Effects of aqueous leaf extract of *Tithonia diversifolia* (Mexican Sunflower) on semen characteristic and morphology in male Wistar albino rats

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Abstract

The objective of the present study was to investigate the effects of the aqueous extract of *Tithonia diversifolia* leaves on semen characteristics and spermatozoa morphology Wistar rats. The rats were divided into three groups (A, B and C) and treated daily with 400, 800 and 1600 mg/kg of the aqueous extract of *Tithonia diversifolia* leaves respectively for 28 days while 5ml/kg of distilled water was given to the control group D. At the end of the experimental period, animals were sacrificed and sperm characteristics, histology of the testes and epididymis were assessed. Aqueous extract of *Tithonia diversifolia* leaves caused a dose- dependent decrease in the progressive sperm motility of the Wistar rats with a significant decrease ($p < 0.05$) in the group dosed with 1600mg/kg (62.50 ± 7.5) when compared with the control (93.75 ± 1.3). It also cause a dose- dependent decrease in the sperm count with a significant ($p < 0.05$) decrease in the group dosed with 1600mg/kg (69.75 ± 0.5) when compared with the control (129.0 ± 5.0). Some sperm abnormalities vise headless tail (321.5 ± 0.3), tailless head (171.00 ± 50.0) and bent tail (3766.0 ± 320.5) were significantly increased in the group dosed with 1,600mg/kg while histopathological evaluation of the testis and epididymis revealed varying degrees of degeneration and necrosis of the germinal epithelia cell of the seminiferous tubules. Spermiostasis and complete epididymal aspermia were also observed. *Tithonia diversifolia* aqueous leaf extract has significant anti-spermatogenic effects on adult male Wistar rats which could impair reproductive activities in these rats.

Key words: *Tithonia diversifolia*, Aqueous Leaf Extract, Semen Characteristics, Wistar

Introduction

Tithonia diversifolia is a medicinal plant that is widely used to treat various illnesses. It is commonly known as Mexican sunflower or Japanese sunflower (English), "sepeleba" (Yoruba), pua renga (Cook Island). It has been discovered that *T. diversifolia* possesses anti-malaria, anti-diarrhoeic, anti-inflammatory, anti-bacterial, anti-proliferation, cytotoxic properties (Coyle *et al.*, 1994; Kuo *et al.*, 1998; Rungeler *et al.*, 1998; Tona *et al.*, 1998; Wu *et al.*, 2001) and it is effective in

the treatment of haematomas and wounds (Akobundu and Agyakwa 1998; Goffin *et al.*, 2002). The leaf is reported to contain sesquiterpene lactones taginin C as an active component against *Plasmodium* (Goffin *et al.*, 2002, Ajaiyeoba *et al.*, 2006), diversifolin, diversifolin methyl ether and tirtundin as active components against inflammatory activity (Rungeler *et al.*, 1998).

It is rich in minerals and vitamins especially the B-complex vitamins (Odunsi *et al.*, 1996) and its leafmeal contains 16.61%

crude protein, 12% crude fibre, 5% ether extract, 14% ash and 52.39% Nitrogen free extract (Odunsi *et al.*, 1999). Phytochemical analysis of *T. diversifolia* leaves' aqueous extract gave positive reactions to alkaloids, flavonoids, phlobatannins, tannins, terpenoids, saponins and reducing sugar (Kuo and Chen 1998).

Some anti-malaria agents, like chloroquine and pyrimethamine, have been reported to have anti-spermatogenic and anti-fertility effects (Adeeko and Dada, 1998; Awoniyi *et al.*, 1993) by having negative effect on sperm motility (Okanlawon *et al.*, 1993). Mass atrophy of spermatogenic elements and Leydig cells (Gbile, 1986) and dose dependent changes in the body weight, organ weights and sperm characteristics of male rats and chicken has also been reported in some medicinal plants with potent anti-malaria properties

Since the knowledge of basic characteristics of the semen produced in a male animal is valuable for the evaluation of the breeding soundness, potential fertility and productivity and the need to reduce cost of drugs used in animal production is fundamental, this study attempts to investigate the effect of aqueous leaf extract of *Tithonia diversifolia* on semen characteristic and morphology in male Wistar albino rats.

Materials and Methods

Experimental Animals

Twenty four adult male Wistar rats (mean body weight: 180.0 ± 3.33 g) were used for this study. The rats were housed in the Experimental Animal Unit of the College of Veterinary Medicine (COLVET), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The Experimental Animal Unit of COLVET is situated at Latitude $7^{\circ} 9' 39''$ N and Longitude $3^{\circ} 20' 54''$ E, 76m

above sea level in the rain forest vegetation zone. The area has a humid climate with mean annual rainfall, average relative humidity and temperature of 1037 mm, 82% and 34.7°C respectively (Oyekunle *et al.*, 2010). They were kept in well-ventilated metal cages at ambient temperature and a period of 12 hour light and 12 hour darkness was maintained. The rats were fed standard ration containing 14% Crude Protein and 2550Kcal/Kg Metabolisable Energy manufactured by Vital Feeds Limited, Ibadan. Clean water was served *ad-libitum*.

Plant material and extraction

Plant extraction was done as described by Iwu *et al.*, (1990). Briefly, fresh leaves of *Tithonia diversifolia* were harvested within the Alabata Campus of the Federal University of Agriculture Abeokuta, Abeokuta, Ogun State. Identification and authentication was done at the Forestry Research Institute of Nigeria (FRIN).

The leaf of *Tithonia diversifolia* was air dried, pulverized, finely sieved and 500g of the plant was soaked in 1 litre of distilled water for 24 hours after which it was filtered. Thereafter, the filtrate was evaporated to dryness and 1g of it was dissolved in 20mls of distilled water to give a concentration of 50mg/ml. The process of evaporation is simply by open stainless bow containing the extract in the water bath. The temperature of the water bath is regulated to between 50° – 60° . The extract is left until all its solvent is evaporated. The resulting residue is kept in the refrigerator until it is needed for the work.

Experimental procedure

The rats were randomly divided into four groups (A-D) of 6 rats each. The rats in groups A, B and C were given aqueous extract of *Tithonia diversifolia* leaf extract at 400mg/kg, 800mg/kg and 1600mg/kg body weight respectively once daily orally

for 28 days. Group D rats were the control to which 5ml/kg distilled water was administered orally once daily for 28 days. Thereafter, the rats were euthanized by placing them in a glass chamber containing cotton wool soaked in chloroform till they lost consciousness followed by cervical dislocation. A ventral midline abdominal incision was then made using a scalpel blade size 14 to expose the abdominal organs. The testis and epididymis of each rat were identified, carefully removed and a small incision was made on the caudal epididymis to squeeze out the semen content on a glass slide for semen evaluation. The testis and epididymis were thereafter preserved in Bouin's fluid for histopathology evaluation.

Microscopic Examination of Semen

The microscopic examination of semen includes assessing sperm concentration, motility, vitality, total sperm count and secondary morphological abnormalities and these were done as described by (Blom, 1973; Mortimer, 1994 and WHO, 1987). Briefly, the sperm concentration was determined by harvesting epididymis into a graduated test tube containing 5.0ml of distilled water to obtain a final volume (volume of distilled water + epididymis). The epididymis in distilled water was properly crushed with the pair of mortar and pestle. A drop of the solution formed was placed into haemocytometer with a cover slip. The haemocytometer is made up of 25 counting chambers and sperm cells were counted in the 5 diagonal and inner chambers.

Sperm concentration = $A \times 1000 \times 5$ sperm cells/ml

where A is the number of sperm cell counted.

The sperm morphology involved counting

the abnormalities present in the sperm cells (spermatozoa). This was done by mixing a drop of sperm from the epididymis on a slide with a drop of Wells and Awa stain. The smear of the mixture was then made on the slide and observed under high power (x 40) microscope lens. The sperm abnormalities considered were tailless head, headless tail, rudimentary tail, bent tail, curved tail, curved midpiece and bent midpiece.

Motility was evaluated within 30minutes of collection and was assessed by placing a drop of well-mixed semen under a 22 X 22-mm coverslip. Using a laboratory counter, 10 random fields were counted on a microscope and scored, counting at least 200 spermatozoa.

Statistical Analysis

The mean and standard error of mean were calculated for all the sperm parameters. Analysis of variance (ANOVA) was used to establish any significant difference in all the stated parameters. P value less than 0.05 was considered significant in all cases.

Results

There was a dose- dependent decrease in the progressive sperm motility of the Wistar rats. There was a significant ($P < 0.05$) decrease in sperm motility (62.50 ± 7.5) in the group dosed with 1600mg/kg when compared with the control (93.75 ± 1.3) (Table 1). There was non -significant ($P > 0.05$) change in the percentage life sperm cells/ death ratio in the rats treated with 400mg/kg, 800mg/kg and 1600mg/kg doses when compared with the control value (Table 1). A similar non significant ($P > 0.05$) change was observed for sperm volume in the rats treated with 400mg/kg, 800mg/kg and 1600mg/kg doses when compared with the control value (5.18 ± 0.0) (Table 1). However, there was a dose-dependent decrease in the sperm count of

Table 1: effects of 400, 800 and 1,600mg/kg doses of the aqueous extracts of *t. Diversifolia* on sperm characteristics of male wistar rats

Treatment	Sperm motility	Live/dead	Sperm counts
400mg/kg	90.00±0.0	95.8±0.8	97.25±2.1
800mg/kg	70.00±11.6	98.00±0.0	77.0±1.7
1,600 mg/kg	62.50±7.5*	97.25±0.1	69.75±0.5*
Control	93.75±1.3	98.00±0.0	129.00±5.0

Superscripted values are significant at $P<0.05$

the Wistar rats following oral administration of aqueous extract of *T. diversifolia* leaves. The decrease in sperm counts value of (69.75±0.5) in the group dosed with 1600mg/kg was significant ($P<0.05$) when compared with the control (129.0±5.0) (Table 1).

There was no significant ($P<0.05$) changes in the total number of abnormal spermatozoa in the group treated with 400mg/kg when compared with the control. The abnormalities observed were mainly secondary, characterized by tailless head, headless tail, rudimentary tail, bent tail, curved tail, curved midpiece, bent midpiece and looped tail. Also, there was no significant ($P<0.05$) change in the total number of abnormal spermatozoa in the group treated with 800mg/kg except in the secondary abnormality of the curved midpiece value (3981.7±330.3) when

compared with the control group (2696.25±300.0). The other secondary abnormalities observed were, characterized by tailless head, headless tail, rudimentary tail, bent tail, curved tail, bent midpiece and looped tail (Table 2).

Similarly, there was no significant ($P>0.05$) change in the total number of abnormal spermatozoa in the group treated with 1600mg/kg when compared with the control. However there were significant ($P<0.05$) increases observed in the following abnormalities: headless tail (321.5±0.3), tailless head (171.00±50.0) and bent tail (3766.0±320.5) whereas, others like rudimentary tail, curved tail, curved midpiece, bent midpiece and looped tail did not exhibit any statistically significant ($P>0.05$) changes when compared to the control values (Table 2). Histopathology of the testis of rats

Table 2: effects of 400, 800 and 1,600mg/kg doses of the aqueous extracts of *t. Diversifolia* on sperm morphology of rats

	Headless Tail	Tailless Head	Rudi-mentary Tail	Bent Tail	Curved Tail	Bent mid Piece	Curved Mid Piece	Looped Tail	Coiled Tail	Total Abnormal
400 mg/kg	191.35±62.3	143.75±74.4	13.50±4.8	2461.8±249.7	1464.3±748.4	2727.3±288.3	2991.75±261.1	11.00±4.1	6.00±5.0	401.3±1.3
800mg/kg	146.33±63.7	147.67±63.3	17.67±3.3	3217.7±3.3	2882.33±329.7	3965.00±267.00	3981.7±330.3*	14.33±3.3	1.00±0.00	405.00±2.9
1,600mg/kg	321.5±0.3*	171.00±50.0*	13.50±2.5	3766.0±320.5*	4072.00±811.3	4052.00±280.00	3792.00±526.7	18.50±2.50	11.00±5.8	400.00±0.00
Control	116.25±54.9	68.75±47.5	8.50±4.8	2492.00±279.0	2767.00±290	3494.25±240.0	2696.25±300.0	13.50±4.8	6.00±2.9	403.75±2.4

Superscripted values are significant at $P<0.05$

Atrophy of the Leydig cells was observed when the leaf extracts of *Azadirachta indica* was administered to rats (Aladakatti and Ahamed, 2005). Gossypol treatment reduces the level of serum testosterone and luteinizing hormones in dose and duration dependent manner (Zhuang *et al.*, 1986; Udoh *et al.*, 1992). *Carica papaya* damages the acrosome membrane damage resulting in bent mid piece, coiled tail, detached head and arrest of spermatogenesis beyond the level of spermatocytes (Lohiya *et al.*, 1999). Piperine in *Piper longum* interferes with acrosome reaction through the inhibition of calcium influx by stimulation of efflux (Piyachaturawat *et al.*, 1991). Further studies should therefore be carried out on *T. diversifolia* to know the specific reproductive organ and hormone directly affected by this extract.

It can be concluded that the continuous administration of *T. diversifolia* extract can induce oligospermia in animals having significantly ($p < 0.05$) reduced sperm motility and sperm concentration. The plant can reduce fertility in male animals and is therefore higher doses of it not recommended for medicinal purpose in male animals especially those used for breeding.

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