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Original Research Article

SEMEN CHARACTERISTICS AND FERTILITY OF MALE WISTAR RATS ADMINISTERED *EUPHORBIA HIRTA* ETHANOL LEAF EXTRACT

***AIYEYIKA, M.K., AKOMAS, S.C. & EGU, U.N.**
Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria.

**Correspondence:* moseskola96@gmail.com +2347030380742

ABSTRACT
Euphorbia hirta has been used in folk medicine for the treatment of disease like respiratory and digestive problem, however, its effect on reproductive performance have not been fully studied. Hence, this study on the effect of *Euphorbia hirta* ethanol extract on reproductive parameters of male albino rats (Wistar strain) was studied. Forty-eight adult male Wistar rats were randomly divided into four groups and were administered 0 (control), 50, 100, and 150 mg/kg body weight of the ethanol leaf *Euphorbia hirta* extract orally for 66 days. The result showed that the extract significantly altered the pH of the epididymal reserve, sperm color, and livability and increased the number of twisted tail spermatozoa at the highest dose. However, the extract did not significantly affect sperm motility, and concentration. Furthermore, the fertility test showed no significant differences in the total number of pups per litter, number of dead pups and average birth weight of the pups delivered by female Wistar rats mated with the treated male rats. The result suggests that the ethanol leaf of *Euphorbia hirta* have adverse effects on male reproductive performance, particularly at dose of 150 mg/kg and above. So caution should be exercise.

Keywords: *Euphorbia hirta*, Fertility, Semen characteristics, Wistar rats

INTRODUCTION
The rise in infertility cases amongst males globally, has been attributed to environmental hazard, diseases, radiation and drug abuse which has resulted to decline in semen quality (Abarikwu, 2013). Infertility may be caused by low sperm count, inadequate sperm motility, abnormal morphological structures or a combination of these factors (Kumar *et al*., 2015). Plants and derivatives of plant have been used globally as a safe source of medicine. Humans and animals have not only relied on plants for healing ailments but also on meeting their necessities such as food and shelter (Shical *et al*., 2010).). For several hundred years, people around the world have used locally grown plants as supplements to energize, vitalize, and eventually improve male sexual functions, although many numerous plants are known historically for their ability to negatively affect male fertility; Jahan *et al*. (2009) reported the anti-fertility effects of some tested medicinal plants. Antifertility effects of plants may be due to ecobolic, estrogenic and spermaticidal properties from the toxic effects of the extracts on the reproductive organ (Orieke *et al*., 2019).

Euphorbia hirta has a worldwide distribution, and it is found throughout the temperate parts of India, Asia, Australia, and Africa, often found in low land, paddy fields, garden, waste places and on the road side (Ghosh *et al.*, 2019). They prefer dry and humid condition (Ghosh *et al*., 2019). Its common name is asthma weed and milk weed (Al-Snafi, 2017). *Euphorbia hirta*, is known as Egele in Yoruba, Obu ani in Igbo and Noonon Kurciya in Hausa (Asha *et al*., 2014). It is a medicinal plant that has been long used as traditional medicine (Asha *et al*., 2014). *Euphorbia hirta* is a plant belonging to the phylum Angiosperm and family *Euphorbiaceae.* It is an annual, branched herb with branches up to 50cm long *Euphorbia hirta* has been a subject of phyto-chemical investigation and compounds including flavenoids, triterpenoids, alkanes, amino acid and alkaloids have been isolated (Asha *et al*., 2014). *Euphorbia hirta* as a weed is considered as beneficial for its diverse application in traditional medicine. Although the herbal medicine play an

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important role as source of medicine and widely used for prevention and treatment of diseases, its impact on fertility parameters of male albino rats are not always considered especially if consumed above permissible levels.

This study evaluated the effect of ethanol leaf extract of *Euphorbia hirta* on reproductive parameters of male albino rats and to provide more knowledge on the extent to which the extract affect reproductive parameters at lower doses considering the fact that literature in this regard is quite scanty.

METHODOLOGY

ETHICAL CONSIDERATION

All procedure in this study were performed according to the Animal Ethic Committee, Michael Okpara University of Agriculture, Umudike (MOUAU/CVM/REC/202322).

EXPERIMENTAL ANIMALS

Forty-eight (48) male albino rats (wistar strain) weighing between 90 – 180g within 8 weeks of age were used for the study, were used for this experiment. They were divided into four treatment groups T_1, T_2, T_3 and T_4. Each treatment group consists of 12 rats replicated 3 times with 4 rats in a Completely Randomized Design (CRD) with four levels of the *Euphorbia hirta* ethanol extracts as treatment.

The study was carried out in the Research and Teaching Laboratory Unit, Department of Veterinary Physiology Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike (MOUAU), Nigeria. Situated between latitude of 5°29'N and longitude 7°32'E and altitude of 123m above the sea level with an annual rainfall of 2177m, average ambient temperature of 22.32°C and relative humidity of above 50-90 (NRCRI – Umudike, 2011).

PLANT COLLECTION AND IDENTIFICATION

Fresh leaves of *Euphorbia hirta* were harvested at various part of the University environment, Michael Okpara University of Agriculture, Umudike, Abia State. The plant was identified and authenticated at the Taxonomy Section, Forestry department, Michael Okpara University of Agriculture, Umudike by Prof M.C. Dike.

PLANT PREPARATION

The leaves were washed to remove dirt and air-dried for 3- 4 days until a constant weight was attained, it was then blended into fine powder using a mechanical grinder and stored in air tight plastic containers.

The ethanol leaf extract of *Euphorbia hirta* (ELEEH) were prepared by method described by Azwainida (2015), and administered at 50mg/kg, 100mg/kg and 150mg/kg respectively.

ACUTE TOXICITY

Acute and sub-chronic oral toxicity of *Euphorbia hirta* was evaluated in Sprague Dawley rats by Yuet *et al*. (2013) 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 any animals tested during the 14 days 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.

STUDY DESIGN

Forty-eight (48)male albino rats (Wistar strain) weighing between 90 – 180g within 8 weeks of age were divided into four treatment groups. Each treatment group consist of twelve (12)rats replicated 3 times with 4 rats per replicate in a Completely Randomized Design (CRD) with four levels of the *Euphorbia hirta* ethanolic extract as treatment. Rats in group 2, 3 and 4 received 50mg/kg, 100mg/kg and 150mg/kg of the extract once daily respectively. The control rats in group 1 received an equal volume of distilled water/kg body weight.

The extract was administered orally with gavage for 66 days. At the end of the experiment, 3 rats were randomly selected from each replicate and kept off feed for 24 hours, but not without water and weighed before sacrifice, while the remaining treated male rats were transferred to individual cages and mated with untreated and sexually mature females in the ratio of one male to a female.

SPERM SAMPLE COLLECTION AND ANALYSIS

After the rats were sacrificed, sperm sample were collected from the caudal epididymis and analyzed as modified by Ajani and Oyeyemi (2014) to determine the sperm motility, livability and sperm count.

SPERM SAMPLE COLOUR AND CONSISTENCY

The colour of the sperm samples and consistency were evaluated by physical observation and recorded. The consistency scale (1-4) adopted by Chibundu (2013) was used. Colour scale (1-3) adopted was 1(white) 2 (milky white) and 3 (creamy white). For the consistency, the scale was 1 (watery), 2 (slightly thick), 3 (thick) and 4 (very thick).

SPERM MOTILITY

Drop of sperm sample 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 percentage 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 (Pant *et al*., 2003).

SPERM VIABILITY (LIVE RATIO)

The proportion of sperm cells that are viable (alive) were determined by staining a drop of collected semen with Eosin-Negrosin stain. According to Oyeyemi *et al*. (2008), the stained glass slide were allowed to dry for 30 seconds before being fixed with ethanol and viewed under light microscope at x100 magnification (oil immersion), and the proportion of the sperm cells that were viable was counted with a hand held stop watch manual counter.

For the study, a total of 300 cells were counted and the number of viable sperm cells was expressed as a percentage of the total counted. The sperm cells that were alive (viable) did not pick the stain while those that were dead did (Oyeyemi *et al*., 2008).

SPERM CONCENTRATION

This was determined in a haemocytometer in accordance with the method described by Ukar *et al*. (2016). A dilution of 1:200 was made using a red blood cell platelet. 10% buffered formalin solution was used as the semen diluting fluid to immobilize the sperm cells.

The haemocytometer was then charged with a drop of the sperm solution and allowed to stand for 2 minutes on a wet paper (for the sperm to settle) before mounting on a light microscope stage and viewed at x40 magnification. Sperm concentration per ml = Number of cells counted x dilution factor x 0.04 x 10^6 (Egbuka, 1995).

ABNORMAL SPERM PROPORTION

The percentage of abnormal sperm proportion was determined by the method described by Oyeyemi *et al*., (2008).

A drop of the semen was stained using Eosin- Negrosin stain and the mixture smeared on a glassed slide and viewed under a lower magnificationof x40 to check for primary and secondary abnormal sperm cells. Percentage of the differential abnormalities such as head, tail, and mid-piece abnormalities were determined (Oyeyemi *et al*., 2008).

THE SPERM SAMPLE PH

It was determined using ExTech$^{(R)}$ (China - 277836) for each of the treatment group.

FERTILITY TEST

Male rats treated for 66 days were introduced to parous female in the ratio of 1:1 for a period of 13 days (covering 3 estrous cycles) according to method reported by Rath *et al*. (2010) and Raichir *et al*. (2014) with modifications.

The day of parturition was designated as the first day of post natal life. After delivery, the litter size, average litter weight and percentage survival were determined and the values compared with that of the control. The percentage survival was calculated thus:

$$\% \text{ survival} = \frac{\text{Total liter size} - \text{Number of dead}}{\text{Total liter size}} \times \frac{100}{1}$$

STATISTICAL ANALYSIS

The values are reported as mean±SEM and analyzed using the student's t-test and ANOVA where necessary. $P< 0.05$ was accepted as significant.

RESULT

EFFECT OF ETHANOL LEAF EXTRACT OF *Euphorbia hirta* ON SPERMIOGRAM OF ALBINO RATS

The result of the spermiogram as presented in Table I showed significant ($p < 0.05$) impact of the graded doses of ELEEH on the pH of the epididymal reserve, when compared with the control group.

The colour of the epididymal reserve of the group treated with 150 mg/kg changed from creamy white (2.00±0.00) to milky white (1.33±0.33) after 66 days of treatment, when compared with the control group.

Although, there were more ($p < 0.05$), live spermatozoa in the 50 mg/kg ELEEH treated group than in 100, and 150 mg/kg ELEEH treated groups, however, ELEEH at the graded doses did not produce any significant ($p > 0.05$) effect on the consistency, progressive motility, epididymal and testicular counts of sperm reserve when compared with the control group.

EFFECT OF ETHANOL LEAF EXTRACT OF *Euphorbia hirta* ON SPERM MORPHOLOGICAL ABNORMALITIES OF ALBINO RATS

The result of the effect of ethanol extract of *Euphorbia hirta* on the sperm morphological abnormalities of Wistar rats in table II. The ELEEH at 150 mg/kg significant ($P<0.05$) increased the number of twisted tail.

However no significant difference was observed ($P>0.05$) on spermatozoa head, cytoplasmic droplets and mid-piece when compared with the control, though, 50 mg/kg ELEEH treated rats recorded less abnormal cells and more of normal spermatozoa morphology when compared with the 150 mg/kg treatment dose.

TABLE I: EFFECT OF ETHANOL LEAF EXTRACT OF *E HIRTA* ON SPERMIOGRAM OF ALBINO RATS

Parameters	Treatment groups			
	Distilled water	**50 mg/kg**	**100 mg/kg**	**150 mg/kg**
Colour	2.00±0.00[a]	2.00±0.00[a]	2.00±0.00[a]	1.33±0.33[b]
Consistency	3.66±0.33	4.00±0.00	3.66±0.33	3.33±0.33
pH	7.06±0.00[b]	7.11±0.01[a]	7.10±0.15[a]	7.12±0.08[a]
Progressive motility (%)	82.38±3.81	87.25±2.88	78.54±2.07	74.66±5.30
Live proportion (%)	88.87±2.78[ab]	92.62±2.14[a]	82.69±1.13[b]	82.61±3.87[b]
Sperm count ($\times 10^6$)	118.85±12.42	124.74±6.13	114.45±2.29	109.11±5.59
Testicular count ($\times 10^6$)	156.00±8.99	242.66±72.24	158.33±11.92	142.00±7.54

Values are presented as Mean ± SEM (Standard Error of Mean). Different superscript letters across treatment groups show significant ($p < 0.05$) differences

TABLE II: EFFECT OF ETHANOL LEAF EXTRACT OF *E HIRTA* ON SPERM MORPHOLOGICAL ABNORMALITIES OF ALBINO RATS

Parameters	Treatment group			
	Distilled water	**50 mg/kg**	**100 mg/kg**	**150 mg/kg**
Relative number of twisted tail (%)	1.37±0.03[b]	1.31±0.02[b]	1.42±0.04[b]	1.56±0.05[a]
Relative number of detached head/tailless (%)	1.77±0.11[ab]	1.54±0.10[b]	1.79±0.12[ab]	1.93±0.06[a]
Relative number with cytoplasmic droplets %	1.46±0.05	1.45±0.07	1.54±0.04	1.64±0.06
Relative number with bentmid-piece (%)	1.09±0.01	1.09±0.00	1.11±0.15	1.13±0.07
Total abnormal spermatozoa (%)	5.68±0.04[b]	5.40±0.19[b]	5.86±0.09[ab]	6.27±0.17[a]
Proportion with Normal morphology (%)	94.31±0.04[ab]	94.59±0.19[a]	94.13±0.09[a]	93.72±0.17[b]

Values are presented as Mean ± SEM (Standard Error of Mean). Different superscript letters across treatment groups show significant ($p < 0.05$) differences

TABLE III: EFFECT OF ETHANOL LEAF EXTRACT OF *E. HIRTA* ON MALE FERTILITY OF ALBINO RATS.

Parameters	Treatment group			
	Distilled water	**50 mg/kg**	**100 mg/kg**	**150 mg/kg**
Total number of pups per liter	7.50±1.32	8.50±0.28	8.00±0.40	9.00±0.81
Number of dead pups	0.00±0.00	0.25±0.25	0.25±0.25	0.25±0.25
Average birth weight (g)	6.04±0.47	6.15±0.29	6.03±0.34	5.78±0.44

Values are presented as Mean ± SEM (Standard Error of Mean). No significant ($p > 0.05$) differences across the groups

EFFECT OF ETHANOL LEAF EXTRACT OF *Euphorbia hirta* ON MALE FERTILITY OF Albino RATS

The effect of the *E. hirta* ethanol extract on male fertility is presented in table III. The non-significant ($p>0.05$) difference in the total number of pups per liter, number of dead pups and the average birth weight of the pups delivered by female albino rats mated with the ELEEH treated male albino rats when compared with the control group, showed no significant ($p>0.05$) effect of ELEEH on the fertility of the treated male albino rats at the various doses when compared with the fertility of the control male Wistar rats.

DISCUSSION

The study was designed to evaluate the fertility potential of male albino rats (Wistar strain) treated with ethanol leaf extract of *Euphorbia hirta*. Semen characteristics, sperm morphological abnormalities and fertility index were evaluated. These parameters are important reproductive indices that described male fertility.

The Sperm sample color was significantly change from creamy white to milky white at the dose of 150mg/kg of the ELEEH treated group when compared with the control group. This suggests that the ELEEH alters sperm sample color at dose of 150mg/kg and above. This could have negative effect on reproductive performance, sperm sample

color is expected to be creamy for better reproductive performance. This is not in consistent with the previous study by Orieke *et al.* (2019) on the effect of *Corchorus olitorius* leaf extract on sperm sample color of male wistar rats, Who reported that the sperm sample colour did not significantly differs among test groups and control.

Sperm sample pH is an important parameter in the evaluation of sperm sample quality, it is a well determinant of the survival rate of sperm cell. Acidic media, are known to increased sperm mortality and inhibit sperm performance and conception and are among the causes of infertility (Zhoun *et al*., 2015). Alkaline medium is the most ideal environment for sperm performance (Guyton & Hall, 2006). The result of the semen qualities as presented in Table I showed significant ($p<0.05$) impact of the graded doses of ELEEH on the pH of the epididymal reserve, when compared with the control group. Therefore the alkaline semen environment observed in the treated groups suggests that the ELEEH at the investigated doses favours sperm survival and this is comparable with the report of Orieke *et al*., 2019.

It is important to note that the extract does not alter the progressive motility of the epididymal reserve when compared with the control. This suggest that the extract may not negatively affect reproductive performance in male rats at the administered dose. Studies have linked progressive sperm motility with high fertility index (Love, 2011). The findings in this study disagrees with a previous report from an *in vivo* study, where oral dosing of WAD rams with *Euphorbia hirta* aqueous leaf extract for 14 days causes a reduction in sperm motility from 83 to 48 % (Oyeyemi *et al*., 2011). Abnormal and low sperm motilities are known to significantly lower fertility.

The finding at lower dose (50mg/kg) of the extract was seen to have a more pronounced effect on sperm viability, resulting in a higher live spermatozoa count. Conversely, the higher doses (100mg/kg and 150mg/kg) had a slight lower effect on sperm viability. This suggest that the administration ELEEH at 50 mg/kg will improve fertility of male rats but have a slight negative effect as the dosage increases. There was noticeable decline in the sperm viability in the extract treated rats as the dose increased. This finding is comparable with the reports of Oyeyemi *et al*. (2009) in WAD rams treated with *Euphorbia hirta*. The decline may be attributed to some toxic properties ELEEHwhich inhibit spermatozoa survival.

The result of the spermatozoa morphology as presented in Table II indicates that the *Euphorbia hirta* ethanol extract at 150mg/kg significantly increased the number of twisted tail and total abnormal spermatozoa following 66 days of treatment, compared to the control group. This agrees with the observation of Oyeyemi *et al*. (2011) in WAD bucks treated with the *Barbadensis miller*, and Wahab *et al*. (2022) in Male albino rats treated with *Euphorbia hirta* extract. Percentage abnormalities increased with increasing dosage of the ELEEH, thereby precipitate infertility.

On the fertility index of treated abino rats mated with the parous female rats, it showed that there was non-significant ($p>0.05$) difference in the total number of pups per liter, number of dead pups and the average birth weight of the pups delivered by female albino rats mated with the ELEEH treated male albino rats when compared with the control group. Thus there is no significant ($p>0.05$) effect of ELEEH on the fertility index of the treated male albino rats at the various doses when compared with the fertility of the control male albino rats.

CONCLUSION

The study revealed that ethanol leaf extract of *Euphorbia hirta* is beneficial to sperm sample characteristic at 50 mg.kg but could be detrimental as the dosage increases especially when taken above 150 mg/kg, so caution should be exercise. Further studies are required to elucidate the impact of the ELEEH on hormone and gonadosomatic index of male albino rats or other animal model.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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