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Pro-Fertility Evidence of *Euphorbia hirta* (Euphorbiaceae) Crude Aqueous Extract in Normal Male Albino Rats

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Abstract

According to folk users in Cameroon, about 30 fresh leaves of *Euphobia hirta* are macerated into 250ml of water and given once a day to an adult male (human) for 21 days to treat fertility deficiencies. Extensive studies available online provide more quantifiable insights into its pharmacological activities and safety profile. In a separate study, we evaluated the copulatory potentials of the aqueous extract of *Euphobia hirta* in sexually naïve normal albino male rats (article in Press). The present study was designed to evaluate the effects of *E. hirta* aqueous extract on some reproductive indices including hormonal profile, relative mass of reproductive organs (RMO) and sperm characteristics in normal male albino rats, with the goal of determining its potential as a natural therapeutic agent to enhance male reproductive health. A total of 25 male albino rats aged 12 to 13 weeks and weighing 150 to 200 grams each were subdivided into 5 groups and treated as follows: rats of groups 1, 2 and 3 were administered 300 mg/kg, 600 mg/kg and 1200 mg/kg (EH_{ae1}, EH_{ae2} and EH_{ae3}) of the plant extract respectively, those of group 4 received 10 ml/kg distilled water, while those of group 5 received sildenafil citrate: Erekta (5mg/kg); these were administered orally using an Oro-pharyngeal cannula once daily, for a period of 52 days. The animals were terminated on day 53 and blood and organs collected for the assessment of hormonal profile, RMO and sperm characteristics. Extract-treated animals recorded a significant increase in plasma testosterone concentration (9.14 ± 2.44, 10.89 ± 1.64, 8.23 ± 1.40 ng/ml for EH_{ae1}, EH_{ae2} and EH_{ae3} respectively) compared to that of DW (3.49 ± 0.69) and Erekta (5.39 ± 1.00). Also, EH_{ae3} showed a remarkably low "No head" percentage of 0.38 ± 0.04 compared to DW (2.35 ± 0.69) and Erekta (2.27 ± 0.51). Furthermore, extract-treated rats registered significant values in sperm motility and concentration unlike non-significant values in RMO. Results obtained from our study enables us to say that the aqueous leaf extract of *E. hirta* enhances fertility in male animals through biosynthesis of reproductive hormones, enhancing sperm motility and by reducing the amount of abnormally formed spermatozoa.

Keywords

Euphorbia hirta, Reproductive Hormones, Abnormal Sperm, Sperm Concentration, Sperm Motility

1. Introduction

According to [1], sexual health is an important component of an individual's quality of life and well-being and sexual relationships remain the most important social and biological relationships in human life. One of the main aims of marriage is procreation (reproduction) to ensure the continuity of an individual's lineage and**,** more importantly, for sexual fulfillment of both partners. For life to continue, an organism must reproduce itself before it dies [2]. Consequently, any individual or couple who is unable to conceive after one year of intercourse without the use of contraception is termed infertile [3]. Infertility is estimated to impact about 10% - 25% of couples of reproductive age [4]. It is classified into two categories: primary and secondary, with secondary infertility more common in Africa, while primary infertility is higher in other regions of the world. Infertility rate is very high in Cameroon and it ranges from 15% to 30% depending on the age and the socio-economic level of couples. In females, it occurs in about 37% of all infertile couples with ovulatory disorders accounting for more than half of the causes of female infertility [5].

Statistically, about 30% - 50% of infertility within a couple is related to the male with 30% - 40% of the causes of male infertility related to sperm disorders [6]. The most common cause of infertility in men remains their inability to produce enough healthy, active and highly motile sperm [7] which results from lack of testicular development, diseases of the reproductive system, increased scrotal temperature, immunological problems, endocrine disorders, lifestyle choices, environmental and nutritional factors which all have been shown to have a negative effect on sperm parameters [8]-[11]. In addition, other factors including genitourinary tract infections, endocrine disorders, immunological factors and drug-related issues, affect the male reproductive system and cause infertility [12]-[16]. Disorders affecting spermatogenesis, hormone regulation, oxidative stress and regulation of spermatogenesis-related genes also cause infertility [17]-[19]. Furthermore, infertility can be due to excessive consumption of natural plant compounds (phytoestrogens), which can affect the reproductive system and reduce fertility [20].

Infertility is a medical condition that can inflict psychological, physical, mental, spiritual, social and medical detriments to the patient. This condition can be accompanied by feelings of shame, guilt and low self-esteem which in turn may lead to anxiety, distress, poor quality of life as well as varying degrees of depression. The condition remains unique in its quality because it affects both the patient and the patient's partner within a couple [21].

Infertility treatment options depend on the type and its possible cause(s). Some treatment options include pro-fertility drugs like clomiphene citrate which stimulates the pituitary gland to release more FSH and LH, thus, enhancing ovulation; Gonadotropins, such as human menopausal gonadotropin or hMG (Menopur) and FSH (Gonal-F, Follistim AQ, Bravelle) that stimulate the ovary to produce multiple eggs; Metformin (Fortamet), used in insulin resistance conditions and helps improve insulin resistance, which can increase the chances of ovulation; Letrozole (Femara), an aromatase inhibitor, has an action similar to that of clomiphene; and utrogestan, which can be taken through the oral route. Infertility can also be remedied through surgery including laparoscopic or hysteroscopic surgery and tubal surgeries, as well as through assisted conception (intrauterine insemination, assisted reproductive technology and in vitro fertilization). These drugs have numerous side effects like bloating, headache, gastric upset, mood swings and developing ovarian hyper-stimulation syndrome.

The use of medicinal plants either as therapeutic alternatives or food additives has run from time immemorial. For several decades, plants have been used to manage illnesses because they are readily or cheaply available in healthcare [22] [23]. Presently, the use of medicinal and aromatic plants for the development and preparation of alternative traditional medicine and food additives has gained much interest [24]. Medicinal plant-based drugs have possible therapeutic properties in the treatment of several diseases like infertility [25] [26]. Although other options exist for the treatment of infertility with pro-fertility drugs as mentioned above, medicinal plants still remain the best as they are relatively cheap and easily accessed with no or minimal side effects. Due to the clear negative effects of chemical drugs on humans, the tendency to use herbal medicines is increasing among women and men. We need to study the use of biologically active plant materials in the field of male fertility and to identify natural plant materials with estrogenic and anti-estrogenic properties [8]. Due to men's fear of infertility [27], it is very important to pay attention to medicinal plants that affect male fertility [16]. In a separate study, we evaluated the copulatory potentials of the aqueous extract of *Euphobia hirta* in sexually naïve normal albino male rats (article in Press). The present study was designed to evaluate the effects of *E. hirta* aqueous extract on some reproductive indices including hormonal profile, relative mass of reproductive organs (RMO) and sperm characteristics in normal male albino rats, with the goal of determining its potential as a natural therapeutic agent to enhance male reproductive health.

2. Materials and Methods

2.1. Plant Material

2.1.1. Collection

Fresh leaves of *Euphorbia hirta* plant were harvested around the Buea locality (southwest region of Cameroon) and were prepared based on a specific extraction process to obtain its aqueous extract.

2.1.2. Preparation of Extract

The harvested leaves were thoroughly washed and allowed to air-dry for approximately two weeks at room temperature. The leaves were then ground using an electric blender in order to produce a fine powder. Following the protocol of [28], 100 g of the powder obtained was macerated into 1000 ml of distilled water and kept for 72 hours. This was accompanied by mechanical agitation by simple manual shaking of the container for about 50 times in 20 minutes daily at 24-hour intervals. Seventy-two hours from the date of maceration, it was filtered using a laboratory sieve of 80μm pores diameter. The filtrate was later evaporated in an oven at 50˚C for a period of 2 days to obtain a semi-solid crude residue from which the yield of extraction was calculated to be 5%.

2.1.3. Administrative Doses

The choice of administrative doses of the extract was guided by the information obtained from folk users of this plant and the tradi-practitioners as well as a comprehensive review of available literature as discussed in our previous study (article in Press). Briefly, according to folk users, about 30 fresh leaves are macerated into 250ml of water [29] and given once a day to an adult male (human) for 21 days to treat fertility deficiencies. Extensive reports from studies available online provide more quantifiable insights into its pharmacological activities and safety profile. Acute and sub chronic toxicity studies in rodents have generally indicated a high safety margin for *E. hirta* extracts. For instance, sub chronic toxicity studies involving repeated oral administration for 90 days at doses up to 1000 mg/kg, have also shown no significant changes in body weight, food/water consumption, haematological and biochemical parameters, or organ histopathology, further supporting its relatively safe profile [30] [31]. Therefore, the chosen doses were 300, 600 and 1200 mg/kg labelled EH_{ae1}, EH_{ae2} and EH_{ae3}, respectively and representing a range that gathers traditionally used concentrations while also exploring higher, yet still ostensibly safe, therapeutic levels based on documented scientific findings, aiming to thoroughly investigate dose-dependent effects.

2.2. Animals

2.2.1. Experimental Animals and Ethical Considerations

All experiments were performed according to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH publication No. 85-23, revised 1996) and the Cameroon National Ethical Committee (Yaoundé, Cameroon) for animal handling and experimental procedure: Reg No. FW-IRB00001954 [32]. The project was approved by the University of Buea Institutional Animal Care and Use Committee with a reference number: UB-IACUC-22/2025.

2.2.2. Raising of Animals

Animals used in this study were male albino rats of the Wistar strain, raised in the Animal Facility of the Department of Animal Biology and Conservation of the Faculty of Science, University of Buea under standard conditions of temperature (25˚C ± 1˚C) and humidity (50% - 80%) with a 12/12 hours light/dark cycle and in standard cages. They had free access to food and water. Each animal was used only once. Their laboratory diet was composed as shown in **Table 1**.

Table 1. Food composition for 1 kg standard laboratory diet for laboratory rats.

Nutrient	Quantity (g)
Corn flour	600
Roasted soy bean flour	200
Fish flour	150
Bone flour	20
Table salt	10
Vitamin	10

2.3. Reagents or Chemicals: Preparation of Other Solutions

Products used in this study included sildenafil citrate (Erecta®; strides Pharma Science limited); oestradiol (Hebei New Century Pharmaceutical Co, Ltd); progesterone (Synergon®); Diclofenac; Bioassay kits (DRG instruments GmbH) for FSH and testosterone were all purchased and kept under recommended conditions until used.

Preparation of the penicillin-G solution was done by dissolving 600 mg of powder penicillin-G 1000000IU into 2 ml of distilled water. It was properly homogenised and then used on the ovariectomised females to prevent post-surgical infection.

Preparation of the diclofenac solution administered consisted in dissolving one diclofenac tablet (50 mg) into 20 ml of distilled water. The mixture was properly homogenised and then administered to the animals at the dose mentioned earlier.

2.4. Experimental Design

2.4.1. Partitioning and Treatment of Animals

A total of 25 adult males obtained from our breed and weighing between 150 and 200 grams each were subdivided into 5 groups of 5 males each and treated as follows. Rats of groups 1, 2 and 3 were administered 300 mg/kg (EH_{ae1}), 600 mg/kg (EH_{ae2}) and 1200 mg/kg (EH_{ae3}) of the plant extract respectively, while those of group 4 received 10 ml/kg distilled water (negative control) and those of group 5 received sildenafil citrate (Erekta) at a dose of 5 mg/kg; various doses of the plant extract, distilled water and Erekta (standard drug) were administered orally using an Oro-pharyngeal cannula once daily, for a period of 52 days which approximately corresponds to the duration of one spermatogenic cycle.

2.4.2. Sacrifice, Blood and Organ Collection

On day 53 from the beginning of treatment, the animals were sacrificed for the assessment of non-sexual parameters. To this effect, each animal was starved on day 52. The following day, after rendering the animal unconscious through an overdose of anaesthesia, the thoracic region was rapidly dissected, the heart identified, blood collected through cardiac puncture using a 5ml syringe and immediately transferred into EDTA-coated test tubes [33]. Immediately after blood collection, the EDTA tubes were centrifuged for 15 minutes at 2500 rpm. At the end of this, the supernatant (plasma) was collected with the help of a micropipette, transferred into Eppendorf tubes and stored in a refrigerator for future biochemical analyses.

2.4.3. Determination of Relative Mass of Sex and Accessory Organs

Following sacrifice of the animals, the organs of interest (penis, testes, epididymis, vas deferens, prostate and seminal vesicle) of each rat was identified, isolated properly without any fat around them and weighed in order to calculate the corresponding relative mass of organ (RMO). The following formula was used for this purpose:

$$\text{RMO} = \frac{\text{organ mass}}{\text{animal mass}} \times 100$$

2.4.4. Sperm Analysis

Sperm characteristics were also evaluated following sacrifice of animals. Sperm motility and concentration analyses were done blindly and according to the procedures described by [34]. Subsequent to the sacrifice of animals and collection of organs, the cauda epididymis was carefully separated, weighed and placed in a Petri dish containing 10 ml of normal saline warmed to 37˚C, pH 7.2. After which, its content was minced out in order for it to move into the normal saline forming a solution. Thereafter, the petri dish was placed into the water bath kept at 37˚C in order to mimic the normal body temperature thereby sustaining the life of the sperm cells. With the help of a micropipette, about 15 μl from this suspension was loaded into a Neubauer cell-counting chamber until it was filled by capillary action.

- Sperm motility was determined by counting the number of spermatozoa displaying any movement on the field of observation. This was done using a 10X eyepiece and a 40X objective of the light microscope (OLYMPIA, USA) and counting the motile spermatozoa. For samples having a very high number of sperm cells, the suspension was diluted using a dilution factor of 25 that is 0.2ml of the suspension was mixed in 4.8ml of normal saline.
- The number of sperm cells was obtained using the following formula.

$$\text{Total motile sperm} = \text{N° motile sperms counted in one chamber} \times \text{df} \times 10^6$$

$$\text{sperm concentration} = \text{total N}^\circ \text{ sperm cell counted in one chamber} \times \text{df} \times 10^6$$

Sperm motility expressed in percentage was calculated as follows:

$$\text{sperm motility} = \frac{\text{total motile sperm cells}}{\text{sperm concentration}} \times 100$$

- Sperm morphology was also assessed by keen observation of each sample under the microscope and by noting the number of sperm cells abnormally formed. Some of the parameters for this assessment were: sperm cells with no head, sperm cells with no tail and counting the number of normally formed sperms as well. The following formula was used:

$$\text{number of sperms} = \text{N}^\circ \text{ sperms with specified parameter counted in one chamber} \times \text{df} \times 10^6$$

2.4.5. Hormonal Assay

Hormonal concentration in the plasma obtained from the sacrificed rats was determined using DRG testosterone and FSH ELISA kits following the procedure described by the manufacturer. A summary of each procedure is as follows:

1) Testosterone evaluation

- The desired number of microtiter wells in the frame holder was secured
- 25 µl of each standard, control and sample were dispensed into appropriate wells with new disposable tips.
- 200 µl Enzyme Conjugate was then dispense into each well.
- This was followed by incubation for 60 min at room temperature.
- The contents of the wells were briskly shaken out and were then rinsed 3 times with 300 µl diluted wash solution per well. The wells were sharply strike on absorbent paper to remove residual droplets.
- 200 µl of substrate solution were added to each well.
- Then incubated for 15min at room temperature.
- The enzymatic reaction was then stopped by adding 100 µl of stop solution to each well.
- Thereafter, optical density of the solution in each well was determined at 450 nm with a microliter plate reader. This was done within 10 min after adding the stop solution.

2) FSH evaluation

- The desired number of microliter wells in the frame holder was secured.
- 25 µl of each standard, controls and samples was dispensed into appropriate wells with new disposable tips.
- 100 µl enzyme conjugate was dispensed into each well and was thoroughly mixed for 10 seconds.
- This was followed by incubation for 30 min at room temperature.
- The contents of the wells were briskly shaken out, then rinsed 5 times with aqua dest (400 µl per well). The wells were sharply strike on absorbent paper to remove residual droplets.
- Thereafter, 100 µl of substrate solution was added to each well.

- Followed by incubation for 10 min at room temperature.
- The enzymatic reaction was stopped by adding 50 µl of stop solution to each well.
- Finally, optical density of the solution in each well was determined at 450 nm with a microliter plate reader. This was done within 10 min after adding the stop solution.

2.5. Statistical Analyses

Values were expressed as Mean ± SEM. Mean values were calculated for each animal and quantitative comparisons between groups established from those means. Analysis of Variance (ANOVA) followed by Duncan test was used in the SPSS for windows version 20.0 software. Significant levels were tested at $p < 0.05$.

3. Results

3.1. Effects of the Aqueous Leaf-Extract of *E. hirta* on Relative Weight of Sex and Accessory Organs

As seen in Table 2, treatment of normal male rats with the leaf aqueous extract of *E. hirta* at 300, 600 and 1200 mg/g (EH_{ae1}, EH_{ae2} and EH_{ae3}) doses for a period of 52 days induced a nonsignificant ($p < 0.05$) increase in relative organ weight (%) compared to the Erekta-treated group.

Table 2. Effects of the aqueous leaf-extract of *E. hirta* on the relative weight of sex and accessory organs of normal male rats.

Organ	Treatment				
	DW	Erekta®	EH_{ae1}	EH_{ae2}	EH_{ae3}
Testes	1.45 ± 0.15	0.92 ± 0.06	0.96 ± 0.06	1.19 ± 0.15	1.22 ± 0.11
Epididymis	0.81 ± 0.11	0.49 ± 0.06	0.67 ± 0.10	0.63 ± 0.05	0.65 ± 0.08
Vas deferens	0.10 ± 0.01	0.08 ± 0.004	0.06 ± 0.01	0.08 ± 0.01	0.07 ± 0.01
Prostate	0.12 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.17 ± 0.05	0.13 ± 0.01
Sem. Vesicles	0.21 ± 0.04	0.18 ± 0.03	0.17 ± 0.03	0.31 ± 0.06	0.25 ± 0.06
Penis	0.17 ± 0.03	0.11 ± 0.01	0.07 ± 0.04	0.17 ± 0.02	0.13 ± 0.01

Values presented as Mean ± SEM; DW: distilled water; EH_{ae1}: *Euphobia hirta* aqueous extract dose 1 (300 mg/kg); EH_{ae2}: *Euphobia hirta* aqueous extract dose 2 (600 mg/kg); EH_{ae3}: *Euphobia hirta* aqueous extract dose 3 (1200 mg/kg); Sem. vesicles: seminal vesicles.

3.2. Effects of the Aqueous Leaf-Extract of *E. hirta* on Sperm Characteristics

3.2.1. Effects on Sperm Concentration and Motility

Subjection of normal male rats to various doses of the aqueous leaf extract of *E. hirta* for a period 52 days caused a significant increase in percentage motility and sperm concentration ($p < 0.05$) compared to either control group (DW and Erekta) (Table 3).

Table 3. Effects of the aqueous leaf-extract of *E. hirta* on sperm motility and concentration of normal male rats.

Sperm characteristics	Treatment				
	DW	Erekta®	EH_{ae1}	EH_{ae2}	EH_{ae3}
Concentration ($\times 10^6$/ml)	6.53 ± 2.98	13.70 ± 4.75	13.20 ± 4.3	12.72 ± 3.60	21.19 ± 2.03
Motility (%)	65.08 ± 4.72	81.99 ± 6.04	95.29 ± 11.44	94.27 ± 8.85	97.19 ± 7.02

Values presented as Mean ± SEM; DW: distilled water; EH_{ae1}: *Euphobia hirta* aqueous extract dose 1 (300 mg/kg); EH_{ae2}: *Euphobia hirta* aqueous extract dose 2 (600 mg/kg); EH_{ae3}: *Euphobia hirta* aqueous extract dose 3 (1200 mg/kg); µl: microliter.

3.2.2. Effects on Sperm Morphology

Treatment of normal male rats to various doses of the aqueous leaf extract of *E. hirta* for a period of 52 days yielded a significant increase in the number of normally formed sperms ($p < 0.05$) compared to either control group (DW and Erekta). In the same line, the administration of these doses of plant extract produced a significant decrease in the number of abnormally formed spermatozoa specifically spermatozoa with no head (Table 4).

Table 4. Effects of the aqueous leaf-extracts of *E. hirta* on sperm morphology of normal male rats.

Characteristics ($\times 10^6$)	Treatment				
	DW	*Erekta*	EH_{ae1}	EH_{ae2}	EH_{ae3}
Normal	6.25 ± 1.27	8.04 ± 2.96	9.33 ± 2.42	14.04 ± 3.69	10.75 ± 1.59
No head	2.35 ± 0.69	2.27 ± 0.51	1.13 ± 0.62	1.56 ± 0.36	0.38 ± 0.04
No tail	1.00 ± 0.08	0.85 ± 0.04	0.05 ± 0.04	0.04 ± 0.02	0.03 ± 00

Values presented as Mean ± SEM; DW: distilled water; EH_{ae1}: *Euphobia hirta* aqueous extract dose 1 (300 mg/kg); EH_{ae2}: *Euphobia hirta* aqueous extract dose 2 (600 mg/kg); EH_{ae3}: *Euphobia hirta* aqueous extract dose 3 (1200 mg/kg).

3.3. Effects of the Aqueous Leaf-Extract of *E. hirta* on Plasma Concentrations of FSH and Testosterone

Analysis of the hormonal content of the plasma obtained from the blood of sacrificed animals produced results that are compiled in Table 5. Administration of aqueous leaf extract of *E. hirta* induced a significant increase in testosterone plasma concentration compared to the control groups (DW and Erekta). On the other hand, FSH plasma concentration was statistically non-significant in extract-treated animals compared to those of the control groups.

4. Discussion

In this study, we measured the weights of testis, epididymis, vas deferens, seminal vesicles, prostate glands and the penis of the animals under experimentation and

Table 5. Effects on plasma concentrations of FSH and testosterone of normal male rats.

Hormone	**Treatment**				
	DW	**Erekta**	EH_{ae1}	EH_{ae2}	EH_{ae3}
Testosterone (ng/ml)	3.49 ± 0.69	5.39 ± 1.00	9.14 ± 2.44	10.89 ± 1.64	8.23 ± 1.40
FSH (mIU/ml)	88.19 ± 1.62	86.2 ± 1.33	89.68 ± 0.95	84.9 ± 1.61	87.2 ± 1.16

Values presented as Mean ± SEM; DW: distilled water; EH_{ae1}: *Euphobia hirta* aqueous extract dose 1 (300 mg/kg); EH_{ae2}: *Euphobia hirta* aqueous extract dose 2 (600 mg/kg); EH_{ae3}: *Euphobia hirta* aqueous extract dose 3 (1200 mg/kg); FSH: Follicle stimulating hormone; mIU/ml: micro international units/millilitre; ng/ml: nanogram/millilitre.

the relative mass of each organ (RMO) was determined. The weight of the testes is a useful index in evaluating the efficiency of steroidogenesis [32]. Extract-treated rats recorded an increase in the relative weights of the sex and accessory organs compared to control groups-rats, thus, exhibiting androgenic effects. Androgens, including testosterone, have been reported to be useful for the histo-morphometric development and maintenance of the testes and ultimately the biochemical process of sperm production [35] [32]. The enhancements in the weights of sex and accessory organs of male rats are usually associated with androgenic activity and anabolic function. Androgens can stimulate the growth of accessory sexual organs (e.g., testis, seminal vesicles and prostate) and increase their weights [36]. Drugs or natural compounds are considered to possess androgenic properties if they can increase the weights of accessory sexual organs [37]. This increase in the relative weight of sex and accessory organs recorded in extract-treated rats, though in a non-significant manner, could reflect the increased plasma levels of testosterone.

Our study was aimed amongst others, at evaluating the pro-fertility potentials of the aqueous extract of *E. hirta* to determine if its ability to facilitate copulation can match with the ability to favour fertilization, hence, pregnancy at the doses studied. In other words, we intended to determine if the extract could enhance efficient spermatogenesis. As mentioned earlier, about 30% - 50% of infertility within a couple is related to the male with 30% - 40% of the causes of male infertility related to sperm disorders [6]. The most common cause of infertility in men remains their inability to produce enough healthy, active and highly motile sperm [7]. Unfortunately, the process of spermatogenesis is significantly affected by free radicals which cause oxidative stress leading to lipid peroxidation of sperm membranes, impairing their motility and viability. Sperm cell membranes are exposed to oxidative damage due to large amounts of unsaturated fatty acids, which ultimately decrease sperm motility and viability. They also induce DNA damage in sperm, compromising genetic integrity and increasing the risk of abnormal embryonic development. Furthermore, excessive free radicals can trigger apoptosis (programmed cell death) of sperm, reducing overall sperm count and quality [38] [39]. Consequently, medicinal plants with antioxidant compounds increase sperm function and can improve fertility [40]. Studies have shown that some medicinal plants may have fertility-enhancing properties in men by improving antioxidant activity, prevent the formation of free radicals and lipid peroxidation, and reduce oxidative stress, preventing damage to sperm cells [41]. They also increase the number of testicular vessels, the lifespan and number of sperm, increasing sperm quality and protecting germ cells. *E. hirta* exhibits strong antioxidant properties both in animal models and in vitro. It has been shown to be able to neutralise free radicals in various experimental models using hydroxyl radical scavenging, ABTS, and DPPH assays [42]. A study by [43] displayed the antioxidant activities for three types of extractions from *E. hirta* including the aqueous extract through superoxide, DPPH and hydroxyl radical scavenging assays. After 52 days of administration, the extract significantly increased both sperm concentration and percentage motility ($p < 0.05$) compared to both the distilled water (DW) and Erekta control groups. Results show a dose-dependent positive effect of *E. hirta* on key fertility parameters (sperm concentration and percentage motility), suggesting a pro-fertility effect that could contribute to or be associated with enhanced sexual function. The aqueous extract of *Cyperus esculentus*, administered to Wistar rats for nine weeks, increased testicular and epididymal weight, increased sperm count and motility. These studies show that the medicinal properties of these plants are related to polyphenolic components, especially flavonoids, which can neutralize free radicals, the source of oxidative stress [44]. Flavonoids in plant samples have antioxidant power and can optimize the function of the body's antioxidant system. Reactive oxygen species are produced in a chain of reactions that alter male reproductive cells quantitatively and qualitatively; antioxidants act by interrupting these chain reactions [44]. Again, the effects produced may be due to phytoconstituents such as phenols, saponins and alkaloids present in the aqueous extract of *E. hirta* which exhibit an antioxidant activity and as a result have contributed in the elimination of free radicals leading to an improvement in percentage motility and sperm concentration. These results are in line with the findings of [45], who upon testing the effects of aqueous and methanol extracts of *E. hirta* for a period of 14 days obtained satisfactory results concerning the percentage motility and sperm concentration in the treated animals.

The administration of various doses of the aqueous leaf extract of *E. hirta* to normal male rats for 52 days demonstrated significant effects on sperm morphology, which is a crucial reproductive index. Firstly, there was a significant ($p < 0.05$) increase in the number of normally formed sperm cells in the treated groups compared to both the distilled water (DW) and Erekta control groups. This finding is highly relevant, since improved sperm morphology is linked to male fertility and reproductive success. The increased percentage of normal sperm suggests a beneficial effect of the *E. hirta* extract on spermatogenesis, the process of sperm development. Secondly, the plant extract administration led to a significant decrease in the number of abnormally formed spermatozoa, specifically those with no head. The "No head" abnormality is a severe morphological defect that renders spermatozoa incapable of fertilization. The reduction in such abnormalities further reinforces the positive impact of the *E. hirta* extract on sperm quality. This is particularly evident when comparing the "No head" values produced by the extract treated groups to the DW and *Erekta*. For instance, EH_{ae3} shows a remarkably low "No head" percentage of 0.38 ± 0.32 compared to DW's (2.35 ± 0.59) and Erekta's (2.27 ± 0.51). Similarly, *Cocos nucifera* water improved reproductive indices in Wistar rats [46]. A study conducted in Iraq showed that *Cyperus esculentus* has a protective effect on testicular and sperm abnormalities caused by lead acetate in Wistar rats [47]). This may be due to phyto-constituents such as flavonoids, phenols and tannins found in the aqueous extract, which have been reported to exhibit strong antioxidant activity [48]. Free radicals significantly impair spermatogenesis by causing oxidative stress which leads to lipid peroxidation of sperm membranes, impairing their motility and viability, discussed earlier. They also induce DNA damage in sperm, compromising genetic integrity and increasing the risk of abnormal embryonic development. Furthermore, excessive free radicals can trigger apoptosis (programmed cell death) of sperm, reducing overall sperm count and quality, as mentioned earlier [38] [39]. These phyto-constituents through their activity, they scavenge free radicals and reduce oxidative stress, thereby protecting developing spermatozoa from damage and increasing the percentage of normally formed spermatozoa and reducing abnormalities. The effect of the plant extract on spermatogenesis could also be via a luteinizing hormone (LH)-related mechanism, even though we did not assay plasma LH in our study. LH stimulates Leydig cells (LC) to release testosterone, control LC differentiation, proliferation and biological rhythm and ultimately control spermatogenesis as well [49]-[51]. In addition, LH has been shown to be associated with sperm quality, sperm motility and sperm capacitation. There is mounting evidence to support the notion that LH is critical to spermatogenesis. critical for the proliferation and maturation of Leydig cells after birth and during puberty, enabling the initiation of spermatogenesis.

Medicinal plants can also enhance the activity of the hypothalamic-pituitary-gonadal axis at different levels, affecting the secretion of follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone [34]. The administration of the aqueous leaf extract of *E. hirta* significantly increased the plasma concentration of testosterone after 52 days of treatment, similar to another study which found that an injection of *Cissus populnea* root into male Wistar rats increased the secretion of male sex hormones such as testosterone and gonadotropins, thereby increasing the fertility of these rats [52]. The significant increase in testosterone noted in our study could be attributed to the action of the LH as explained earlier and reported in many studies, although the plasma level of the latter was not measured in our experiment. The enhanced sperm characteristics of extract-treated rats observed in this study and discussed above, may be attributed to the increase in serum testosterone concentration as revealed in this study. Testosterone being a crucial factor for germ cell differentiation, its increase in extract-treated animals explains why sperm parameters (concentration, motility and morphology) were improved in these animals. This suggests that the various phyto-constituents found in the extract act indirectly on these sexual parameters by increasing the secretion of testosterone.

5. Conclusion

Euphobia hirta increases the weights of sex and accessory sexual organs through its androgenic properties and improves sperm quality through its antioxidant properties induced by phyto-constituents such as flavonoids, phenols, saponins and alkaloids present in its aqueous extract and which contributed to the elimination of free radicals leading to an improvement in percentage motility and sperm concentration as well as sperm morphology.

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Authors' Contributions

1) Study concept and design: Egbe Ben Besong and Nguedjang Nzepang Mesack Alassane.

2) Data acquisition: Nchegang Benjamin and Nguedjang N. M. Alassane.

3) Data analysis: Bertrand Yuwoung Wanyu and Germain Sotoing Taiwe.

4) Drafting of manuscript: Egbe Ben Besong and Nchegang Benjamin.

5) Critical revision of the manuscript: Egbe Ben Besong, Ngwasiri Nancy.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] World Health Organisation (2002) Working Document on Sexual and Reproductive Health.

[2] Yakubu, M.T., Akanji, M.A. and Oladiji, A.T. (2007) Male Sexual Dysfunction and Methods Used in Assessing Medicinal Plants with Aphrodisiac Potentials. *Pharmacological Reviews*, **1**, 49-56.

[3] Kim, S.J., Kim, M.R., Hwang, S.Y., Bae, W.J., Kim, S., Hong, S.H., *et al.* (2013) Preliminary Report on the Safety of a New Herbal Formula and Its Effect on Sperm Quality. *The World Journal of Men's Health*, **31**, 254-261. https://doi.org/10.5534/wjmh.2013.31.3.254

[4] Vander Borght, M. and Wyns, C. (2018) Fertility and Infertility: Definition and Epidemiology. *Clinical Biochemistry*, **62**, 2-10. https://doi.org/10.1016/j.clinbiochem.2018.03.012

[5] Hasanpoor-Azghdy, S.B., Simbar, M. and Vedadhir, A. (2014) The Emotional-Psy-

chological Consequences of Infertility among Infertile Women Seeking Treatment: Results of a Qualitative Study. *International Journal of Reproductive Medicine*, **12**, 131-138.

[6] Godmann, M., Lambrot, R. and Kimmins, S. (2009) The Dynamic Epigenetic Program in Male Germ Cells: Its Role in Spermatogenesis, Testis Cancer, and Its Response to the Environment. *Microscopy Research and Technique*, **72**, 603-619. https://doi.org/10.1002/jemt.20715

[7] Oyeyemi, M.O., Olukole, S.G. and Esan, O. (2008) Sperm Morphological Studies of the West African Dwarf Buck Treated with Pumpkin Plant (*Cucurbita pepo*). *International Journal of Morphology*, **26**, 121-123. https://doi.org/10.4067/s0717-95022008000100020

[8] Sharpe, R.M. and Franks, S. (2002) Environment, Lifestyle and Infertility—An Inter-Generational Issue. *Nature Cell Biology*, **4**, S33-S40. https://doi.org/10.1038/ncb-nm-fertilitys33

[9] I. Mar been, M.r., E. AI-Snafi, A., M. Marbut, M. and Y. Allahwerdy, L. (2023) The Probable Therapeutic Effects of Date Palm Pollen in the Treatment of Male Infertility. *Tikrit Journal of Pharmaceutical Sciences*, **1**, 30-35. https://doi.org/10.25130/tjphs.2005.1.4.30.35

[10] Low, B., Das, P.K. and Chan, K. (2013) Standardized Quassinoid-Rich *Eurycoma longifolia* Extract Improved Spermatogenesis and Fertility in Male Rats via the Hypothalamic-Pituitary-Gonadal Axis. *Journal of Ethnopharmacology*, **145**, 706-714. https://doi.org/10.1016/j.jep.2012.11.013

[11] Singh, G.K. and Jemal, A. (2017) Socioeconomic and Racial/Ethnic Disparities in Cancer Mortality, Incidence, and Survival in the United States, 1950-2014: Over Six Decades of Changing Patterns and Widening Inequalities. *Journal of Environmental and Public Health*, **2017**, Article ID: 2819372. https://doi.org/10.1155/2017/2819372

[12] Jiang, D., Coscione, A., Li, L. and Zeng, B. (2017) Effect of Chinese Herbal Medicine on Male Infertility. *International Review of Neurobiology*, **135**, 297-311. https://doi.org/10.1016/bs.irn.2017.02.014

[13] Agarwal, A., Prabakaran, S. and Allamaneni, S.S. (2006) Relationship between Oxidative Stress, Varicocele and Infertility: A Meta-Analysis. *Reproductive BioMedicine Online*, **12**, 630-633. https://doi.org/10.1016/s1472-6483(10)61190-x

[14] Kesari, K.K., Agarwal, A. and Henkel, R. (2018) Radiations and Male Fertility. *Reproductive Biology and Endocrinology*, **16**, Article No. 118. https://doi.org/10.1186/s12958-018-0431-1

[15] Wang, S., Wang, S., Li, C., Lin, C., Huang, H., Tsai, L., *et al.* (2018) The Therapeutic Effects of Traditional Chinese Medicine for Poor Semen Quality in Infertile Males. *Journal of Clinical Medicine*, **7**, Article 239. https://doi.org/10.3390/jcm7090239

[16] Boroujeni, S.N., Bossaghzadeh, F., Malamiri, F.A., Esmaeili, A. and Moudi, E. (2021) The Most Important Medicinal Plants Affecting Sperm and Testosterone Production: A Systematic Review. *JBRA Assisted Reproduction*, **26**, 522-530. https://doi.org/10.5935/1518-0557.20210108

[17] Moghbelinejad, S., Mozdarani, H., Ghoraeian, P. and Asadi, R. (2018) Basic and Clinical Genetic Studies on Male Infertility in Iran during 2000-2016: A Review. *International Journal of Reproductive BioMedicine*, **16**, 131-148. https://doi.org/10.29252/ijrm.16.3.131

[18] Zhou, S.H., Deng, Y.F., Weng, Z.W., Weng, H.W. and Liu, Z.D. (2019) Traditional Chinese Medicine as a Remedy for Male Infertility: A Review. *The World Journal of Men's Health*, **37**, 175-181. https://doi.org/10.5534/wjmh.180069

[19] Zhang, K., Fu, L., An, Q., Hu, W., Liu, J., Tang, X., *et al.* (2020) Effects of Qilin Pills on Spermatogenesis, Reproductive Hormones, Oxidative Stress, and the TSSK2 Gene in a Rat Model of Oligoasthenospermia. *BMC Complementary Medicine and Therapies*, **20**, Article No. 42. https://doi.org/10.1186/s12906-019-2799-7

[20] Csupor-Löffler, B., Hajdú, Z., Zupkó, I., Réthy, B., Falkay, G., Forgo, P., *et al.* (2008) Antiproliferative Effect of Flavonoids and Sesquiterpenoids from *Achillea millefolium* S.L. on Cultured Human Tumour Cell Lines. *Phytotherapy Research*, **23**, 672-676. https://doi.org/10.1002/ptr.2697

[21] Ventura, C., Nieto, M.R.R., Bourguignon, N., Lux-Lantos, V., Rodriguez, H., Cao, G., *et al.* (2016) Pesticide Chlorpyrifos Acts as an Endocrine Disruptor in Adult Rats Causing Changes in Mammary Gland and Hormonal Balance. *The Journal of Steroid Biochemistry and Molecular Biology*, **156**, 1-9. https://doi.org/10.1016/j.jsbmb.2015.10.010

[22] Gwet-Bell, E., Gwet, B.B., Akoung, N. and Fiadjoe, M.K. (2018) The 5 Main Challenges Faced in Infertility Care in Cameroon. *Global Reproductive Health*, **3**, e16. https://doi.org/10.1097/grh.0000000000000016

[23] Cabada-Aguirre, P., López López, A.M., Mendoza, K.C.O., Garay Buenrostro, K.D., Luna-Vital, D.A. and Mahady, G.B. (2023) Mexican Traditional Medicines for Women's Reproductive Health. *Scientific Reports*, **13**, Article No. 2807. https://doi.org/10.1038/s41598-023-29921-1

[24] Bertrand, P., Ahmed, H., Ngwafor, R. and Frazzoli, C. (2016) Toxicovigilance Systems and Practices in Africa. *Toxics*, **4**, Article 13. https://doi.org/10.3390/toxics4030013

[25] Unuane, D., Tournaye, H., Velkeniers, B. and Poppe, K. (2011) Endocrine Disorders & Female Infertility. *Best Practice & Research Clinical Endocrinology & Metabolism*, **25**, 861-873. https://doi.org/10.1016/j.beem.2011.08.001

[26] Hossain, M.A., AL-Raqmi, K.A.S., AL-Mijizy, Z.H., Weli, A.M. and Al-Riyami, Q. (2013) Study of Total Phenol, Flavonoids Contents and Phytochemical Screening of Various Leaves Crude Extracts of Locally Grown *Thymus vulgaris*. *Asian Pacific Journal of Tropical Biomedicine*, **3**, 705-710. https://doi.org/10.1016/s2221-1691(13)60142-2

[27] Abdi, F. and Hosseini, H. (2012) Experiences of Vasectomy: A Phenomenological Study. *North American Journal of Medical Sciences*, **4**, 619-623. https://doi.org/10.4103/1947-2714.104311

[28] Besong, E.B., G., A., Kamanyi, A. and Moumbock, A.F.A. (2019) Aphrodisiac Effects of Methanolic Leaf Extract of *Pseudopanax arboreus* (Araliaceae) (L.F. Phillipson) In Normal Male Rats. *African Journal of Traditional, Complementary and Alternative Medicines*, **16**, 24-33. https://doi.org/10.21010/ajtcam.v16i1.3

[29] Besong, E.B., Sylvain, B.M.O., Mbi, T.K., Wanyu, B.Y., Taiwe, G.S. and Marinash, B.B. (2024) *Dicliptera Laxata* (Acanthaceae) (C. B. Clark) Unblocks an Abamectin-Blocked Estrous Cycle and Restores Cyclicity in Female Albino Rats. *European Journal of Zoology*, **3**, 1-10. https://doi.org/10.24018/ejzoo.2024.3.2.27

[30] Ajayi, E.I.O., Adeleke, M.A., Adewumi, T.Y. and Adeyemi, A.A. (2017) Antiplasmodial Activities of Ethanol Extracts of *Euphorbia hirta* Whole Plant and *Vernonia amygdalina* Leaves in *Plasmodium berghei*-Infected Mice. *Journal of Taibah University for Science*, **11**, 831-835. https://doi.org/10.1016/j.jtusci.2017.01.008

[31] Maya, D. (2018) Phytochemical Composition, Toxicity, Antioxidant and Lactogenic Activities of *Euphorbia hirta* (L.). *International Journal of Advanced Research*, **6**, 322-335. https://doi.org/10.21474/ijar01/7524

[32] Besong, E.B., Ateufack, G., Babiaka, S.B. and Kamanyi, A. (2018) Leaf-Methanolic Extract of *Pseudopanax arboreus* (Araliaceae) (L. F. Phillipson) Reverses Amitriptyline-Induced Sexual Dysfunction in Male Rats. *Biochemistry Research International*, **2018**, Article ID: 2869727. https://doi.org/10.1155/2018/2869727

[33] Njoku-Oji, N., Osaji, D., Ifegwu, N., Uchefuna, R. and Ezejindu, D. (2015) Effects of Aqueous Extract of Ficus Capensis Leaf on Some Reproductive Parameters in Normal Adult Male Wistar Rats. *Indian Journal of Multilingual Research and Development*, **2**, 577-583.

[34] Ralebona, N., Sewani-Rusike, C.R. and Nkeh-Chungag, B.N. (2012) Effects of Ethanolic Extract of Garcinia Kola on Sexual Behaviour and Sperm Parameters in Male Wistar Rats. *African Journal of Pharmacy and Pharmacology*, **6**, 1077-1082. https://doi.org/10.5897/ajpp11.652

[35] Adimoelja, A. (2000) Phytochemicals and the Breakthrough of Traditional Herbs in the Management of Sexual Dysfunctions. *International Journal of Andrology*, **23**, 82-84. https://doi.org/10.1046/j.1365-2605.2000.00020.x

[36] Brandes, D. (2012) Male Accessory Sex Organs: Structure and Function in Mammals. Elsevier.

[37] Chung, F., Rubio, J., Gonzales, C., Gasco, M. and Gonzales, G.F. (2005) Dose-Response Effects of *Lepidium meyenii* (Maca) Aqueous Extract on Testicular Function and Weight of Different Organs in Adult Rats. *Journal of Ethnopharmacology*, **98**, 143-147. https://doi.org/10.1016/j.jep.2005.01.028

[38] Aprioku, J.S. (2013) Pharmacology of Free Radicals and the Impact of Reactive Oxygen Species on the Testis. *Journal of Reproduction & Infertility*, **14**, 158-172.

[39] Ilieva, I., Sainova, I. and Nikolov, B. (2019) Free Radicals, Male Reproductive Diseases and Male Infertility: A Review. *Canadian Journal of Biomedical Research*, **1**, 1-28.

[40] Kooti, W., Mansouri, E., Ghasemiboroon, M., Harizi, M., Ashtary-Larky, D. and Afrisham, R. (2014) The Effects of Hydroalcoholic Extract of *Apium graveolens* Leaf on the Number of Sexual Cells and Testicular Structure in Rat. *Jundishapur Journal of Natural Pharmaceutical Products*, **9**, e17532. https://doi.org/10.17795/jjnpp-17532

[41] Moher, D., Liberati, A., Tetzlaff, J. and Altman, D.G. (2009) Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLOS Medicine*, **6**, e1000097. https://doi.org/10.1371/journal.pmed.1000097

[42] Khursheed, A., Jain, V. and Wani, A.R. (2022) Euphorbia Hirta as a Gold Mine of High-Value Phytochemicals: A Comprehensive Review of Its Pharmacological Activities and Possible Role against SARS-CoV-2. *Biomedical Research and Therapy*, **9**, 4930-4949. https://doi.org/10.15419/bmrat.v9i2.728

[43] Asha, S., Thirunavukkarasu, P., Mani, V. and Sadiq, A. (2016) Antioxidant Activity of Euphorbia Hirta Linn Leaves Extracts. *European Journal of Medicinal Plants*, **14**, 1-14. https://doi.org/10.9734/ejmp/2016/24952

[44] Methorst, C. and Huyghe, E. (2014) Stress Oxydant et Infertilité Masculine: Physiopathologie et Intérêt Thérapeutique des Antioxydants. *Progrès en Urologie*, **24**, 4-10.

[45] Ganiyu, Y.A., Temitayo Wahab, A. and Olug-benga Oyeyemi, M. (2022) Fertility Assessment of the Male Albino Rats (Wistar Strain) Treated with Aqueous and Ethanol Leaf Extracts of Euphorbia Hirta Linn. *Acta Scientific Veterinary Sciences*, **4**, 63-73. https://doi.org/10.31080/asvs.2022.04.0515

[46] Kunle-Alabi, O.T., Akindele, O.O., Oyovwi, M.O., Duro-Ladipo, M.A. and Raji, Y. (2014) *Cocos nucifera* L. Water Improves Reproductive Indices in Wistar Rats. *African Journal of Medicine and Medical Sciences*, **43**, 305-313.

[47] Al-Shaikh, M.N., Wahab, T.A.L.A., Kareem, S.H.A. and Hamoudi, S.R. (2013) Protective Effect of Chufa Tubers (*Cyperus esculentus*) on Induction of Sperm Abnormalities in Mice Treated with Lead Acetate. *International Journal of Drug Development & Research*, **5**, 387-392.

[48] Nwozo, O.S., Effiong, E.M., Aja, P.M. and Awuchi, C.G. (2023) Antioxidant, Phytochemical, and Therapeutic Properties of Medicinal Plants: A Review. *International Journal of Food Properties*, **26**, 359-388. https://doi.org/10.1080/10942912.2022.2157425

[49] Sriraman, V., Rao, V.S., Sairam, M.R. and Rao, A.J. (2000) Effect of Deprival of LH on Leydig Cell Proliferation: Involvement of PCNA, Cyclin D3 and igf-1. *Molecular and Cellular Endocrinology*, **162**, 113-120. https://doi.org/10.1016/s0303-7207(00)00201-x

[50] Sriraman, V., Sairam, M. and Rao, A. (2003) Evaluation of Relative Roles of LH and FSH in Regulation of Differentiation of Leydig Cells Using an Ethane 1,2-Dimethylsulfonate-Treated Adult Rat Model. *Journal of Endocrinology*, **176**, 151-161. https://doi.org/10.1677/joe.0.1760151

[51] Baburski, A.Z., Andric, S.A. and Kostic, T.S. (2019) Luteinizing Hormone Signaling Is Involved in Synchronization of Leydig Cell's Clock and Is Crucial for Rhythm Robustness of Testosterone Production. *Biology of Reproduction*, **100**, 1406-1415. https://doi.org/10.1093/biolre/ioz020

[52] Olaolu, T., Rotimi, D. and Olaolu, A. (2018) Effect of Alcohol Infusion of Cissus Populnea Root on Testicular Function and Serum Hormone of Male Wistar Rats. *Asian Pacific Journal of Reproduction*, **7**, 117-122. https://doi.org/10.4103/2305-0500.233572