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Research Article

Fertility Assessment of the Male Albino Rats (Wistar Strain) Treated with Aqueous and Ethanol Leaf Extracts of Euphorbia Hirta Linn

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Abstract

Euphorbia hirta has been used in traditional medicine for the treatment of respiratory, digestive problems as well as many other diseases. However, its effects on fertility have not been fully studied. Hence, this study on the effect of the aqueous and ethanol extract of *Euphorbita hirta* on of the male albino rats (Wistar strain) was studied.

Aqueous and ethanol extract of *Euphorbia hirta* leaf was prepared to qualitative phytochemical analysis using standard methods. Sixty-six (66) rats assigned into six groups A-F with 11 rats each were used in this study. Rats in groups A and B received 500mg/kg and 1000mg/kg of aqueous extract respectively for 14 days, those in groups C and D received similar dosage for ethanol extract respectively while group E and F received distilled water and 10% DMSO+90% distilled water respectively for 14days. On days 1, 7 and 14 post treatments, three rats were sacrificed from across the group. The testes, epididymis and semen samples were collected for analysis and the Data collected were analyzed.

Phytochemical analysis revealed the presence of saponins, tannins, flavonoids, cardiac glycoside, anthraquinone, terpenoid, phenols, alkaloid and steroids in the ethanol extract of the plant while terpenoid and steroid were absent from the aqueous extract. There were significant increases in the sperm percentage motility, livability at day 14 post treatment compared to days 1 and 7 post treatments. Increase in the total number of morphologically abnormal sperm cells observed across the treatment groups at day 1 post treatment falls within the normal range (20%). Gonadometric indices (testicular and epididymal biometry) showed no significant differences Histological features of the gonads (testes and epididymis) showed no observable lesion.

Oral treatment of rats with 1000mg/kg aqueous leaf extract *Euphorbia hirta* increased the sperm motility, livability and count continuously for 14 days of treatment. Therefore, the aqueous plant extract can be used to improve the fertility of male breeding stock at 1000mg/kg bodyweight.

Keyword: *Euphorbia Hirta;* Semen Characteristics; Haemogram; Wistar Rats

Introduction

The use of herbal medicine in treating human ailments and animal diseases has gained a wide acceptable globally in the recent past. It is the oldest form of practice known to mankind especially in developing countries -like Nigeria where several medicinal plants are used to treat or cure specific diseases locally with little or no scientific knowledge [1].

Euphorbia hirta linn is one of the medicinal plants that are widely used traditionally in Nigeria, Africa and other parts of the world. It has a worldwide distribution and its common name in-

clude asthma weed, milkweed [2]. It is named locally in Nigeria as *ẹmile* in Yoruba, *nóónòn kúrcíyáá* in Hausa and ọ̀bụ ànị̀ in Igbo [15]. This important plant grows up to 40cm tall and its mostly found growing in open spaces, path sides, roadsides and gardens. It is widespread throughout the West African sub-region and dispersed pan-tropically and sub-tropically around the world.

The medicinal potentials of *Euphorbia hirta* against certain disease conditions have been studied by different researchers such as Ali., *et al*. [3] who reported that the crude extract of *Euphorbia hirta* possesses antidiarrheal effect, as well as anticancer activity. The aqueous extract significantly reduced the release of prostaglandin I_2 (also called Prostacyclin), prostaglandin E_2 (Dinoprostone) and prostaglandin D_2 (PGD_2) [4]. Aflatoxin contamination in rice, wheat, maize, and mustard crops is also reduced by the aqueous extract as affirmed by Singh and Sinha [5]. Methanolic leaf extract of *Euphorbia hirta* has an antifungal and antibacterial property. The leaves are warmed and rubbed on itchy soles after being pounded with turmeric and coconut oil. To treat eye sores, it was reported by Kumar., *et al*. [4] that *Euphorbia hirta* latex is applied to the lower eyelids, similar to surma. The root exudate has nematocidal activity against *Meloidogyne incognita* juveniles [4].

The aqueous extract of *Euphorbia hirta*, showed an antioxidant effect and a free radical scavenging activity in various *in vitro* models like total antioxidant and total ferric reducing power determination, assay for free radical-scavenging activity using ABTS, DPPH, and hydroxyl radical scavenging assays. It showed maximum antioxidants and free radical scavenging activities, at 0.25 mg/ml. The free radical scavenging effect on DPPH and hydroxyl was found as 68.80 ± 5.21 and 73.36 ± 5.21%, respectively [6]. However, its effect on male animal fertility is unknown, therefore this study is designed to investigate the effect of aqueous and ethanol leaf extract of *Euphorbia hirta* leaf on fertility indices in the male albino rats (Wistar strain).

Materials and Method

- **Experimental Animals:** Sixty-six male albino rats weighing between 150 and 200g were used for this study. They were randomly assigned into 6 groups A-F (n = 11). The study was carried out at the Laboratory Animal House of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria situated between longitude 3.895^0E and latitude 7.379^0N with relative humidity from 46.3% in the dry season to 80.1% in the rainy season [7].

- **Plant collection and Identification:** Fresh leaves of *Euphorbia hirta* were harvested from the botanical garden of the University of Ibadan and identified at the herbarium of the Department of Botany, University of Ibadan Nigeria with a voucher number UIH-22998.

- **Plant Preparation and Phytochemical Screening:** The plant extracts and phytochemical screening were carried out at the Organic Laboratory of the Department of Pharmaceutical Chemistry Faculty of the Pharmacy University of Ibadan Nigeria. The aqueous leaf extract of *Euphorbia hirta* (ALEEH) and Ethanol leaf extract of *Euphorbia hirta* (ELEEH) were prepared by methods described by Oyeyemi., *et al*. [8], Omotayo., *et al*. [9] respectively and administered orally at 500mg/kg and 1000mg/kg for 14 days.

- **Acute Toxicity:** Acute and sub-chronic oral toxicity of *Euphorbia hirta* was evaluated in Sprague Dawley rats by Yuet Ping., *et al*. [10] and reported that the plant extract at a single dose of 5000 mg/kg did not produce treatment-related signs of toxicity or mortality in any of the animals tested during the 14-day observation period. Therefore, the LD 50 of this plant was estimated to be more than 5000 mg/kg and this was adopted for the present study, Yuet Ping., *et al*. [10].

- **Study Design:** Sixty-six (66) rats assigned into six groups A-F with 11 rats each were used in this study. Rats in groups A and B received 500mg/kg and 1000mg/kg of aqueous extract respectively for 14 days, those in groups C and D received similar dosage for ethanol extract respectively while group E and F received distilled water and 10% DMSO+90% distilled water respectively for 14days. On days 1, 7 and 14 post treatments, three rats were sacrificed from across the group. The testes, epididymis and semen samples were collected for analysis.

Live body weights were taken every week with the use of a digital weighing scale and the weight of each rat was recorded.

Three (3) rats from each Group (A-F) were sacrificed on days 1, 7 and 14 post- treatments.

Following which blood, semen, testes and epididymal samples were harvested for semen and gonadometric studies.

- **Collection of the testes and epididymis:** The animals were humanely sacrifices after which a caudoventral mid-abdominal incision was made using a sterilized scissors in order to access the internal organs. The testes were located once pushed upward from the scrotum. Then, the testes were detached from the epididymis and harvested using the method described by [7].

- **Semen Collection and Analysis:** After the animals were sacrificed, semen sample was collected from the caudal epididymis and analyzed as modified by Ajani and Oyeyemi [11] to determine the sperm motility, livability and the sperm count.

- **Gonadometric Assessment:** the testicular and epididymal biometry was done as described by Oyeyemi and Ajani, [12]. This includes weight of left testis, weight of right testis, length of left testis, length of right testis, diameter of left testis, diameter of right testis and weight of epididymis.

- **Sperm Motility**: Drop of semen was placed on a warm microscopic slide mixed with drop of sodium citrate and covered with a cover slip. The sample was observed under microscope at X 10 magnification and the percentages recorded; only sperm cells moving in a unidirectional motion were included in the count, while cells moving in circles, backward direction or pendulous movements were excluded [17].

- **Percentage livability**: drop of semen was placed on microscopic slide in 1% eosin nigrosin stain solution, cells were distinguished by adding one drop of the stain to one drop of the semen at room temperature and smearing the mixture on microscopic slide, membrane permeability was used as basis for differentiation [16].

- **Sperm Concentration*:*** The concentration was determined by the use of the improved Neubauer hemocytometer. Semen was pipetted to the 0.5 mark using the blood cell pipette and this was made up to1.0 mark with normal saline. The normal saline serves both to dilute the semen and fix the spermatozoa present. The pipette was introduced into a pipette shaker and allowed to mix. About 2 or 3 drops diluted sperm was discarded from the pipette before been introduced under the cover slip on the counting chamber from each side of the hemocytometer. The hemocytometer was carefully placed in a closed pre-wetted chamber for five minutes before been viewed under light microscope at X40 objective. Sperm heads that have more than half the sperm head within the large five squares that formed the diagonal segment of squares of the haemocytometer chambers was counted. The sperm concentration was determined and calculated as Concentration/ml = (Dilution Factor) (Count in 5 squares) ($0.05 \text{ X } 10^6$) [12].

Data analysis

Data were analyzed by descriptive statistics using One-way Analysis of variance (ANOVA) and the mean; Standard Deviation and coefficient of variation and regression were calculated and compared by using Duncan Multiple Range Test (DMRT) at a 5% probability level on IBM SPSS Version 20.0 Statistical package. The results were expressed as Mean ± SD.

Results

The result of phytochemical composition of aqueous and ethanol Leaf extract of *Euphorbia hirta* obtained (Table 1) showed that the quality of saponins, tannins, cardiac glycoside and phenols were higher in ethanol leaf extract of *Euphorbia hirta* (ELEEH) than aqueous leaf extract of *Euphorbia hirta* (ALEEH), whereas alkaloid was lower in ELEEH compared to ALEEH concentrations. While terpenoid and steroids were absent in the aqueous leaf extract, both extracts showed flavonoids and anthraquinone of close values.

Result obtained at day 1 post-treatment (Figure 3) showed that the percentage (%) sperm liveability was lower across the groups except for group B compared to the controls (Groups E and F). Group B's value was higher significantly compared to other test groups.

On day 7 post-treatment (Figure 3) there was no significant changes ($P > 0.05$) in the mean values of percentage (%) liveability. However, there was an increase in the mean values in groups A and B and a decrease in groups C and D but are not significant when compared to the control.

Phytochemical	Aqueous Leaf Extract	Ethanol Leaf Extract
Saponins	+	++
Tannins	+	++
Flavonoid	++	++
Cardiac glycosides	+	++
Anthraquinone	++	++
Terpenoid	_	++
Phenol	+	++
Alkaloid	++	+
Steroids	_	++

Table 1: Phytochemical composition of Aqueous and ethanol Leaf extract of *Euphorbia hirta.*

Sperm quality assessment

On day 1 post-treatment the sperm motility decreased across the groups compared to the control (Figure 1) the mean sperm motility values decreased across the groups compared to the control. The differences were significant ($P < 0.05$) for groups A, B and D compared to the control.

The result obtained on day 7 (Figure 1) shows that there were no significant changes ($P > 0.05$) in the mean values of percentage (%) sperm motility across the group. However, there was a decrease ($P > 0.05$) in the mean values in A, B, C and D compared to the controls.

On day 14 post-treatment (Figure 1) showed that the percentage (%) sperm motility values increased across the groups except for group D when compared to it control (Group F).

The time dependent effect (Figure 2) showed that the percentage sperm motility increased progressively as days of treatment increased in each of the group A, B, C and D. Group B and C showed the highest increase at days 7 and 14 post-treatment.

The result obtained at day 14 post-treatment (Figure 3) showed that the mean values of percentage (%) liveability increased across the group A and B when compared with the control in group E. The difference observed in group A and B were significant ($P < 0.05$) while the increase in groups C and D were not significant compared to the control in group F.

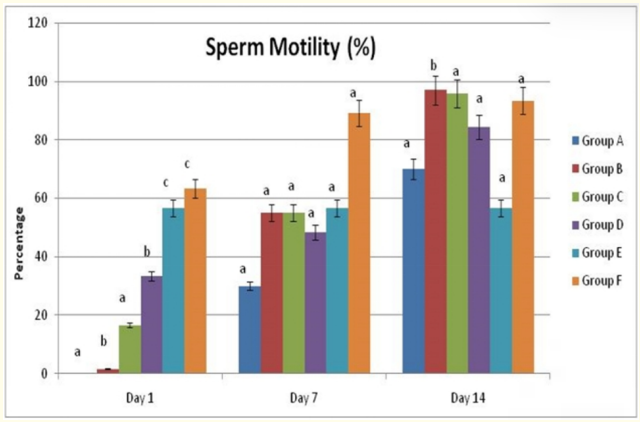

Figure 1: Sperm motility of the male albino rats treated with aqueous and ethanol leaf extracts of *Euphorbia hirta* on days 1, 7 and 14 post-treatments.
abc: values with different superscript differ significantly.

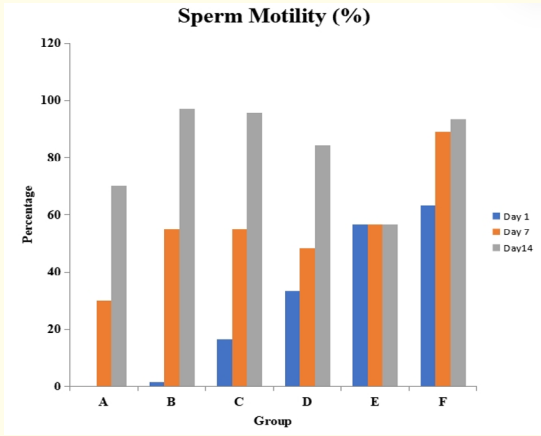

Figure 2: Time-dependent effect on Sperm motility of the male albino rats treated with aqueous and ethanol leaf extracts of *Euphorbia hirta* on days 1, 7 and 14 post-treatments.

The time dependent effect (Figure 4) showed that the percentage sperm liveability increased progressively as days of treatment increased in each of the group A, B, C and D. At day 1 post-treatment, group B showed the highest percentage sperm liveability. Groups B and C showed the highest increase at day 14 post-treatment.

The result obtained in (Figure 5) shows at day one post-treatment there was no significant change in the sperm count across the groups. The mean values of groups C and D were higher than groups A and B but the differences were not significant (P > 0.05) compared to the controls (Group F) and (Group E) respectively.

On day 7 there were no significant changes (P > 0.05) in the mean values of sperm concentration across the groups. However, there was a decrease in the mean values of sperm concentration in groups B, C, D and increase in group A compared to the controls.

On day 14 post-treatment the mean values of sperm count increased across the groups except in group D that there was a significant (P < 0.05) decrease compared to control (group F). The increase observed in groups A and B were significant (P < 0.05) when compared to the control (Group E).

The time dependent effect (Figure 6) showed that the sperm concentration increased progressively as days of treatment increased in groups A and B. The sperm concentration decreased in groups C and D at day 7 post-treatment compared to day 1. The sperm concentration values increased across the groups at day 14 post-treatment and Group B and C showed the highest increase.

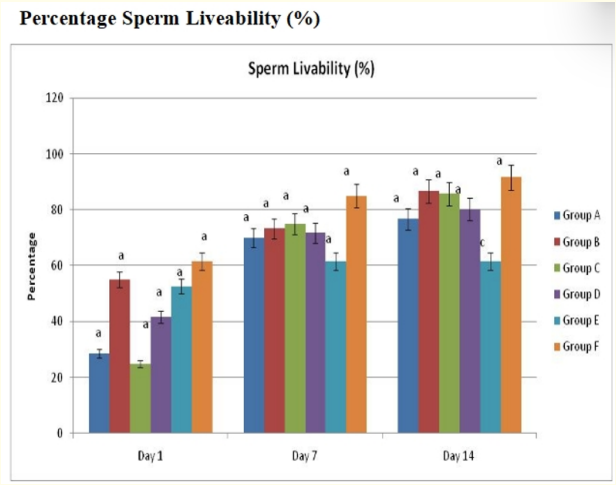

Figure 3: Percentage Sperm liveability of the male albino rats treated with aqueous and ethanol leaf extracts of *Euphorbia hirta* on days 1, 7 and 14 post-treatments.
abc: values with different superscript differ significantly.

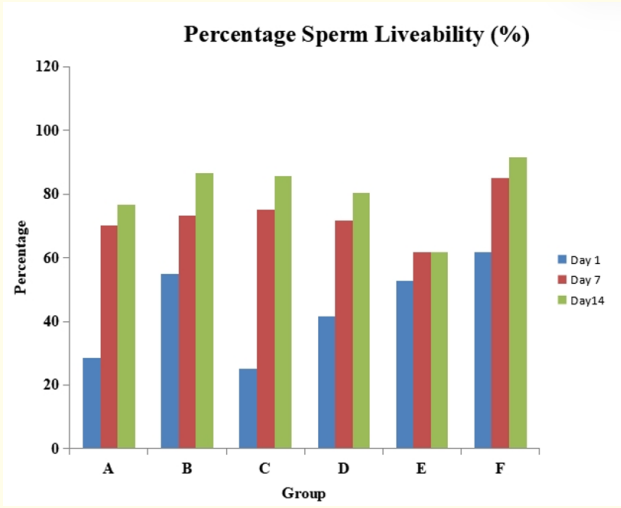

Figure 4: Time-dependent effect on Percentage Sperm liveability of the male albino rats treated with aqueous and ethanol leaf extracts of *Euphorbia hirta* on days 1, 7 and 14 post-treatments.

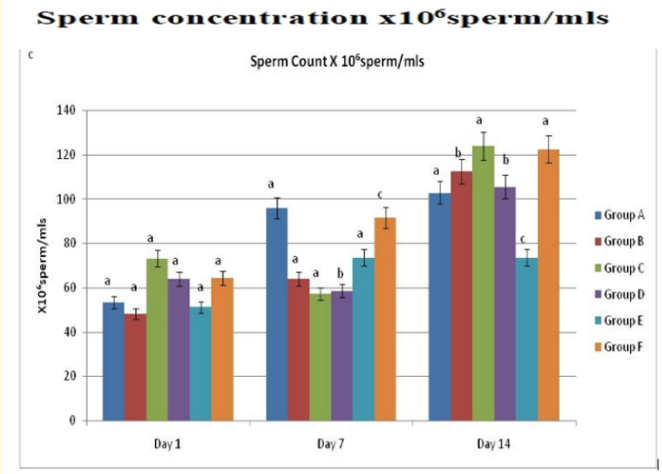

Figure 5: Sperm concentration of the male albino rats treated with aqueous and ethanol leaf extracts of *Euphorbia hirta* on days 1, 7 and 14 post-treatments.
abc: values with different superscript differ significantly.

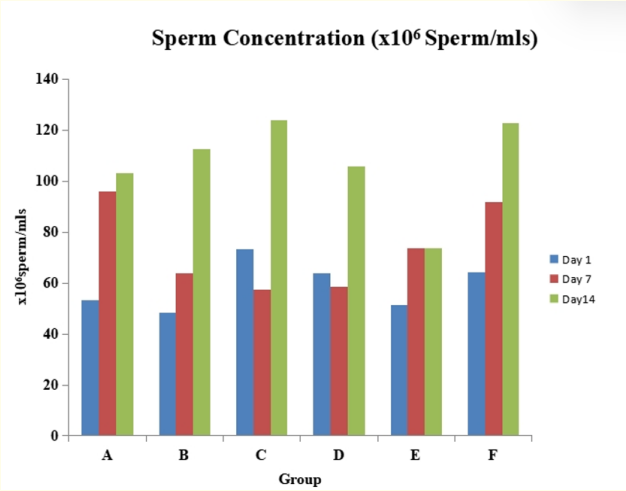

Figure 6: Time-dependent effect on Sperm concentration of the male albino rats treated with aqueous and ethanol leaf extracts of *Euphorbia hirta* on days 1, 7 and 14 post-treatments.

Sperm morphology characteristics

The result obtained (Table 2) shows there were increases in total sperm abnormalities in groups A, B, C compared to the control which were not significant. However, the mean values of the total abnormal cell (TAC) for groups A and B were higher than C and D.

The result obtained (Table 3) shows that the mean total abnormal cell (TAC) of groups A, B, C and D increased compared to the control. The difference was significant in group D and also group D had the highest mean Total abnormal cell (TAC), however, results obtained on day 14 post-treatment shows no significant changes in the TAC.

Gonadometric Indices

Biometrical assessment of the testes and epididymis: Result obtained (Table 5) shows that there was no significant changes in the mean body weight and testicular biometrical values at Day 1 post-treatment except for a significant reduction in the Epididymal weight of group B rats.

Group (%)	Group A (500mg/kg aqueous extract)	Group B (1000mg/kg aqueous extract)	Group C (500mg/kg ethanol extract)	Group D (1000mg/kg ethanol extract)	Group E (Control for aqueous extract)	Group F (Control for ethanol extract)
Coiled tail	1.00 ± 1.00 [a]	4.00 ± 0 [a]	2.00 ± 1.00 [a]	1.33 ± 0.67 [a]	3.00 ± 0.58 [a]	3.00 ± 0.58 [a]
Doubled head	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 ±
Double tail	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 ± [a]	0.00 ±
Abnormal head	4.00 ± 0.58 [a]	1.67 ± 0.88 [a]	3.33 ± 0.33 [a]	3.00 ± 0.58 [a]	3.00 ± 0.58 [a]	3.00 ± 1.53 [a]
Headless tail	0.67 ± 0.67 [a]	2.00 ± 0.00 [b]	1.00 ± 1.00 [a]	0.67 ± 0.67 [a]	0.00 ± 0.00 [a]	0.00 ± 0 [a]
Tailless head	0.67 ± 0.67 [a]	2.00 ± 0 [c]	1.00 ± 1.00 [a]	0.67 ± 0.67 [a]	0.00 ± 0 [a]	0.00 ± 0 [a]
Bent tail	3.00 ± 0.58 [a]	3.67 ± 0.33 [a]	3.67 ± 0.88 [a]	3.33 ± 0.33 [b]	3.00 ± 0.58 [a]	3.33 ± 0.33 [b]
Looped tail	1.67 ± 0.88	1.00 ± 1.00	2.33 ± 0.33	1.00 ± 0.58	2.67 ± 0.33	3.33 ± 0.30
Rudimentary tail	1.67 ± 0.88 [a]	1.67 ± 0 [a]	1.33 ± 0.67 [a]	0.33 ± 1.00 [a]	0.00 ± 0.58 [a]	1.00 ± 0.67 [a]
Curved mid piece	3.67 ± 0.88 [a]	0.00 ± 0 [a]	0.67 ± 0.66 [a]	1.00 ± 1.00 [a]	2.00 ± 0.58 [a]	0.67 ± 0.67 [a]
Total abnormal cell	16.33 ± 1.33 [a]	16.00 ± 2.00 [a]	15.33 ± 4.16 [a]	11.33 ± 1.15 [a]	13.67 ± 3.21 [a]	14.33 ± 2.33 [a]
Percentage abnormality %	3.69 ± 0.21 [a]	3.89 ± 0.29 [a]	3.18 ± 0.35 [a]	2.52 ± 0.34 [a]	3.15 ± 0.43 [a]	3.34 ± 0.55 [a]
Total cell count	441.67 ± 18.35 [a]	411.67 ± 0.88 [a]	477.67 ± 30.99 [a]	460.33 ± 40.92 [a]	433.67 ± 11.26 [a]	430.33 ± 8.74 [a]

Table 2: Sperm morphology abnormalities of the male albino rats treated with aqueous and ethanol leaf extracts of *Euphorbia hirta* on day 1 post-treatment.

Values are reported as mean ± SEM.

abc: Means in the same row with different superscript differ significantly ($P < 0.05$).

Group (%)	Group A (500mg/kg aqueous extract)	Group B (1000mg/kg aqueous extract)	Group C (500mg/kg ethanol extract)	Group D (1000mg/kg ethanol extract)	Group E (Control for aqueous extract)	Group F (Control for ethanol extract)
Coiled tail	1.00 ± 1.00 [a]	4.00 ± 0 [a]	2.00 ± 1.00 [a]	1.33 ± 0.67 [a]	3.00 ± 0.5 8 [a]	3.00 ± 0.58 [a]
Doubled head	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 ±
Double tail	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]	0.00 ± [a]	0.00 ±
Abnormal head	4.00 ± 0.58 [a]	1.67 ± 0.88 [a]	3.33 ± 0.33 [a]	3.00 ± 0.58 [a]	3.00 ± 0.58 [a]	3.00 ± 1.53 [a]
Headless tail	0.67 ± 0.67 [a]	2.00 ± 0.00 [b]	1.00 ± 1.00 [a]	0.67 ± 0.67 [a]	0.00 ± 0.00 [a]	0.00 ± 0 [a]
Tailless head	0.67 ± 0.67 [a]	2.00 ± 0 [c]	1.00 ± 1.00 [a]	0.67 ± 0.67 [a]	0.00 ± 0 [a]	0.00 ± 0 [a]
Bent tail	3.00 ± 0.58 [a]	3.67 ± 0.33 [a]	3.67 ± 0.88 [a]	3.33 ± 0.33 [b]	3.00 ± 0.58 [a]	3.33 ± 0.33 [b]
Looped tail	1.67 ± 0.88	1.00 ± 1.00	2.33 ± 0.33	1.00 ± 0.58	2.67 ± 0.33	3.33 ± 0.30
Rudimentary tail	1.67 ± 0.88 [a]	1.67 ± 0 [a]	1.33 ± 0.67 [a]	0.33 ± 1.00 [a]	0.00 ± 0.58 [a]	1.00 ± 0.67 [a]
Curved mid piece	3.67 ± 0.88 [a]	0.00 ± 0 [a]	0.67 ± 0.66 [a]	1.00 ± 1.00 [a]	2.00 ± 0.58 [a]	0.67 ± 0.67 [a]
Total abnormal cell	16.33 ± 1.33 [a]	16.00 ± 2.00 [a]	15.33 ± 4.16 [a]	11.33 ± 1.15 [a]	13.67 ± 3.21 [a]	14.33 ± 2.33 [a]
Percentage abnormality %	3.69 ± 0.21 [a]	3.89 ± 0.29 [a]	3.18 ± 0.35 [a]	2.52 ± 0.34 [a]	3.15 ± 0.43 [a]	3.34 ± 0.55 [a]
Total cell count	441.67 ± 18.35 [a]	411.67 ± 0.88 [a]	477.67 ± 30.99 [a]	460.33 ± 40.92 [a]	433.67 ± 11.26 [a]	430.33 ± 8.74 [a]

Table 2: Sperm morphology abnormalities of the male albino rats treated with aqueous and ethanol leaf extracts of *Euphorbia hirta* on day 1 post-treatment.

Values are reported as mean ± SEM.

abc: Means in the same row with different superscript differ significantly (P < 0.05).

Group (%)	Group A (500mg/kg aqueous extract)	Group B (1000mg/kg aqueous extract)	Group C (500mg/kg ethanol extract)	Group D (1000mg/kg ethanol extract)	Group E (Control for aqueous extract)	Group F (Control for ethanol extract)
Coiled tail	2.00 ± 0.58 [a]	3.33 ± 1.67 [a]	2.33 ± 0.33 [a]	3.00 ± 0.58 [a]	3.00 ± 0.58 [a]	3.00 ± 0.58 [a]
Doubled head	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]
Double tail	0.67 ± 0.67 [a]	0.67 ± 0.67 [a]	0.00 ± 0.00 [a]	0.67 ± 0.67 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]
Abnormal head	4.00 ± 0.58 [a]	2.33 ± 1.20 [a]	3.00 ± 0.58 [a]	3.33 ± 0.33 [a]	3.00 ± 0.58 [a]	2.00 ± 1.15 [a]
Headless tail	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]
Tailless head	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± .00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]
Bent tail	3.67 ± 1.20 [a]	2.00 ± 1.15 [a]	1.33 ± 0.67 [a]	4.33 ± 0.33 [a]	3.00 ± 0.58 [a]	4.33 ± 0.33 [a]
Looped tail	3.00 ± 0.58 [a]	1.00 ± 1.00 [a]	4.00 ± 0.00 [a]	3.67 ± 0.67 [a]	2.67 ± 0.33 [a]	2.67 ± 0.33 [a]
Rudimentary tail	1.00 ± 0.58 [a]	1.67 ± 0.88 [a]	2.67 ± 0.33 [a]	2.33 ± 1.20 [a]	0.00 ± 0.00 [a]	1.33 ± 0.33 [a]
Curved mid piece	1.00 ± 1.00 [a]	2.00 ± 1.15 [a]	2.00 ± 1.15 [a]	1.67 ± 1.67 [a]	2.00 ± .58 [a]	1.67 ± 0.88 [a]
Total abnormal cell	15.33 ± 1.20 [a]	13.00 ± 1.00 [a]	15.33 ± 2.91 [a]	19.00 ± 1.15 [a]	13.67 ± 0.86 [a]	15.00 ± 0.58 [a]
Percentage abnormality %	3.05 ± 0.19 [a]	3.16 ± 0.23 [a]	3.07 ± 0.48 [a]	3.94 ± 0.36 [a]	3.15 ± 0.43 [a]	3.13 ± 0.24 [a]
Total cell count	502.00 ± 13.58 [b]	411.00 ± 3.61 [a]	494.00 ± 8.48 [a]	485.00 ± 8.25 [a]	433.6711.26 [a]	484.67 ± 39.55 [a]

Table 3: Sperm morphology abnormalities of the male albino rats treated with aqueous and ethanol leaf extracts of *Euphorbia hirta* on day 7 post treatment.

Values are reported as the mean ± SEM.

abc: Means in the same row with different superscript differ significantly (P < 0.05).

Parameters (%)	Group A (500mg/kg aqueous extract)	Group B (1000mg/ kg aqueous extract)	Group C (500mg/ kg ethanol extract)	Group D (1000mg/ kg ethanol extract)	Group E (Control for aqueous extract)	Group F (Control for ethanol extract)
Coiled tail	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	4.00 ± 0.58 [b]	3.00 ± 0.58 [b]	3.00 ± 0.00 [b]
Doubled head	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]
Double tail	1.00 ± 1.00 [a]	1.00 ± 1.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]
Abnormal head	1.67 ± 0.88	1.67 ± 0.88 [a]	3.67 ± 0.33 [a]	2.33 ± 1.20 [a]	3.00 ± 0.58 [a]	4.00 ± 0.00 [a]
Headless tail	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]
Tailless head	0.00 ± 0.00	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]
Bent tail	3.00 ± 1.53 [a]	2.33 ± 1.20 [a]	3.67 ± 0.33 [a]	4.67 ± 0.33 [b]	3.00 ± 0.58 [a]	2.00 ± 0.00 [a]
Looped tail	3.33 ± 0.88 [a]	2.00 ± 1.15 [a]	4.00 ± 0.58 [a]	4.33 ± 0.88 [a]	2.67 ± 0.33 [a]	3.67 ± 0.33 [a]
Rudimentary tail	3.33 ± 0.33 [a]	1.00 ± 1.00 [a]	2.67 ± 0.33 [a]	1.67 ± 0.88 [a]	0.00 ± 0.00 [b]	2.67 ± 0.33 [a]
Curved mid piece	0.67 ± 0.67 [a]	3.00 ± 1.00 [a]	1.67 ± 0.88 [a]	1.33 ± 0.33 [a]	2.00 ± 0.58 [a]	2.67 ± 0.33 [a]
Total abnormal cell	15.67 ± 1.76 [a]	13.67 ± 1.45	18.33 ± 1.67 [a]	18.33 ± 2.19 [a]	13.67 ± 1.86 [a]	18.00 ± 0.58 [a]
Percentage abnormality %	3.43 ± 0.32 [a]	3.23 ± 0.49 [a]	3.66 ± 0.29 [a]	3.49 ± 0.41 [a]	3.15 ± 0.43 [a]	3.68 ± 0.12 [a]
Total cell count	456.00 ± 20.82 [a]	442.33 ± 20.99 [a]	499.33 ± 7.22 [a]	525.33 ± 8.09 [a]	433.67 ± 11.26 [a]	490.33 ± 0.88 [a]

Table 4: Sperm morphology abnormalities of the male albino rats treated with aqueous and ethanol leaf extracts of *Euphorbia hirta* on day 14 post-treatment.

Parameters	Group A (500mg/kg aqueous extract)	Group B (1000mg/ kg aqueous extract)	Group C (500mg/ kg ethanol extract)	Group D (1000mg/ kg ethanol extract)	Group E (control for aqueous extract)	Group F (control for ethanol extract)
Body weight (g)	156.33 ± 5.84 [a]	167.67 ± 14.10 [a]	171.67 ± 4.41 [a]	173.33 ± 7.45 [a]	169.33 ± 12.35 [a]	163.00 ± 1.73 [a]
Left testis weight(g)	0.90 ± 0.07 [a]	1.01 ± 0.05 [a]	0.97 ± 0.05 [a]	1.04 ± 0.04 [a]	0.97 ± 0.12 [a]	0.85 ± 0.03 [a]
Right testis weight(g)	0.95 ± 0.10 [a]	0.96 ± 0.07 [a]	0.97 ± 0.04 [a]	1.02 ± 0.12 [a]	1.00 ± 0.12 [a]	0.85 ± 0.05 [a]
Left testis length (mm)	19.76 ± 0.76 [a]	18.38 ± 0.81 a	16.65 ± 0.81 [a]	19.00 ± 0.26 [a]	17.69 ± 0.26 [a]	17.71 ± 1.12 [a]
Right testis length (mm)	18.35 ± 0.41 [a]	18.03 ± 0.58 [a]	17.03 ± 0.48 [a]	17.63 ± 0.42 [a]	17.87 ± 0.88 [a]	16.72 ± 0.34 [a]
Left testis diameter (mm)	10.08 ± 0.75 [a]	9.68 ± 0.33 [a]	9.14 ± 0.62 [a]	9.47 ± 0.39 [a]	8.64 ± 1.46 [a]	9.09 ± 0.20 [a]
Right testis diameter (mm)	9.69 ± 0.66 [a]	8.59 ± 0.41 [a]	8.71 ± 0.55 [a]	9.45 ± 0.36 [a]	10.43 ± 0.60 [a]	9.11 ± 0.31 [a]
Epididymis weight(g)	0.23 ± 0.05 [a]	0.23 ± 0.04 [b]	0.44 ± 0.10 [a]	0.37 ± 0.06 [a]	0.33 ± 0.02 [c]	0.42 ± 0.06 [a]

Table 5: Testicular and Epididymal Biometrics of the male albino rats treated with aqueous and ethanol leaf extracts of *Euphorbia hirta* on day 1 post-treatment.

Values are reported as mean ± SEM.

abc: Means in the same row with different superscript differ significantly ($P < 0.05$).

The result obtained at day 7 post-treatment (Table 6) shows that there were no significant changes in the mean body weight across the group when compared to the control except for group D in which the bodyweight increase was significant (191.33 ± 3.93) when compared with the control.

Parameters	Group A (500mg/ kg aqueous extract)	Group B (1000mg/ kg aqueous extract)	Group C (500mg/ kg ethanol extract)	Group D (1000mg/ kg ethanol extract)	Group E (Control for aqueous extract)	Group F (Control for ethanol extract)
Body weight (g)	186.33 ± 1.45 [a]	181.67 ± 7.36 [a]	155.33 ± 6.94 [a]	191.33 ± 3.93 [b]	169.33 ± 12.35 [a]	154.00 ± 3.79 [a]
Left testis weight (g)	0.91 ± 0.08 [a]	1.02 ± 0.02 [a]	0.92 ± 0.04 [a]	1.00 ± 0.03 [a]	0.97 ± 0.12 [a]	0.90 ± 0.06
Right testis weight (g)	0.92 ± 0.10 [a]	1.02 ± 0.01 [a]	0.93 ± 0.04 [a]	1.02 ± 0.03 [a]	1.00 ± 0.12 [a]	0.89 ± 0.06 [a]
Left testis length (mm)	17.57 ± 0.21 [a]	18.37 ± 0.31 [a]	17.15 ± 0.36 [a]	17.77 ± 0.26 [a]	17.69 ± 0.26 [a]	16.94 ± 0.30 [a]
Right testis length(mm)	17.50 ± 0.43 [a]	18.29 ± 0.32 [a]	17.88 ± 0.30 [a]	17.65 ± 0.26 [a]	17.87 ± 0.88 [a]	16.50 ± 0.20 [a]
Left testis diameter(mm)	8.84 ± 0.49 [a]	9.55 ± 0.19 [a]	9.09 ± 0.21 [a]	9.64 ± 0.10 [a]	8.64 ± 1.46 [a]	9.12 ± 0.19 [a]
Right testis diameter (mm)	8.52 ± 0.32 [a]	9.76 ± 0.05 [b]	9.24 ± 0.08 [a]	9.55 ± 0.13 [a]	10.43 ± 0.60 [a]	9.13 ± 0.14 [b]
Epididymis weight (g)	0.28 ± 0.01 [a]	0.25 ± 0.01 [b]	0.22 ± 0.01 [a]	0.24 ± 0.02 [b]	0.33 ± 0.02 [a]	0.18 ± 0.03 [a]

Table 6: Testicular and Epididymal Biometry of the male albino rats treated with aqueous and ethanol leaf extracts of *Euphorbia hirta* on day 7 post-treatment.

Values are reported as mean ± SEM.

abc: Means in the same row with different superscript differ significantly ($P < 0.05$).

There was a significant decrease in the mean values of right testes diameter and Epididymal weight for group B compared to the control. Conversely, these values increased significantly ($P < 0.05$) in groups C and D.

The result obtained (Table 7) shows that there were no significant changes in the body weight and the biometrical parameters at Day 14 across the groups except a decrease in the Right testes Diameter for group B which was significant ($P < 0.05$).

Parameters	Group A (500mg/ kg aqueous extract)	Group B (1000mg/ kg aqueous extract)	Group C (500mg/ kg ethanol extract)	Group D (1000mg/ kg ethanol extract)	Group E (control for aqueous extract)	Group F (control for ethanol extract)
Body weight (g)	166.67 ± 4.33[a]	153.00 ± 11.59[a]	156.33 ± 2.03[a]	168.00 ± 6.43[a]	169.33 ± 12.35[a]	145.00 ± 6.56[a]
Left testis weight (g)	1.08 ± 0.03 [a]	0.98 ± 0.03 [a]	1.01 ± 0.05 [a]	0.99 ± 0.03 [a]	0.97 ± 0.12 [a]	0.82 ± 0.04 [a]
Right testis weight (g)	1.06 ± 0.04 [a]	0.95 ± 0.05 [a]	0.98 ± 0.04 [a]	0.99 ± 0.03 [a]	1.00 ± 0.12 [a]	0.85 ± 0.04 [a]
Left testis length (mm)	17.79 ± 0.15[a]	16.85 ± 0.51[a]	15.65 ± 1.86[a]	17.44 ± 0.20[a]	17.69 ± 0.26[a]	15.78 ± 1.11[a]
Right testis length (mm)	18.04 ± 0.11[a]	19.04 ± 0.78[a]	17.24 ± 0.48[a]	17.32 ± 0.08[a]	17.87 ± 0.88[a]	15.97 ± 0.83[a]
Left testis diameter (mm)	9.69 ± 0.24[a]	8.81 ± 0.11[a]	8.99 ± 0.55[a]	9.35 ± 0.13[a]	8.64 ± 1.46[a]	8.38 ± 0.31
Right testis diameter (mm)	9.44 ± 0.26 [a]	8.99 ± 0.40 [a]	8.98 ± 0.19 [a]	9.52 ± 0.22 [a]	10.43 ± 0.60 [a]	8.67 ± 0.41 [a]
Epididymis weight (g)	0.36 ± 0.03 [a]	0.28 ± 0.03 [a]	0.30 ± 0.01 [a]	0.27 ± 0.01 [a]	0.33 ± 0.02 [a]	0.31 ± 0.02 [a]

Table 7: Testicular and Epididymal Biometry of the male albino rats treated with aqueous and ethanol leaf extracts of *Euphorbia hirta* on day 14 post-treatment.

Values are reported as the mean **±** SEM

abc: Means in the same row with different superscript differ significantly ($P < 0.05$).

Discussion

The study was designed to assess the fertility potential of the male Wistar rats treated with aqueous and ethanol leaf extracts of *Euphorbia hirta linn.*

Phytochemical analysis revealed the presence of saponins, tannins, flavonoid, cardiac glycoside, anthraquinone, terpinoid, phenols, alkaloid and steroids in the ethanol extract of the plant while terpinoid and steroid were absent from the aqueous extract. The phytoconstituents in the extracts suggests their fertility regulatory potentials, as a number of plant metabolites – alkaloids, saponins, flavonoids and phenolic acids – have been shown to exert fertility regulatory effect acting as an antioxidant and enhancing male fertility potential by prevention and management of oxidative stress [13].

The initial decrease observed in the sperm motility and liveability across the treatment groups might be attributed to a transient shock triggered by sudden administration of both the ALEEH and ELEEH in the rats. This observation, however, change as the treatment days increased. There were significant increases in the sperm percentage motility, liveability at days 14 post-treatment compared to days 1 and 7 post-treatment. This observation is more prominent in group B and this suggests that administration of ALEEH at a dosage of 1000mg/kg will improve the fertility of male rats, as sperm motility and liveability are major fertility indices in the male animal [14].

It also suggests that prolonged treatment with the ALEEH at a dosage of 1000mg/kg will boost the semen quality of male albino Wistar rats since the sperm parameters (sperm motility, sperm livability and sperm concentration) continued to increase with increasing days of treatment.

There was an initial increase in the total number of morphologically abnormal sperm cells across the treatment groups at day 1 post-treatment. However, this observation falls within the maximum of 20% allowed [8].

The increase in the sperm morphological abnormalities was consistent in groups C and D. This implies that continuous and prolonged administration of 500mg/kg and 1000mg/kg ethanol extract of the plant might be toxic to the male rats, thereby precipitate infertility. This also suggests that the aqueous extract might be safer than the ethanol extract at similar dosages. Overall, evidence from this current study revealed that the aqueous extract of the plant *Euphorbia hirta* has more bioactive advantage than its ethanol extract.

Observation on the testicular and epididymal biometry was not consistent with the results of the semen characteristics. For instance, there was a significant decrease in epididymal weight and Right Testes Diameter of group B that had increased sperm motility, liveability and sperm count. These discrepancies might be attributed to variations in the weights of the experimental rats used in this study. Meanwhile, there were no observable significant changes in the biometrical parameters of other treated groups.

Conclusion

In conclusion, 1000 mg/kg of aqueous leaf extract of *Euphorbia hirta* increased sperm motility, percentage livability and sperm concentration in Wistar rat 14 days post-treatment. These suggest the aqueous extract of the plant possesses a profertility property.

500mg/kg and1000 mg/kg of ethanol leaf extract of *Euphorbia hirta* increased sperm morphological abnormalities as days of treatment increased, this implies that prolonged administration of ethanol leaf extract of the plant at these dosages might be toxic to the male rats, thereby precipitate infertility. Both aqueous and ethanol extract of the plant had no negative effect on gonadometric indices of the male Wistar rats. This study also reveals that oral administration of aqueous and ethanol extracts of *Euphorbia hirta (*AELEHA) has no negative effects on the semen characteristics and the gonadometrics of the male albino rats. Also, oral treatment with 1000 mg/kg of aqueous leaf extract *Euphorbia hirta* (AELEHA) increased sperm motility, liveability and concentration continuously for 14 days of treatment. Therefore, the aqueous leaf extract of *Euphorbia hirta* at an oral dose of 1000 mg/kg possesses pro-fertility properties, howbeit; the ethanol extract of the plant at the same dose may precipitate infertility.

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