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Effects of ethanol extract of *Tridax procumbens* on spermiogram and reproductive hormones in Wistar rat

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Abstract

Therapeutic potentials of medicinal plants in the improvement of semen characteristics and reproductive hormones have been reported by some authors. This study explored such potentials in *Tridax procumbens* which is from the family *Asteraceae*. Twenty-five adult male albino wistar rats were used for this study, divided into five groups (Groups A to E) of 5 rats each receiving 0mg/kg, 100 mg/kg, 200mg/kg, 400mg/kg and 800mg/kg of ethanol extract of *Tridax procumbens* leaf respectively for 28days. The seminal volume and concentration of the ethanol extract treated groups D (5.16±0.05, 94.40±9.58, respectively) and E(5.18 ±0.04, 110.40±4.86, respectively), were higher significantly ($p<0.05$) compared to ethanol extract treated groups B(5.12± 0.08, 91.80±12.72 respectively), C(5.11 ±0.05, 85.60± 8.20 respectively) and control group A(5.02±0.18, 72.80±2.59 respectively). Sperm motility was higher significantly ($p<0.05$) in groups B (65.00±5.00), D (65.00 ±5.00) and E(86.00±6.52) when compared with group C (63.00± 8.3) and control group A (54.00± 4.18). Sperm percentage liveability was not significantly different among all groups ($p> 0.05$) though group E was the highest (97.20±1.64). Sperm morphological abnormality was significantly lower in group E (10.70± 0.54) compared to groups A (14.12 ±0.28), B(12.71 ±0.82), C(12.39± 0.95) and D (12.60± 0.49). Serum testosterone and follicle stimulating hormone concentrations were highest in group E(0.18 ±0.01 and 0.26± 0.07 respectively) and significant when compared with all the other groups A(0.11± 0.01 and 0.05 ±0.16 respectively), B(0.11± 0.01 and 0.07± 0.07 respectively), C(0.14± 0.04 and 0.08± 0.01 respectively) and D(0.15 ±0.03 and 0.22± 0.26 respectively). Serum luteinizing hormone concentration was also highest in group E(0.34± 0.23) but significant when compared with only groups A(0.12 ±0.03), B(0.11± 0.01) and D(0.16± 0.07). This study revealed that ethanol leaf extract of *Tridax procumbens* given up to 800mg/kg could enhance the spermiogram and reproductive hormones of Wistar rats.

Keywords: Phytochemicals, Rats, Reproductive hormones, Spermiogram, *Tridax procumbens*
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Introduction

Infertility is a major problem in up to 15% of the sexually active population and male factor is responsible in 50% of these cases in the world today (Thomas *et al.*, 2013). Recently, there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in alleviating oxidative stress-induced pathologies (Agarwal *et al.*, 2010, D'Cruz *et al.*, 2010, M´ınguez-Alarc *et al.*,

2012). Medicinal plants have active phytochemical components responsible for the various pharmacological properties exhibited by the plants. *Tridax procumbens* of the family *Asteraceae* is a thin, crawling plant regarded as weed found in most parts of the world. It has been reported to have immunomodulatory and antibacterial effect (Diwan *et al.,* 1989; Oladunjoye, 2006) and promotes normal and steroid-depressed wound healing. In Nigeria, *Tridax procumbens* is widely used as feed in rabbits (Olukunle & Abatan 2008). Anthony *et al.* (2014) incorporated *Tridax procumbens* in cassava based diet in rabbits and reported that the *Tridax procumbens* improved the growth of rabbits.

Tridax procumbens had been shown to be a good source of plant protein, it can supplement potassium and is a potential source of provitamin A (Ikewuchi *et al.,* 2009). Habila *et al.* (2010) observed in their study that due to the presence of total phenolic found in *Tridax procumbens* it can serve as a good natural source of antioxidants which can be useful in physiological and pathological medicine and of great interest to food manufacturing industries. Jain & Amita (2012) revealed that *Tridax procumbens* contain biomolecules such as anthraquinone, flavonoids, phenolic compounds, saponins, steroids, tannins and terpenoids. This can make *Tridax procumbens* to exhibit various pharmacological activities like hepatoprotective effect, immunomodulating property, wound healing activity, antidiabetic, antimicrobial, anti-inflammatory and antioxidant properties. *Tridax procumbens* had been shown to reduce lipid profile in rats (Keerthi *et al.,* 2014). Although, *Tridax procumbens* had been shown to have muscle relaxant effect on the isolated rat corpus cavernosum due to the release of nitric oxide from the endothelium that improve erectile dysfunction (Hussein *et al., 2*014), there is dearth of information on effects of *Tridax procumbens* leaf on seminal parameters and concentrations of reproductive hormones in animals. This study investigated the phytochemical contents of ethanol extract of *Tridax procumbens* leaf and evaluated the effects on seminal parameters and some reproductive hormones in adult male wistar albino rat.

Materials and Methods

Plant materials

The plant was collected at Department of Biochemistry University of Ibadan, Nigeria. The authentication of the *Tridax procumbens* leaf was done at the herbarium Unit Botany department, University of Ibadan with voucher number UIH-22443.

Preparation of extracts

Ethanol extract of *Tridax procumbens* leaf was prepared as described by Parekh & Chanda (2007). Briefly, the leaves were carefully removed from the plant, washed thoroughly and air-dried. The dried leaves were reduced to coarse powder by using electric blender. Eighty grams of the powdered sample was soaked in a beaker containing 400ml of 70% ethanol for a period of 48 hours and then filtered with a Whatman filter paper. The filtrate was subsequently concentrated using a rotary evaporator for 36 hours. The weight of residue obtained was 7.56g. The yield was found to be 9.45% w/w. The leaf ethanol extract of *Tridax procumbens* obtained was stored in refrigerator for subsequent use. The ethanol extraction and phytochemical screening of *Tridax procumbens* leaf were done at the Nutritional and Industrial Biochemistry Laboratory, Department of Biochemistry, University of Ibadan, Nigeria.

Phytochemical screening of ethanol extract of Tridax procumbens leaf

The crude ethanol extract of *Tridax procumbens* leaf obtained from the extraction was subjected to qualitative phytochemical screening of carbohydrates, protein, saponin, steroids and flavonoids.

Test for carbohydrate using molisch's test: 0.2g of crude ethanol extract of *Tridax procumbens* leaf was dissolved in 5ml of ethanol and filtered. 2ml of the filtrate of ethanol extract of *Tridax procumbens* leaf was treated with 2 drops of alcoholic α-naphthalol solution in a test tube. 1ml of conc.H_2SO_4 was added along the side of the glass. There was formation of an interphase with a violet ring colored solution at the junction indicating the presence of carbohydrates (Edogo *et al.,* 2005).

Test for protein: Ninhydrin Test was adopted, 2g of crude ethanol extract of *Tridax procumbens* leaf extract was dissolved in 5ml of ethanol and filtered. 1ml of ethanol extract of *Tridax procumbens* leaf was dissolved in 5 drops of Ninhydrin reagent and boiled for five minutes. The solution turned to light green color indicating the presence of amino acids (Edogo *et al.,* 2005).

Test for saponin:

Two grams of crude ethanol extract of *Tridax procumbens* leaf was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of filtrate of ethanol extract of *Tridax procumbens* leaf was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing solution was mixed with 3 drops of olive oil and shaken vigorously. The solution then formed an emulsion indicating the presence of saponin (Uma & Sekar, 2014).

Test for steroids: Two mililitres of acetic anhydride was added to 0.5 g of crude ethanol extract of *Tridax procumbens* leaf with 2 ml of concentrated H_2SO_4. The solution turned light green indicating the presence of steroids (Uma & Sekar, 2014).

Test for flavonoids: Few drops of conc. HCL was added to 1ml of crude ethanol extract of *Tridax procumbens* leaf. A red colored solution was formed indicating the presence of flavonoids (Momin *et al.,* 2013)**.**

Test for cardiac glycosides (keller-killani test): One gram of crude ethanol extract of *Tridax procumbens* leaf was dissolved in 5ml distilled water, 5ml of the dissolved crude ethanol extract of *Tridax procumbens* leaf was treated with 1ml of glacial acetic acid with one drop of ferric chloride solution. 1 ml of concentrated H_2SO_4 was later added. A brown ring of the interface was observed indicating the presence of a deoxy-sugar. A violet ring appeared below the brown ring on the acetic acid layer, the absence of greenish blue solution indicates the absence of cardiac glycosides (Parekh & Chanda 2007.).

Test for tanin: One gram of crude ethanol extract of *Tridax procumbens* leaf was dissolved in 5ml distilled water. 5ml of the dissolved crude ethanol extract of *Tridax procumbens* leaf was dissolved in 1ml of 5% ferric chloride the solution formed greenish-black precipitate indicating the presence of tanin (Momin *et al.,* 2013).

Acute oral toxicity: Rats were dosed up to 2000mg/kg crude leaf ethanol extract of *Tridax procumbens* in carrying out this test and no death was recorded (Abubakar *et al.,* 2012)

Experimental procedure

Twenty-five male albino wistar rats weighing between 190g and 210g were used for this study. They were divided into five groups (Groups A to E) of 5 rats each. Rats were placed in separate cages at the experimental unit of Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Ogun state Nigeria under natural day and night cycles. The rats had free access to rat feeds (Ladokun feeds®) and water *ad libitum*. They were allowed two weeks of acclimatization and randomly distributed as follows:

a) Group A: Control Group (0mg/kg). Rats received 10ml/kg body weight of normal saline which was used as the vehicle for the crude ethanol extract
b) Group B: Rats received 100mg/kg body weight of crude ethanol extract of *Tridax procunmbens* leaf.
c) Group C: Rats received 200mg/kg body weight of crude ethanol extract of *Tridax procunmbens* leaf.
d) Group D: Rats received 400mg/kg body weight of crude ethanol extract of *Tridax procunmbens* leaf
e) Group E: Rats received 800mg/kg body weight of crude ethanol extract of *Tridax procunmbens* leaf.

The crude ethanol extract of *Tridax procunmbens* leaf was administered orally once daily to the male albino wistar rats for 28 days using oral cannula.

Animal sacrifice and sample collection

On the 29th day of the experiment all the rats were sacrificed by cervical dislocation. Blood sample was collected through cardiac puncture for hormonal analysis. The blood samples were placed in plain sample bottles and subsequently centrifuged at 1500rpm for 5 minutes and serum obtained for assay of some reproductive hormones (Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Testosterone). The serum concentrations of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Testosterone were determined for all experimental rats by the enzyme-linked immunosorbent assay (ELISA) technique using commercially available kits. The hormonal kits used for the assay was produced by Mono-bind Inc. Lake Forest, CA, USA. Hormone levels were determined on the same day of collection of blood samples.

Determination of semen parameters

A midline abdominal incision was made on the abdominal cavity to expose the reproductive organs. The testes and epididymis of the rats were carefully exteriorized. The harvested organs were utilised immediately for determination of semen parameters

percentage liveability and morphological (sperm volume, sperm motility, concentration, abnormality).

Semen collection: The caudal epididymis was carefully separated from the testis and minced in 2ml of normal saline kept at 37^{o}C followed by filtration through a nylon mesh (Narayana *et al.*, 2005). Then there was determination of semen parameters (sperm volume, sperm motility, concentration, percentage liveability and morphological abnormality).

Sperm motility: One drop of the semen suspension was charged into a Makler counting chamber and the number of motile and non-motile spermatocytes was counted in ten random fields. The number of motile spermatocytes was then expressed as a percentage of the total number of the counted spermatocytes (Mahaneem *et al., 2*011).

Sperm concentration: The semen suspension was stained with 2% eosin in normal saline. The spermatocytes heads were counted using a Neubauer haematocytometer. The sperm heads in eight chambers (except the central chamber) were counted, the average determined and expressed as the number of sperm per caudal epididymis (Mahaneem *et al.,* 2011).

Sperm percentage liveability: Semen suspension from the caudal epididymis was carefully dropped on a slide and mixed with a drop of 0.5% eosin solution. After 2 minutes, the slide was examined under a light microscope at X40 magnification.

Total sperm abnormality: This was determined by smearing a drop of the stained semen suspension on a glass slide; the smear was allowed to dry and subsequently examined under the light microscope at X 400 magnification. For each sample, 200 spermatocytes were carefully observed and the percentage of total abnormalities of the spermatocyte head and total abnormalities of the spermatocyte tails were determined (Narayana *et al.*, 2005).

Statistical analysis

All data were expressed as Mean ± standard deviation, (SD). Significant differences were determined using the one way analysis of variance (ANOVA) test followed by the Duncan post hoc tests. A p value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS 17.0 version.

Results

The phytochemical screening of ethanol leaf extract of *Tridax procumbens* revealed presence of carbohydrates, protein, flavonoids, saponin, tannin, steroids and absence of cardiac glycosides (Table 1).

The seminal volume of groups D (5.16 ± 0.05ml), E (5.18 ± 0.04ml) B, 5.12 ± 0.08ml; and C, 5.11 ± 0.05ml) were not significantly different (p>0.05) when compared with seminal volume of group A (5.02 ± 0.18ml which is the control group. The sperm concentration of groups D (94.40 ± 9.58x10^{6}) and E (110.40 ± 4.86 x10^{6}) were significantly higher (p<0.05) compared with groups B (91.80 ± 12.72 x10^{6}), C(85.60 ± 8.20 x10^{6}) and A(72.80 ± 2.59 x10^{6}) of the ethanol extract (Table 2). Sperm motility (%) was significantly higher (p<0.05) in group E (86.00 ± 6.52%) compared with group C (63.00 ± 8.37%) and control group A(54.00 ± 4.18%). Sperm percentage liveability (%) was not significantly different (p> 0.05) among all groups. Sperm morphological abnormality (%) was significantly lower (p<0.05) in group E (10.70 ± 0.54%) compared with groups (A,14.12 ± 0.28%, B,12.71 ± 0.82%, C,12.39 ± 0.95% and D,12.60 ± 0.49%) (Table 2).

The serum testosterone concentration was significantly higher (p<0.05) in group E, 0.18 ±0.01 ngml^{-1} when compared with other groups (A,0.11 ± 0.01 ngml^{-1}; B,0.11 ± 0.01 ngml^{-1}; C, 0.14 ± 0.04 ngml^{-1}; D, 0.15 ± 0.03 ngml^{-1}) also the follicle stimulating hormone concentration was significantly higher (p<0.05) in group E,0.26 ± 0.07 miuml^{-1} when

Table 1: Phytochemical contents of ethanol extract of *Tridax procumbens* leaf

Phytochemical Components	
a. Carbohydrate	+
b. Protein	+
c. Saponin	+
d. Steroids	+
e. Flavonoids	+
f. Cardiac glycosides	-

g. Tanin	+

+ present - absent

Table 2: Effect of ethanol extract of *Tridax procumbens* leaf on semen parameters in male wistar albino rats

Group	Sperm Vol., (ml)	Sperm Motility, (%)	Sperm count, (X 10^6)(unit)	Sperm Viability, (%)	Sperm morphology (Total Sperm Abnormality) TSA, (%)
A, 0mg/kg	5.02 ±0.18 [a]	54.00± 4.18[a]	72.80±2.59[a]	88.80± 3.96	14.12 ±0.28[a]
B,100mg/kg	5.12± 0.08 [a]	65.00 ±5.00[b]	91.80±12.72[a]	89.60 ±5.32	12.71 ±0.82[a]
C,200mg/kg	5.11±0.05[a]	63.00± 8.37[a]	85.60± 8.20[a]	91.80± 7.22	12.39± 0.95[a]
D,400mg/kg	5.16± 0.05[b]	65.00±5.00[b]	94.40 ±9.58[b]	91.60±6.58	12.60± 0.49[a]
E,800mg/kg	5.18 ± 0.04[b]	86.00±6.52[b]	110.40±4.86[b]	97.20±1.64	10.70± 0.54[b]

n=5 Mean ± SD with different superscript were significantly ($p<0.05$) different

Table 3: Effect of ethanol extract of *Tridax procumbens* leaf on some reproductive hormones in male wistar albino rats

Group	Testosterone, (ng/ml)	Follicle stimulating hormone, (miu/ml)	Leutinizing hormone, (miu/ml)
A,0mg/kg	0.11 ± 0.01[a]	0.05 ± 0.06[a]	0.12 ± 0.03[a]
B,100mg/kg	0.11 ± 0.01[a]	0.07 ± 0.07[a]	0.11 ± 0.01[a]
C,200mg/kg	0.14 ± 0.04[a]	0.08 ± 0.01[a]	0.18 ± 0.01[b]
D,400mg/kg	0.15 ± 0.03[a]	0.22 ± 0.26[a]	0.16 ± 0.07[a]
E,800mg/kg	0.18 ± 0.01[b]	0.26 ± 0.07[b]	0.34 ± 0.23[b]

n=5 Mean ± SD with different superscript were significantly ($p<0.05$) different

compared with all other groups (A,0.05±0.06 miuml^{-1}; B,0.07 ± 0.07 miuml^{-1}; C,0.08 ± 0.01 miuml^{-1} and D,0.22 ± 0.026 miuml^{-1}). Serum luteinizing hormone concentration was significantly higher ($p<0.05$) in group E,0.34 ± 0.23 miuml^{-1} when compared with groups (A,0.12 ± 0.03 miuml^{-1}; B,0.11 ± 0.01 miuml^{-1} and D,0.16 ± 0.07 miuml^{-1}) (Table 3).

Discussion

The significant increase of sperm concentration and sperm motility in male wistar rats by ethanol extract of *Tridax procumbens* observed in this study showed that there was dose dependent increase in the semen parameters of extract treated (Mekasha *et al.,* 2007). This may be due to the presence of flavonoids and steroid found in the ethanol extract of *Tridax procumbens* (Jain & Amita 2012). Spermatogenesis is regulated by the pulsatile release of gonadotropin-releasing hormone (GnRH) from the arcuate nucleus of the hypothalamus, which stimulates the anterior pituitary to release follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Herbison 2006).The gonadotropin-releasing hormone (GnRH) stimulates production of LH which acts on the Leydig cells to produce testosterone (Mekasha *et al., 2*007) which has a local effect on the interstitial and seminiferous tubules and results in sperm production and maturation while FSH exerts its effect directly on the Sertoli cells that in turn promote and sustain spermatogenesis (Akingbemi, 2005). The ethanol leaf extract of *Tridax procumbens* increased the serum level of testosterone as the concentration increased, attributable perhaps to the steroidal contents, leading to increased efficiency of spermatogenesis machinery and increased number of germ cells in the seminiferous tubules (Mekasha *et al., 2*007). Steroid found in *Tridax procumbens* had been reported to have good pharmacological effects in rats (Diwan *et al.,* 1989).

This work also revealed that increasing doses of ethanol extract of *Tridax procumbens* leaf led to increased sperm motility and decreased total sperm abnormality in the sperm cells. This result agreed with the works done by Carpino *et al.* (1998), Narayana *et al.* (2005) and Mínguez-Alarcón *et al.,* 2012). The steroid found in ethanol extract of *Tridax procumbens* leaf at higher doses has positive effects on sperm motility (D'Cruz *et al.,* 2010).

The phytochemical content of *Tridax procumbens* validated in this study (Table 1) had been shown to have high medicinal values (Sharma & Kumar 2009; Jain & Amita 2012). Keerthi *et al*. (2014), revealed that ethanol extract of *Tridax procumbens* had low lipid profile activity in rats. The spermatogenetic properties of ethanol extract of *Tridax procumbens* on male wistar rats in this study may also be due to

the presence of phytochemicals (carbohydrates, flavonoids and steroid) found in the ethanol extract of *Tridax procumbens*. This could also account for improvement in the sperm cells over those in the control group.

In conclusion, this study revealed that ethanol extract of *Tridax procumbens* leaf could improve semen qualities (sperm motility, sperm count, sperm morphology) and enhance release of testosterone, follicle stimulating hormone, FSH and luteinizing hormone, LH in male wistar rats as the dose of ethanol extract *Tridax procumbens* increased to 800mg/kg. This may be due to the presence of phytochemicals (carbohydrates, flavonoids and steroid) found in the ethanol extract of *Tridax procumbens* leaf. *Tridax procumbens* leaf therefore could ultimately improve livestock production when fed to animals.

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