

Reproductive Profile of Male Wistar Rats Treated with Graded Doses of Aqueous Extract of *Titonia diversifolia* Leaf

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ABSTRACT [ENGLISH/ANGLAIS]

The effect of an aqueous extract of *Titonia diversifolia* (AETD) was investigated on morphometric of testis, hormonal assay and semen analysis of caudal epididymes of 24 male Wistar rats. They were divided into four groups of six rats each, Group A served as the control and was administered distilled water while groups B, C and D were treated with 50mg/kg, 100mg/kg and 200mg/kg body weight of aqueous extract of *Titonia diversifolia* respectively for 28 days. The result of the study showed that aqueous extract of *Titonia diversifolia* affect the morphometric of testis showing a significant ($p < 0.05$) decrease in width, length and weight at 100mg/kg AETD, the right epididymidis was also significantly reduced in weight at 100mg/kg of AETD. Plasma testosterone significantly increased at 50mg/kg of AETD. The highest sperm count, motility, viability was also recorded at a dose of 50mg/kg. Phytochemical screening of the extract showed the presence of tannins and saponins. Aqueous extract of *Titonia diversifolia* at a dose of 100mg/kg and 200mg/kg possesses the potential to reduce sperm count, motility, morphology, and viability. While a dose of 50mg/kg increased the sperm count, motility and viability, and the plasma testosterone was also increased at 50mg/kg of AETD. Therefore, 50mg/kg of the extract will increase the testosterone level, thereby improving spermatogenesis and other reproductive profile.

Keywords: Aqueous extract, *Titonia diversifolia*, Caudal epididymes, spermatogenesis

RÉSUMÉ [FRANÇAIS/FRENCH]

L'effet d'un extrait aqueux de *Titonia diversifolia* (AETD) a été étudiée sur morphométrie du testicule, le dosage hormonal et une analyse du sperme de épидидymes caudales de 24 rats mâles Wistar. Ils ont été divisés en quatre groupes de six rats chacun, Groupe A a servi de témoin et a été administré de l'eau distillée tandis que les groupes B, C et D ont été traités avec 50mg/kg, 100mg/kg et 200mg/kg de poids corporel de l'extrait aqueux de *Titonia diversifolia* respectivement pour 28 jours. Le résultat de l'étude a montré que l'extrait aqueux de *Titonia diversifolia* affecter la morphométrie du testicule montrant une augmentation significative ($p < 0,05$) diminution de la largeur, la longueur et le poids à 100mg/kg AETD, l'épididyme droit a également été significativement réduite dans le poids à 100 mg / kg de AETD. Testostérone plasmatique significativement plus élevée à 50mg/kg de comptage AETD. The plus de sperme, la motilité, la viabilité a également été enregistrée à la dose de 50mg/kg. Criblage phytochimique de l'extrait a montré la présence de tanins et des saponines. L'extrait aqueux de *Titonia diversifolia* à une dose de 100mg/kg et 200mg/kg possède le potentiel de réduire le nombre de spermatozoïdes, la motilité, la morphologie et la viabilité. Bien que la dose de 50mg/kg a augmenté la numération des spermatozoïdes, la motilité et la viabilité, et la testostérone plasmatique a également été augmenté à 50mg/kg de AETD. Therefore, 50mg/kg de l'extrait va augmenter le niveau de testostérone, ce qui améliore la spermatogenèse et autres problèmes de reproduction le profil.

Mots-clés: Extrait aqueux, *Titonia diversifolia*, épидидymes caudales, la spermatogenèse

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INTRODUCTION

Titonia diversifolia (Helms A. Gray) is commonly called wild sunflower or Mexican sunflower. They are mostly found on fields and roadside in western part of Nigeria

[1].The plant has been used for treatment of stomach pains, indigestion, sore throat and liver pain by the Lino tribe of Kenya [2].This wild sunflower is of high nutritional value containing all the essential amino acid,

also rich in minerals and vitamins especially the B complex vitamins, and can be used as manure for farming [3].

Earlier studies on the extracts of *Titonia diversifolia* was based on the treatment of hepatitis [4], wounds [5], control of amoebic dysentery [6], also have medicinal value for treatment of type two diabetes [7]. Both the aqueous and methanolic extracts of *T. diversifolia* leaf contain antimalarial substances with properties that showed both preventive and curative effects on malaria parasites [8], while the Soap made from *Titonia diversifolia* plant extract was found to be effective against *E. coli*, [9]. Other studies have focused on its feeding values and activities on genitalia morphometric in Isa Brown cocks [10], showing a dearth of information on the effect of the plant on reproduction.

Cavazos and Melampy [11] have observed that epididymis of rat may need higher levels of testosterone for maintaining its weight and secretory activity than the other accessory glands. Various plants and their active substance have been extensively tested for spermatogenesis and accessory reproductive organs profile in different animals [12]. Since *Titonia diversifolia* has a high nutritional value which is useful in animal production, and it is of great medicinal importance in man. It could also have a positive or negative effect on reproduction. However, much attention has not been given to the effect of this useful plant on reproduction.

This study is aimed at investigating the effects of aqueous extract of *Titonia diversifolia* on reproductive profile of male rats by examining the morphometric of the testis, level of testosterone and semen analysis. The outcome of this study will constitute a source of baseline data and basis for advising the ethno medical practitioner and the public on the usage of this herb.

MATERIALS AND METHODS

Plant material

Wild sunflower leaves were harvested from Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Nigeria. The plant was authenticated by Dr. A.T. Ogunkunle, a botanist in the department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Nigeria, and a Voucher specimen (Voucher number: LAH 105) was kept at the same University Herbarium. The leaves were air dried for seven days, the air dried leaves were then milled using electric blender.

Plant extract preparation

Milled leaves weighing 100 grams was soaked in 750mls of distilled water for extraction and was kept at 60°C temperature for 24 hours after which it was filtered, the filtrate was poured into a capped bottle and then stored in a refrigerator at 4°C. The resultant residue was air-dried and weighed, calculations were done to determine the dosage of the filtrate that will be administered.

$$\text{Concentration} = \frac{X-Y}{Z}$$

Where; X=weight of blended leaves (g)
Y=weight of leaves residue after drying (g)
Z=volume of filtrate (ml).

Animals

Twenty-four Adult Male Wister rats weighing 120-170g were purchased from the Laboratory animal farm of Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Nigeria, where the experiment was carried out. The animals were kept in identical cages in a well ventilated room; food and water were given *ad libitum*.

Experimental design

The animals were randomly allocated into four groups each group composing of six rats each. Group A was given distilled water i.e., the control, while groups B, C and D were treated with 50, 100, 200 mg/kg doses of aqueous extract of *Titonia diversifolia* (AETD) respectively for 28 days. The rats were sacrificed by cervical dislocation at the end of the treatment period.

Estimation of testis morphometry and caudal epididymal sperm characteristics

The scrotum of the rats was incised, after which the testes were removed; the weight and length were recorded. The caudal epididymis of the experimental rats were separated and then transferred into specimen bottles with the addition of 0.9mls of normal saline and 2.9% buffer sodium citrate. The morphology and motility of the sperms were determined by using specific method [13]. While the sperm counts were studied by using the Neubauer hemacytometer counting chamber and the counting done under the microscope at a magnification of x 40.

Serum testosterone

Serum levels of testosterone were assayed in duplicate using specific RIA method [14]. Serum samples were separated by standard procedure and stored at -20°C for subsequent analysis.

Phytochemical Screening;

Chemical tests were carried out on the aqueous extracts and on the powdered specimens using standard procedures to identify the constituents [15, 16].

Test for Alkaloids

About 0.5 g of each plant extract was stirred with five mL of 1% aqueous hydrochloric acid on a steam bath. One milliliter of the filtrate was treated with a few drop of Dragendorff's reagent. Turbidity or precipitation with this reagent showed the presence of alkaloids in the extracts.

Test for Saponins

About 0.5 g of each plant extract was shaken with water in a test tube. Frothing which persisted on warming showed the presence of saponins.

Test for Phlobatannins

Aqueous sample of the plant extracts was boiled with 1% aqueous hydrochloric acid. Deposition of a red precipitate showed the presence of phlobatannins.

Test for Tannins

About five gram of each plant extracts was stirred with ten mL of distilled water. It was filtered and ferric chloride reagent was added to the filtrate. A blue-green, green or blue-black precipitate showed the presence of tannins.

This experiment was carried out, at the Laboratory, department of pharmacognosy, faculty of pharmacy, university of Ibadan, Nigeria.

Statistical analysis

Results obtained were expressed as the mean \pm SD. Means were analyzed using one way analysis of variance, followed by the Duncan Multiple Range Test to determine significant differences between pairs. Differences with values of $p < 0.05$ were considered statistically significant [17].

RESULTS

At the end of the 28 day treatment period, the following results were obtained; serum testosterone level of rats given 50mg/kg of AETD (4.07 ± 0.41) was significantly

greater than that of the control (3.33 ± 0.41). increasing the dose of AETD to 100mg/kg reduces testosterone level to 3.37 ± 0.31 and a dose of 200mg/kg further reduced the testosterone level to 2.10 ± 0.37 . showing that the variation in testosterone level is dose dependent as presented in Table 1.

The caudal epididymal sperm characteristics of rats treated with AETD is shown in Table 2, there was significant ($p < 0.05$) increase in sperm count, increase in sperm motility and viability in group treated with 50mg/kg of AETD when compared with other groups. Increase in the dose of AETD decreased the caudal epididymal characteristics. Therefore sperm count, and morphology were significantly ($p < 0.05$) reduced at a dose of 200mg/kg of AETD when compared with other treatments

The result of the morphometric of the testis and caudal epididymis is presented in Table 3, the weight, length and width of the left testis of rats treated with 100mg/kg of AETD (Group C) was significantly ($p < 0.05$) lower than that of the other groups, while the result obtained for the rats given 50mg/kg body weight of AETD was higher than that of the other groups.

Phytochemical screening of the extract showed the presence of tannins and saponins (Table 4).

DISCUSSION

The result of the study showed that rats treated with 50mg/kg body weight of AETD had a significant ($p < 0.05$) increase in caudal epididymal sperm motility, count, viability, testes weight, length, epididymal weight and testosterone level. Alteration in sperm count suggests alteration in sperm production (spermatogenesis), the increase in sperm count shows that aqueous extract of *Titonia diversifolia* leaf promote the process of spermatogenesis in testes at a dose of 50mg/kg body weight for 28 days. Increase in the amount of sperm count may be associated with the increase in serum levels of testosterone. Testosterone was necessary for the development, growth and normal functioning of the testes and male accessory reproductive glands [18]. Increase in testosterone level could arise from increase in gonadotropin releasing hormone, which result in increase in pituitary luteinizing hormone and follicle stimulating hormone biosynthesis. In as much as testicular function is controlled by gonadotropin [19], the implication of this cascade of endocrine manipulation for testicular steroidogenesis, could be an increase in testosterone

biosynthesis thus leading to the increase in testosterone level.

Furthermore, the extract could increase testicular steroidogenesis by increasing the activity of membrane receptors in leydig cells, decreasing the synthesis of sex hormone binding globulin (SHBG) [20]. Therefore decrease in synthesis of SHBG will lead to increase in testosterone level, and this could be traced to the presence of Saponins in the aqueous extract of *Titonia diversifolia*. Saponins have been shown to have both positive and negative effects on the viability of human sperm cells in vitro with some ginseng saponins increasing motility as well as progression of sperm [21], while *Sesbania sesban* saponins were spermicidal at 1.0–1.3 mg/ml [22]. Salvati and his co-workers [23] reported that *Panax Ginseng* C.A. Meyer saponins extract showed an increase in spermatozoa number/ml and progressive oscillating motility, an increase in plasma total and free testosterone, Dihydrotestosterone, Follicle stimulating hormone and Luteinizing hormone levels, but decreases in mean Prolactin. Suggesting that the extract may have an effect at different levels of the hypothalamus-pituitary-testis axis,

Table 2: This table shows caudal epididymal sperm characteristics in the experimental rats

Parameter	Group A (0mg/kg AETD)	Group B (50mg/kg AETD)	Group C (100mg/kg AETD)	Group D (200mg/kg AETD)
Sperm count(%)	38.67±1.23 ^a	42.53±1.20 ^c	37.47±1.27 ^a	30.67±1.44 ^b
Sperm motility(%)	70.4±0.74 ^a	76.6±0.74 ^c	66.70±0.89 ^b	66.01±0.76 ^b
Sperm Morphology (%)	76.6±1.10 ^a	71.67±0.72 ^b	65.0±1.24 ^c	61.67±0.72 ^d
Sperm viability(%)	40.8±1.06 ^a	53.2±1.24 ^c	33.4±1.25 ^b	33.97±1.08 ^b
Progressivity	C	D	D	D

Values carrying superscripts different from the control for each parameter are significantly different ($p < 0.05$).

Table 3: This table shows morphometric of the testis and caudal epididymis in the experimental rats

Parameter	Group A (0mg/kg AETD)	Group B (50mg/kg AETD)	Group C (100mg/kg AETD)	Group D (200mg/kg AETD)
Weight of LT(g)	1.07±0.35 ^a	1.31±0.12 ^c	1.15±0.20 ^b	1.34±0.31 ^c
Length of LT(mm)	1.70±0.18 ^a	1.73±0.09 ^a	1.33±0.26 ^c	1.68±0.15 ^b
Width of LT(mm)	0.82±0.11 ^a	0.85±0.12 ^a	0.37±0.18 ^c	0.73±0.15 ^b
Weight of LE(g)	0.11±0.01 ^a	0.13±0.05 ^b	0.11±0.07 ^a	0.11±0.06 ^a
Weight of RE(g)	0.15±0.03 ^a	0.13±0.05 ^b	0.12±0.05 ^b	0.13±0.11 ^b

Values carrying superscripts different from the control for each parameter are significantly different ($p < 0.05$). LT=Left Testes, LE=Left Epididymis, RE=Right Epididymis.

while increase in sperm motility suggests increase of sperm maturation in the epididymides [24]. The increase in weight of epididymis may also show the increase amount of sperm produced by testes, [25]. The amount of

Table 1: This table shows serum testosterone level in the experimental rat groups

Groups	Testosterone levels
A (0mg/kg AETD)	3.33±0.41 ^a
B (50mg/kg AETD)	4.07±0.41 ^c
C (100mg/kg AETD)	3.37±0.31 ^a
D (200mg/kg AETD)	2.10±0.37 ^b

Values carrying superscripts different from the control for each parameter are significantly different ($p < 0.05$)

Table 4: This table shows the phytochemical constituents of Aqueous Extract of *Titonia diversifolia* (Helms A. Gray) Leaf (AETD).

Plant constituents	Levels
Alkanoids	±
Saponins	++
Phlobatannins	-
Tannins	++

++ Present in abundance; - Absent; ± present/absent

sperm viability also increased, this may be associated with the improvement of epididymal function of maturation [26]. Similar result were obtained for *Ficus deltoidea* stem extract which improved sperm quality [27]. Also similar

dose dependant results were obtained for aqueous leaf extract of *Vernonia amygdalina*, and the extract contains

CONCLUSION

It is concluded that 50mg/kg of the extract is recommended as the dose capable of improving reproductive profile, while dose above this will lowers reproductive profile of the male Wistar rat and so it is advised that the continual usage of aqueous leaf extracts of *Tithonia diversifolia* should be with caution particularly above the recommended dose.

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CONFLICT OF INTEREST

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