildlife Shanahan *et al.*, (2001). Some cultures employ ficus extract for its therapeutic properties, while others use it as a religious object (Egbuna *et al.*, 2011). *Ficus mucoso* is a member of the fig commonly found in the Guinea savannah vegetation belt of West and Central Africa (Shi *et al.*, 2018). One of the common leafy green vegetables used in various local dishes in Ikwo culture is the leaves of the *Ficus mucoso* plant. In Ikwo culture, the species is called “*ogbuenugu*” according to reports by ethno-medical practitioners (Amaechi and Daniel, 2016). The extract of *Ficus mucoso* leaf is used locally for the management of anaemia, diabetes, wounds, malaria and testicular dysfunction Olaoluwa *et al.*, 2022).

Therefore, this study is aimed at determining the effect of ethanolic leaf extract of *Ficus mucoso* on full haematological parameters in adult male albino Wistar rats and male fertility by evaluating some andrological parameters of Wistar rats, such as, the morphology of spermatozoa, sperm count and motility, to elucidate the ethnomedical claim of the use of the plant in the treatment of testicular dysfunction and anaemia.

METHODS

Collection of Plant: Fresh leaves of *Ficus mucoso* were harvested from a farm in Ikwo Local Government Area of Ebonyi State. The plant was authenticated by a botanist in the Department of Botany, Ebonyi State University (EBSU). The leaves were air dried at room temperature (25-28°C) for two weeks at the laboratory, Department of Physiology, AE-FUNAI. The dried leaves were blended into a powdery form using a hand mill to afford 500g of the sample. It was stored in a well-labeled polyethylene bag under refrigeration until required.

Preparation of Plant Extract: Five hundred (500) grams of the powdered leaves were soaked for 72 hours in 95% ethanol. The mixture was filtered with cheesecloth and re-filtered with Whatmann No. 1 filter paper to obtain a homogenous clear filtrate. The filtrate was concentrated *in vacua* into a solid paste (2.84g) at 45°C using a rotary evaporator. The dry extract was stored in a refrigerator at 4°C before use.

Phytochemical Analysis of Ethanolic Leaf Extract of *Ficus Mucoso*

Alkaloids: The aqueous extracts were evaporated to dryness, the residue was heated in a boiling water bath with 2N HCl (5 ml). After cooling, the mixture was filtered, and the filtrate was divided into two equal portions. One portion of each mixture was treated with a few drops of Mayer's reagent and the other with equal amounts of Wagner's reagent (Rizk, 1982). The samples were observed for the presence of turbidity or precipitation. A letter sign probably (S+) score was used if the reagent produced only a slight opaqueness; (E+) score was used if a definite turbidity, but no flocculation was observed and (E+++) score if a definite heavy precipitate or flocculation was produced (Salehi-Surmaghi *et al.*, 1992).

Flavonoids: 5 ml of the extracts were treated with a few drops of concentrated HCl and magnesium turnings. The presence of flavonoids was confirmed if a pink or magenta-red colour developed within 3 minutes (Somolenski *et al.*, 1972).

Phenols: 1 ml extract was added to 5ml Folin-Ciocalteu reagent and 4ml of NaCO₂. The blue colour indicates the presence of phenols (Singleton and Rossi, 1965).



Saponin: About 2.5 g of the extract was extracted further with boiling water. After cooling, the extract was shaken thoroughly to froth and allowed to stand for about 15-20 min. The saponin content classification was done as follows: No froth = negative, froth less than 1 cm = weakly positive, froth 1.2 cm high = positive, and froth greater than 2 cm high = strongly positive (Somolenski *et al.*, 1994).

Tannins: The extract was extracted further by adding 10 ml of hot 0.9% NaCl solution. Then they were filtered and divided into three (3) equal portions. One part of each extract was mixed with a sodium chloride solution, another with a 1% gelatin solution, and a third with the gelatin-salt reagent. Precipitation with the latter reagent or with both the second and third reagent was a sign of the presence of tannins. Positive tests were confirmed by the addition of FeCl solution to the extract which resulted in a characteristic blue, blue-black, green or blue-green colour and precipitate (phenolic compounds) (Segelman *et al.*, 1969).

Experimental Animals: Fifteen (15) male albino Wistar rats weighing 108 – 131g were procured and acclimatized for two weeks in the Animal Holdings of the Department of Physiology, Faculty of Basic Medical Sciences, AE-FUNAI. The animals were allowed free access to rat chow and water *ad libitum* throughout the study. The rats were housed in well-ventilated aluminium cages under clean conditions. The rats were allowed to acclimatize with the environment at ambient temperature under natural daylight/night conditions for two weeks before the start of the experiment.

Experimental Design: Fifteen (15) male albino rats were randomly selected and divided into groups A, B and C of five rats each. They were fed as follows: Group 1 (Control) was fed only normal rat pellets, clean water and normal saline orally daily. Group 2 (High Dose) was fed normal rat pellets, clean water, plus 500 mg/kg body weight (bwt) of *Ficus mucoso* extract orally once daily. Group 3 (Low Dose) was fed normal rat pellets, clean water, plus 200 mg/kg body weight (bwt) of *Ficus mucoso* extract orally once daily. All rats were allowed free access to drinking water. The feeding regimens through oral gavage lasted for three (3) weeks (21 days). At the end of the feeding period, blood samples were collected for analysis.

Ethical Consideration: The rats were handled humanely in consistent with the approved animal experimental procedures of the ARRIVE guidelines and were conducted in compliance with established ethical standards, including the NIH Publication (NIH Publication No. 85–23, revised 1996) on Guide for the Care and Use of Laboratory Animals and the Faculty of Basic Medical Sciences Research Ethics Committee authorized the study protocol, which was assigned protocol number 02/02/2024.

Collection of Blood Samples: The animals were anaesthetized with 2% sodium pentobarbital (75mg/kg) intraperitoneally and blood was collected via cardiac puncture using a 2-ml syringe attached to a needle. The blood was collected into capped bottles containing ethylenediamine tetra acetate (EDTA) by a modified method of Ohwada (Ohwada, 1986). The samples were analyzed immediately for the haematological parameters. Rats were sacrificed by decapitation for organ removal.

Sperm Analysis: For epididymal sperm preparation, all the rats were sacrificed by cervical dislocation. The right and left epididymis were excised and dissected in 1.5mL pre-warmed phosphate-buffered saline (PBS, pH = 7.4) at 37°C. Gentle agitation of the tissue was applied to enable spermatozoa to disperse for about 20–25 minutes. Semen samples were incubated at 37°C for 20–25 minutes. Sperm parameters were analyzed according to the criteria of the World Health Organization (fifth edition) with some modifications under a light microscope at a magnification of ×400 (Khaki *et al.*, 2009).

Assessment of Sperm Motility: The percentage of motile spermatozoa was determined after the preparation of sperm suspension by pipetting. A drop of the suspension was transferred on a clean glass slide, and a 15–20 second film was recorded using a video camera in five fields from each slide. Sperm motility was assessed by counting progressive, non-progressive and immotile spermatozoa after analyzing the recorded films.

Assessment of Sperm morphology: Sperm morphology was observed under a microscope at 1000×. A total of 200 sperm in samples from each animal were assessed for macrocephaly, microcephaly, pinhead, no-hook, curved midpiece, bent midpiece, coiled tail, looped tail, tailless, bent tail, and curved tail, which are all abnormal sperm. We only calculated the percentage of abnormal sperm as the proportion of abnormal sperm to total observed sperm (%).



Assessment of Sperm Count: Sperm count was determined by diluting 1mL of the sperm suspension with 1mL of 10% FA fixative. Ten microliters of the mixture were transferred into a haemocytometer, and sperm count was evaluated per 250 small squares of a haemocytometer.

Laboratory analysis (Measurement) of Blood Parameters: Blood samples (complete blood count) were analyzed using an automated cell counter with standard calibration (Bc-5300 Mindray Hematology auto-analyzer) according to the manufacturer's instructions.

Statistical Analysis: The data obtained was analyzed using Statistical Package for Social Sciences (SPSS) (version 20). Data was expressed as mean $\pm$ SEM (Standard error of mean). The significance of differences in mean values between groups was analyzed using Student's (independent) t-test, while the significance of the differences in mean values among different groups was evaluated using one-way ANOVA. A *p*-value of <0.05 was considered statistically significant.

RESULTS

Table 1 presents the phytochemicals detected in the ethanolic leaf extract of *Ficus mucoso*, including alkaloids, tannins, phenols, saponins, and flavonoids, along with their respective quantitative analysis.

Table 1: Phytochemical Composition and Quantitative Analysis of Ethanolic Leaf Extract of *Ficus mucoso*

Phytochemical	Quantitative Amount (if present)
Alkaloids	+++ (>80%)
Tannins	+ (<50%)
Phenols	+ (<50%)
Saponins	++ (50% < 80%)
Flavonoids	++ (50% < 80%)

Keys: (+) = <50% compared with control; (++) = 50% < 80%; (+++) = >80%.

Total White Blood Cell count and differential counts results showed no significant difference in all groups when compared with the control group (Table 2).

Table 2: Effect of ethanolic extract of *Ficus mucoso* on white blood cell parameters

Blood Parameters	Group 2 (N=5)	Group 3 (N=5)	Group 1 (N=5)	f-value	p-value
TWBC (X $10^9/l$)	8.687 $\pm$ 4.046	7.913 $\pm$ 1.210	7.607 $\pm$ 1.162	0.048	0.953
Lymphocyte (%)	0.475 $\pm$ 0.202	0.654 $\pm$ 0.057	0.773 $\pm$ 0.086	1.312	0.337
Monocyte (%)	0.052 $\pm$ 0.026	0.182 $\pm$ 0.024	0.115 $\pm$ 0.069	2.078	0.206
Neutrophil (%)	0.204 $\pm$ 0.094	0.157 $\pm$ 0.029	0.104 $\pm$ 0.020	0.734	0.519
Eosinophil (%)	0.004 $\pm$ 0.003	0.004 $\pm$ 0.004	0.003 $\pm$ 0.002	0.071	0.932
Basophil (%)	0.009 $\pm$ 0.006	0.002 $\pm$ 0.001	0.005 $\pm$ 0.003	0.893	0.458
Neutrophil#	2.533 $\pm$ 1.993	1.193 $\pm$ 0.150	0.823 $\pm$ 0.229	0.600	0.579
Lymphocyte#	5.747 $\pm$ 2.054	5.300 $\pm$ 1.139	5.890 $\pm$ 1.173	0.041	0.960
Monocyte#	0.300 $\pm$ 0.125	1.387 $\pm$ 0.068	0.843 $\pm$ 0.484	3.484	0.099
Eosinophil#	0.063 $\pm$ 0.063	0.020 $\pm$ 0.020	0.020 $\pm$ 0.015	0.404	0.684
Basophil#	0.043 $\pm$ 0.019	0.013 $\pm$ 0.003	0.030 $\pm$ 0.015	1.151	0.378

Keys: P-value is significant at $p<0.05$. TWBC=Total White Blood Cell count, g/dl= gram per decilitre, /l= per litre, (%) = percentage, # = Absolute Count, * =significant at $p<0.05$.



Red Blood Cell count, Haemoglobin, Packed Cell Volume, mean corpuscular volume, Mean Corpuscular Haemoglobin and Red Cell Distribution width results showed no significant difference in all groups when compared with the control group (Table 3).

Platelet count and plateletcrit results showed no significant difference in all groups when compared with the control group (Table 4), while Mean platelet volume and platelet distribution width showed a significant increase ($p>0.05$) in group 2 when compared with control group (Table 4).

Abnormal morphology showed a significant increase ($p>0.05$) in group 2 and 3 when compared with control group (Table 5), while Sperm motility and Sperm count showed a significant decrease ($p>0.05$) in group 2 and 3 when compared with control group (Table 5).

Table 3: Effect of ethanolic extract of *Ficus mucuso* on Red Blood Cell Parameters

Blood Parameters	Group 2 (N=5)	Group 3 (N=5)	Group 1 (N=5)	f-value	p-value
RBC ($\times 10^{12}/l$)	8.24 $\pm$ 0.63	8.39 $\pm$ 0.78	7.76 $\pm$ 0.61	0.93	0.42
HB level (g/dl)	14.65 $\pm$ 0.53	14.32 $\pm$ 1.36	14.07 $\pm$ 1.25	0.26	0.77
PCV (%)	51.70 $\pm$ 2.06	50.62 $\pm$ 6.27	50.25 $\pm$ 2.00	0.14	0.86
MCV	61.87 $\pm$ 1.88	61.33 $\pm$ 0.32	66.43 $\pm$ 1.09	4.88	0.06
MCH	17.53 $\pm$ 0.49	17.10 $\pm$ 0.31	18.33 $\pm$ 0.43	2.25	0.19
RDWS	44.53 $\pm$ 0.913	43.57 $\pm$ 1.27	49.47 $\pm$ 4.98	1.10	0.39

Keys: P-value is significant at $p<0.05$. RBC= Red blood cells, HB= Haemoglobin, PCV= Packed Cell Volume, MCV=mean corpuscular volume, MCH= Mean Corpuscular Haemoglobin, RDWS= red cell distribution width, * =significant at $p<0.05$.

Table 4: Effect of ethanolic extract of *Ficus mucuso* on Platelet Parameters

Blood Parameters	Group 2 (n=5)	Group 3 (n=5)	Group 1 (n=5)	f-value	p-value
PLT ($\times 10^9/l$)	840.00 $\pm$ 128.30	820.50 $\pm$ 127.91	731.25 $\pm$ 126.00	0.82	0.46
MPV	7.37 $\pm$ 0.18*	6.57 $\pm$ 0.12	6.60 $\pm$ 0.15	8.92	0.02*
PDW	15.67 $\pm$ 0.07*	15.43 $\pm$ 0.03	15.40 $\pm$ 0.06	7.13	0.03*
PCT	5.91 $\pm$ 0.45	5.51 $\pm$ 0.49	4.65 $\pm$ 0.51	1.75	0.25

P-value is significant at $p<0.05$. PLT = Platelet Count, MPV= Mean Platelet volume, PDW= Platelet distribution width, PCT = Plateletcrit, * =significant at $p<0.05$.

Table 5: Effect of ethanolic extract of *Ficus mucuso* on some andrological parameters

Semen Parameters	Group 2 (n=5)	Group 3 (n=5)	Group 1 (n=5)	f-value	p-value
Sperm motility (%)	67.52 $\pm$ 9.42*	61.00 $\pm$ 8.14*	78.22 $\pm$ 11.50	47.36	0.002*
Abnormal morphology (%)	5.82 $\pm$ 1.23*	6.10 $\pm$ 0.94*	3.60 $\pm$ 0.80	8.24	0.035*
Sperm count ($\times 10^6$)	98.45 $\pm$ 10.03*	95.00 $\pm$ 7.43*	126 $\pm$ 11.27	72.55	0.005*

P-value is significant at $p<0.05$. Key: GROUP 2= albino Wistar rats that were administered 500 mg of the extract of *Ficus mucuso*, GROUP 3 = albino Wistar rats that were administered 200 mg of the extract of *Ficus mucuso*. GROUP 1 = albino Wistar rats that were administered normal saline (control).

DISCUSSION

The results of this investigation shed important light on how *Ficus mucuso* leaf extract in ethanol affects male fertility and hematological parameters in albino Wistar rats. Numerous physiological effects have been reported for bioactive



compounds like alkaloids, tannins, phenols, saponins, and flavonoids, which were confirmed to be present by the phytochemical analysis (Perez-Vizcaino and Fraga, 2018; Vicente and Boscaiu, 2018). Although these substances are well-known for their possible therapeutic benefits, there is still worry about how they may affect reproductive health.

The fact that platelet count, red and white blood cell indices, and other associated parameters did not significantly change between the experimental groups, aligns with the assertion that medicinal plants may not have a negative impact on haematological indices (Uboh *et al.*, 2010; Chanda *et al.*, 2015). This implies that *Ficus mucuso* may not cause haematotoxicity.

On the other hand, *Ficus mucuso* showed notable negative impacts on male reproductive parameters. When comparing the treated groups to the control, a dose-dependent decrease in sperm motility and count was noted, along with an increase in aberrant sperm morphology. According to these results, *Ficus mucuso* may interfere with spermatogenesis and sperm maturation, possibly by influencing the seminiferous tubules (Yoshida, 2016). Other medicinal plants with high levels of alkaloids and flavonoids, which are known to disrupt testicular function and androgenic regulation, have also been shown to exhibit similar patterns of reproductive toxicity (Dewal *et al.*, 2018; Sharma and Mali, 2016).

Ficus mucuso's disruption of the hypothalamic-pituitary-testicular axis is one tenable explanation for the reproductive toxicity seen. According to earlier research, bioactive substances like flavonoids and alkaloids may interfere with endocrine signaling, changing the release of gonadotropin and testosterone levels (Oduwole *et al.*, 2018). By reducing the availability of vital epididymal secretions required for sperm maturation and functional competence, a decrease in testosterone can impair sperm motility and viability (Zhou *et al.*, 2018; Gervasi and Visconti, 2017).

The idea that *Ficus mucuso* interferes with sperm maturation processes is further supported by the observed rise in sperm abnormalities, such as midpiece and tail defects. These abnormalities are a sign of damage from oxidative stress or impaired development of spermatogenic cells, both of which have been linked to extended exposure to phytochemicals that may be harmful to reproduction (Oridupa *et al.*, 2018; Wang *et al.*, 2018). Such morphological abnormalities are known to compromise sperm fertilization capacity, potentially leading to subfertility or infertility (de Boer *et al.*, 2015).

It is crucial to think about whether *Ficus mucuso*'s effects on reproduction are reversible or if prolonged exposure could lead to irreversible fertility impairment. It has been discovered that certain plant extracts with well-known antifertility effects only have temporary effects, with normal sperm parameters returning after exposure ends (Kalita *et al.*, 2016; Mali *et al.*, 2015). In order to fully understand the impact of *Ficus mucuso* on male reproductive health, more research is required to assess the reversibility of these effects, hormonal changes, and potential histopathological changes in testicular tissue.

In conclusion, *Ficus mucuso* administration has a negative effect on male fertility by decreasing sperm motility and count and increasing sperm morphological abnormalities, even though it has no negative effect on haematological parameters. These results point to a possible toxicity to reproduction linked to the plant extract. The safety profile of *Ficus mucuso* for medicinal use and its effects on male reproductive health should also be determined in large part by further studies.

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CONFLICT OF INTEREST

None declared by the authors.