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SEMEN CHARACTERISTICS OF WISTAR RATS TREATED WITH METHANOLIC EXTRACT OF JATROPHA GOSSYPIFOLIA

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ABSTRACT

Aim: The study was designed to determine the effect of methanolic leave extract of *Jatropha gossypifolia* on semen characteristics in Wistar rats.

Methods: Twenty-five male Wistar rats were divided into 5 groups (A, B, C, D and E) comprising 5 rats each. Rats in group A served as control, and were administered distilled water *per os* for 21 days, while rats in groups B, C, D and E were administered oral 100, 300, 500 and 1000 mg/kg bodyweight of *Jatropha gossypifolia* leaf extract for 21 days, respectively. At the end of the treatment period, the rats were humanly sacrificed and semen was collected by open castration and evaluated.

Results: There was a dose dependent decrease in the mass activity, sperm motility, sperm concentration and percentage live sperm cells of the treated groups. These were significantly ($p<0.05$) different from the control group except group B. Percentage abnormalities characterized by detached head, double head, curved head, giant head, coiled tail, curved tail and curved mid-piece were seen during the study. These abnormalities were significantly ($p<0.05$) higher in the treated groups (C – E) than the control.

Conclusion: It was concluded from the study that *Jatropha gossypifolia* leave extract above 100 mg/kg is capable of disrupting the reproductive process of male Wistar rats through poor semen characteristics. However, extract at not more than100 mg/kg body weight may be safe.

Key words: *Jatropha gossypifolia*, Sperm abnormalities, concentration, motility

INTRODUCTION

Jatropha gossypifolia is a multipurpose medicinal plant used for treatment of many diseases (Jain *et al*., 2013; Sabandar *et al*., 2013). The plant is also known as "bellyache-bush" or "black physicnut" or "cotton-leaf physicnut" and belongs to the Euphorbiaceae family that occurs predominantly in subtropical, tropical and tropical semi-arid areas of Africa, Asia and the Americas (Mariz *et al*., 2010; Felix-Silva *et al*., 2014). Extracts from the leaves, stems, roots, seeds and latex have been studied (Sharma and Singh, 2012) and the phytochemical ingredients of alkaloids, coumarins, flavonoids, lignoids, phenols, saponins, steroids, tannins, and terpenoids detected (Zhang *et al*., 2009). The seed and fruits of *Jatropha gossypifolia* have laxative, analgesic, neuropharmacological, anti-diarrheal and anti-influenza properties (Apu *et al*., 2013, Sabandar *et al*. 2013); while the leaves have anti-inflammatory (Nagaharika *et al*., 2013); anti-plasmodial (Jansen *et al*., 2010); anticoagulant (Oduola *et al*., 2005), such as in

nose bleeding (Oduola *et al*., 2007); anti-microbial (Dhale and Birari, 2010) and pesticide properties (Valencia *et al*., 2006). The leaves are also used in managing intermittent fevers, carbuncles, eczema, itches, sores on the tongues of babies, and venereal disease (Oduola *et al*., 2007). The roots and stems have similar activities as the leaf, in addition to having cytotoxic properties (de Almeida *et al*., 2015), while the latex has antibacterial properties (Gaikwad *et al*., 2012). Other uses of the plant are biodiseal production (Ceasar and Ignacimuthu, 2011); vermifuge, ornamentation and religious use (Sabandar *et al*. 2013; Felix-Silva *et al*., 2014). In spite of all the therapeutic properties of the plant, studies have showed some level of toxicity. However, this is influenced by the dose of administration, extract employed, animal model and part of the plant used (Felix-Silva *et al*., 2014). The latex and seeds are probably the most toxic part, producing nervous and digestive disturbances (de Almeida *et al*., 2015). Experimental poisoning of sheep with single dose of fresh *Jatropha gossypifolia* leaves was toxic, with histopathological signs evident in the digestive, respiratory and circulatory systems (Oliveira *et al*., 2008). In rats, signs of ptosis have been observed in single oral dose (Felix-Silva *et al*., 2014), while reduced central nervous system activity and digestive disorders were observed in chronic toxicity studies (Mariz *et al*., 2012). The anti-fertility properties of *Jatropha gossypifolia* plant have been evaluated in females such as rats (Jain *et al*., 2013) and albino mice (Jain *et al*., 2012), and were characterized by alterations in reproductive hormones like the follicular stimulating hormone (FSH), luteinizing hormone (LH) and estrogen. This has prompted the use of the plant in family planning (Yogesh, 2002). However, similar report of the plant on male reproduction is lacking to the best of our knowledge. This study was therefore designed to determine the effect of *Jatropha gossypifolia* on the semen characteristics of Wistar rats.

MATERIALS AND METHODS

Experimental Animals

The animal experiments followed the principles of Laboratory animal care (CACC, 1993). Forty three (43) mature Laboratory bred male Wistar rats weighing between 180 – 220 grams were used for the study. They were housed in wired cages and fed pelleted poultry growers mash and water *ad libitum* throughout the study.

Plant Material and Extraction

Leaves of *Jatropha gossypifolia* were obtained from Chukuku village in Kuje area of Abuja, Nigeria. The plant was identified at the Herbarium centre of the University of Abuja, Nigeria. The leaves were air-dried at room temperature and pulverized by a mechanical grinder to powdered form with initial weight of 680 grams. This was soaked in methanol as the solvent extractor for 24 hours after which it was filtered and concentrated with rotary evaporator to a final weight of 14.30 grams giving a percentage yield of 2.10 %.

Determination of Oral LD_{50}

The LD_{50} was determined using modified Lorke's method (Lorke, 1983). A total of 18 male Wistar rats were divided into 6 groups (A, B, C, D, E and F) comprising 3 rats each. The first 3 groups (A, B and C) were administered 10, 100 and 1000 mg/kg oral extract of the plant, respectively, and monitored for 24 hours. Thereafter, the remaining 3 groups (D, E and F) were administered 1225, 2500 and 5000 mg/kg oral extract, respectively, and also monitored for 24 hours. The LD_{50} was calculated based on rat mortality within 24 hours and obtained by multiplying the square root of maximum tolerated dosage by maximum dosage of dead as described by Walum (1998).

LD_{50} = √(max. dosage tolerated) x √(max. dosage dead).

Semen Characteristic

The remaining 25 male Wistar rats were divided into 5 groups (A, B, C, D and E) comprising 5 rats each. Rats in group A served as control, and were administered distilled water *per os* for 21 days. Those in groups B, C, D and E were administered 100, 300, 500 and 1000 mg/kg bodyweight of methanolic leaf extract of *Jatropha gossypifolia* orally for 21 days, respectively. At the end of the study, the animals were humanly sacrificed and semen collected by open castration and analyzed.

Semen Collection

To collect semen, orchidectomy was performed using open castration by incising the scrotum to milk out the testicles from the incision site. The left and right testicles were popped out as the tunica vaginalis was incised. The caudal and cranial epididymides were detached from the testes, rinsed in warm normal saline at 37^0C and immediately minced in 2 ml normal saline.

Semen Evaluation

Semen was evaluated based on the methods described by Zemjanis (1970). A drop of raw undiluted semen sample was placed on a pre-warmed slide, with a drop of Trisodium citrate buffer then cover-slipped and viewed using a field microscope at x40 magnification to determine the mass activity and motility of each sample. Sperm concentration was estimated with a hemocytometer using the improved Neubauer Chamber as described by Pant and Srivastava, (2003). Morphological abnormalities were determined by diluting the semen with buffered formal saline and staining with eosin-nigrosin stain. The slides were allowed to dry and were viewed under light microscope using x100 oil immersion for percentage live and abnormalities as described by Esteso *et al*. (2006).

Statistical Analysis

Data obtained were analyzed using one way analysis of variance (ANOVA) and expressed as mean ± standard error of mean (SEM). Values of $p < 0.05$ were considered statistically significant.

RESULTS

The LD_{50} studies showed there was no mortality up to 5000 mg/kg dose of the extract used, suggesting it is safe for use at this dose. The result for semen characteristics is presented on table 1. There was a dose-dependent significant ($p<0.05$) difference in the mass activity between the treated groups and the control. Similarly, the sperm motility, concentration, percentage live and abnormalities of the treated group (C-E) were significant ($p<0.05$) different from the control. Sperm abnormalities of double, detached and curved heads; curved mid piece as well as coiled and curved tail. These abnormalities increased as the dose of the extract is increased, with the highest observed at a dose of 1000 mg/kg. Further details are presented on table 2 and figures 1.

Table 1: Semen characteristics of Wistar rats administered leave extract of *Jatropha gossypifolia*

Dosage (mg/kg)	Mass activity	Sperm motility (%)	**Sperm** concentration ($x10^6$ /ml)	**Percentage** live	Percentage Abnormalities
Control (Group A)	+++	88.8 ± 4.7^a	$60.0\ \ 8.3^a$	81.5 ± 0.4^a	1.25 ± 0.4^a
100 (Group B)	++	76.3 ± 2.8^a	52.8 ± 6.7^a	91.3 ± 3.3^a	1.0 ± 0.7^a
300 (Group C)	++	60.0 ± 5.4^b	27.0 ± 4.7^b	57.8 ± 4.7^b	4.75 ± 2.3^b
500 (Group D)	+	38.3 ± 9.5^c	21.7 ± 6.7^c	48.3 ± 4.9^b	5.75 ± 1.2^b
1000 (Group E)	+	27.5 ± 14.5^c	29.3 ± 11.0^b	28.8 ± 5.8^c	12.0 ± 3.8^c

Values are expressed as Mean ± SEM, means with the same superscripts within the same column are not significantly different ($p>0.05$), while means with different superscript within the same column are significantly different ($p<0.05$)

Table 2: Percentage sperm cell abnormalities of Wistar rats administered leave extract of *Jatropha gossypifolia*

Sperm abnormalities	Control (Group A)	100mg/kg (Group B)	300mg/kg (Group C)	500 mg/kg (Group D)	1000mg/kg (Group E)
Detached head	0	0	9	6	13
Double head	0	1	0	3	7
Curved head	2	3	2	1	1
Giant head	0	0	0	2	0
Curved midpiece	**1**	**0**	**1**	**4**	**5**
Coiled tail	1	0	4	5	22
Curved tail	1	0	2	2	0
Total abnormalities	5	4	18	23	48

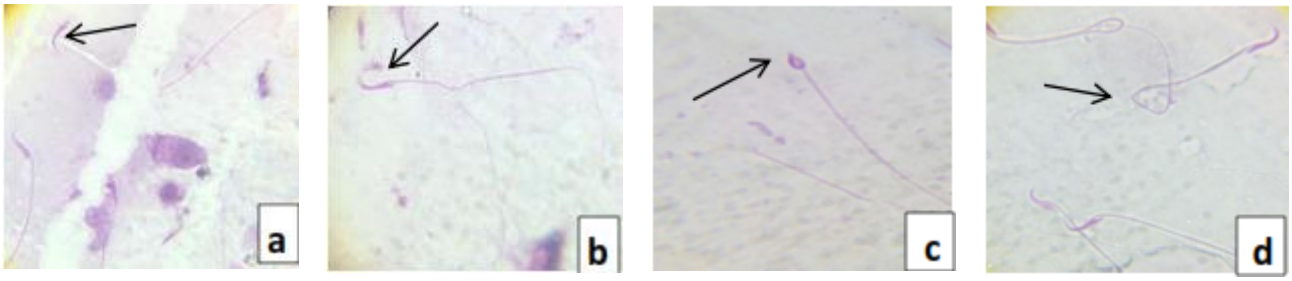

Figure 1: Photomicrograph of stained semen smear (eosin and nigrosin) of Wistar rat showing (a) detached head; (b) curved head; (c) giant head sperm and (d) coiled tail (×100 magnification)

DISCUSSION

Semen characteristics of mass activity, sperm motility, sperm concentration, and percentage live and sperm morphological abnormalities were evaluated in this study. These parameters are important reproductive indices that describes male fertility. The mass activity of semen progressively decrease with increasing dose of the extract. They were considerably lower than those of the control. This disagrees with the report of Oyeyemi *et al*. (2011), who observed insignificant difference in the mass activity of West Africa Dwarf (WAD) rams treated with *Euphorbia hirta*. They used an extract containing tannins, alkaloids and phenols which is similar to the *Jatropha gossypifolia* used in this study. The difference may be attributed to the longer period of treatment (21 days) used in this study, unlike the 14 days used by Oyeyemi *et al*. (2011). Sperm motility decreased in all the treated groups except the group with the lowest dose of 100 mg/kg, which was comparable with the control. This finding is consistent with previous studies of medicinal plants on male fertility (Ahmed *et al*., 2002; Adedapo *et al*., 2007; Manivannon *et al*., 2009; Oyeyemi *et al*., 2009;Oyeyemi *et al*., 2011;Olayemi, 2012). This may be caused by the influence of these plants on the spermatozoa to interfere with its enzymatic activities (Copper and Ignacimuthu, 2011). The sperm concentration in the *J. gossypifolia* treated group decreased and was substantially different from the control except the 100mg group. This is similar to the report of Oyeyemi *et al*. (2011) in WAD buck treated with *Aloe barbadensis miller* extract and Udoh and Kehinde (1999) in rats treated with *Carica papaya* extracts. A dose dependent increase in sperm concentration was reported by Saalu *et al*. (2013) in rats administered *Vernonia amygdalina* extracts. Although they observed that on increasing the dose of the extract and duration of administration; sperm concentration decreased. In a recent review, Felix-Silva *et al*. (2014) demonstrated that the toxicity of *Jatropha gossypifolia* extract depends on the dose, plant part used and animal model. This may account for the reduction in sperm concentration in this study. There was noticeable decline in the percentage live cells 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*, Oyeyemi *et al*. (2011) in WAD buck treated with *Aloe barbadensis miller* and Saalu *et al*. (2013) in rats administered high dose of *Vernonia amygdalina* extract for a prolonged duration. The decline may be attributed to some toxic properties of *J. gossypifolia* which inhibit spermatozoa survival. Percentage abnormalities increased with increasing dosage of the extract. This was significantly different from the control except the group with 100 mg/kg. This is in agreement with the observation made in rats treated with plant extract of *Carica papaya* (Manivannon *et al*., 2009), *Ocimum sanctum* (Ahmed *et al*., 2002) and *Vernonia amygdalina* (Saalu *et al*., 2013). It also agrees with the observation of Oyeyemi *et al*. (2011) in WAD bucks treated with *Aloe barbadnsis miller*. The percentage abnormalities were less than 10 % in the treated groups except the 1000 mg/kg group. This may not be significant in bovine where abnormality level 10 % is accepted (Zemjanis, 1970). However, fertility may be compromised since sperm abnormalities in the semen are usually accompanied by infertility or sterility (Sekoni and Gustafsson, 1987). Abnormalities of detached head, double head, curved head, giant head, coiled tail, curved tail and curved mid-piece were seen. These abnormalities are capable of causing conception failure due to unsuccessful fertilization.

Conclusion

The study revealed that methanolic extract of *Jatropha gossypifolia* is detrimental to semen characteristics of rats and may disrupt reproductive process when taken at a dose above

100 mg/kg. Further studies are required to unravel the effect of the extract on hormonal profile of male Wistar rats or other animal models.

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