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Research Article

Androgenic and Spermatogenic Effects of Methanolic Root Extract of *Waltheria indica* (linn) in Male Wistar Rats

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ABSTRACT

Waltheria indica is a common shrub found in many parts of Northern Nigeria for the management of several diseases. Although the ability of *Waltheria indica* to treat infertility, erectile dysfunction and impotency in male has been reported in ethno-medicine, there is no scientific study to verify its action on androgens and spermatogenic parameters. This study seeks to evaluate the effect of methanolic root extract of *Waltheria indica* on androgens and spermatogenesis in albino rats. Rats were grouped into four: 1, 2, 3 and 4 where 1(the control) received orally 1mL of normal saline. Groups 2, 3 and 4 received orally on daily basis, graded doses of 50, 100 and 200 mg/kg body weight of the plant extract, respectively, for 40 days. Administration of plant extract resulted in enhanced levels of testosterone, follicular stimulating hormone, luteinizing hormone, sperm count, sperm viability, and sperm mobility, testicular gamma glutamyl transferase, testicular alkaline phosphatase, testicular acid phosphatase and testicular cholesterol. The results show that methanolic root extract of *Waltheria indica* has demonstrated androgenic and spermatogenic activity which further substantiates its claimed traditional use in the management of infertility in male folk.

Keywords: Waltheria indica root extract; infertility; erectile dysfunction; impotency in male; androgenic effect; spermatogenic effect

INTRODUCTION

Sexual Dysfunction in males can be defined as impediment of the normal sexual response cycle that causes physical illness, anxiety, depression, psychological toll, and feeling of inadequacy mainly in males.[1,2] The incidence of sexual dysfunction resulting from hormonal imbalance is estimated to be 20–25%.[3] Male Sex disorders are classified into sexual function disorder, sexual orientation, and sexual behavior. Several factors are responsible to maintain normal sexual functions such as intracavernosal nitric oxide system, neural activity, vascular events, and androgens.[4] Androgens vital actions in male cannot be overemphasized as they act centrally and peripherally during initiation of sexual activity and sexual intercourse.[5] One of the most important androgen secreted by the testis in humans is testosterone.[6] A reduction in the level of testosterone at the early developmental phase results in the lack of virilization, sustained height, increase without closure of the epiphysis, lack of pubertal growth spurt, incomplete sexual development and aspermia. In adulthood, it may result in the loss of libido and sexual activity.[3,7] Some medicinal plantsof Africa have been reported to possess enhanced aphrodisiac activities there by significantly improving fertility profile in males.[8] These plants includes *Mondia whitei, Bersama engleriana, Eruca sativa, Nigella sativa* alkaloids found in *Alangium salviifolium* and saponins isolated from *Tribulus terrestris* increases weight of sex accessory organs, concentrations of testosterone and tissue proteins.[3,9]

Waltheria indica linn. also known as sleepy morning is wide spread in West Africa. The Hausas call it Hankufa while the Yorubas call it korikodi. In folkloric medicine, *Waltheria indica* produces anti-inflammatory actions similar to that of aspirin.[10,11]

Waltheria indica whole plant is used in malaria, peptic ulcer, dysentery, cancers, leprosy, epilepsy, infertility, blad-

der ailments, erectile dysfunction and impotence.[12–20]

Even though there are ethno-medicinal claims that *Waltheria indica* has the potentials to treat infertility, erectile dysfunction and impotency in male folks, there is no scientific study to verify its action on androgen. This study seeks to evaluate the activity of methanolic root extract of *Waltheria indica* on androgens and spermatogenesis in albino rats.

MATERIALS AND METHODS

Plant collection authentication and preparation

The roots of *Waltheria indica* were sourced from Geji village in Bauchi State, Nigeria in November 2021. The plant was identified and authenticated at the Department of Horticulture and Landscape Technology was given by Mr. Christopher Abok, a taxonomist with Federal College of Forestry, Jos, Nigeria with assigned voucher numbers of FHJ 273 and deposited in the Federal College of Forestry herbarium. The sourced roots of *Waltheria indica* were cleaned of debris and dried under shade in the department of Pharmacology and Toxicology University of Jos. The dried roots were pulverized using wooden pestle and mortar into coarse powder. Five hundred (500) gram of the pulverized plant material was poured into a 6-L round bottom flask and 3 L of 70% methanol was added to the plant powder to be extracted by maceration. The content of the flask was allowed to stand for 72 h with shaking at intervals of 12 h. After 72 h and was successively passed through sieves of varying pore sizes (800, 500 and 150 μm); followed by filtration using cotton wool plug and finally Whatman No. 1 filter paper to obtain a clear filtrate.

The filtrate was dried by passing a steady stream of air using an exhaust fan to reduce the volume to about one third of the original volume. The remainder was transferred into an oven to dry at a temperature of 40°C after which the dried extract was scrapped and stored in airtight containers at 25 $\pm$ 2°C till use.

Preparation of male rats

Healthy sexually experienced male white albino rats weighing between 200-300 g with age ranging from 20-20.5 weeks were obtained from the Animal House Unit of the Department of Pharmacology; University of Jos, Nigeria and used for the studies. Ethical Clearance Certificate was obtained from Ethical Committee Animal Experimental Unit of Department of Pharmacology, FCULTY OF Pharmaceutical Sciences, University of Jos, Nigeria vide reference UJ/FPS/F17-00379 dated 10.01.2022. The male rats were kept in separate cages (Temperature: 22-28^{0}C; 12 h natural light and 12 h dark; humidity: 50-55 %) with free access to rat pellets. Ethical clearance was obtained from the animal care and use committee of the Department of Pharmacology and toxicology, Faculty of Pharmaceutical Sciences, University of Jos vide Ethical clearance Certificate No. UJ/FPS/F17-00379 dated 10th January, 2022.

Experimental design

The method describe by Yakubuet al., 2012 was employed with slight modification.[21] Male rats in group 1 served as control and orally received10ml/kg of normal saline using oro-pharyngeal cannula while male rats in groups 2, 3 and 4 received 50, 100 and 200mg/kg body weight of the extracts respectively, orally daily for 40 days. Male rats were anesthetized, sacrificed on the 41st day and blood from the rats was collected into clean; dry corked centrifuge tubes. Thereafter, the tubes were centrifuged (Hawksley, England) at 3000 rpm for 15 minutes. The rats were also dissected; testes and epidydimis were collected for testicular studies and sperm analysis

Hormonal assay

The sera wascollected using Pasteur pipettes into clean, dry, sample bottles and then stored frozen overnight before being used for the assay.[21] The concentration of testosterone, follicle stimulating hormone and luteinizing hormone were quantitatively determined using Cobas e411 (Roche, Switzerland) auto-analyzer as outlined in the manufacturer's protocol.

Sperm analysis

The caudal epididymis excised from male rats were sliced into pieces and pulverized with phosphate buffered glucose saline and after being centrifuged a clear suspension devoid of debris was obtained. The total sperm count and motility were calculated according to the method of Besley et al., 1980 using Neubauer's haemocytometer.[22] Smears were prepared from the collected epididymal sample and stained with Eosin-Nigrosin stains to determine the percentage of dead or live sperm cells as described by Ahmed et al., 2014.[23] Similarly, percentage progressive motility was carried out using 2-3 drops of 2.9% warm buffered sodium citrate maintained at body temperature as described by Zemjanis, 1977. The slides prepared were viewed under a microscope as described by Oyeyemi and Ajani, 2015.[24,25]

Testicular analysis

Testes collected were individually weighed, blotted with tissue paper and cut very thinly with sterile blade. The thinly cut pieces were homogenized in ice cold 0.25 M sucrose solution (1:5, w/v). The homogenates were further centrifuged at 1340rpm for 15 min to obtain the supernatant, which was then aspirated with Pasteur pipette into sample bottle to carry out test for testicular total cholesterol, total protein, acid phosphatase, alkaline phosphatase and gamma glutamyl transferase.[26]

Testicular histology

Histological studies was carried out according to the method described by Mehranjani and co-authors in 2009 with slight modifications.[27] The testes were carefully dissected and fixed in10% buffered formalin solution. Thereafter, the testis was embedded in paraffin, followed by preparation of 5 μm thick sections using a rotary microtome. The sections were then stained with hematoxylin and eosin and observed under a light microscope at $\times$100.

Stastical Analysis

The data were subjected to analysis of variance (ANOVA) using Dunnett's test to determine the level of statistical significance. Significance level was set at $p<0.05$ and confidence level at 95%. Statistical analysis was carried out using Graph pad Prism 7.0. Data obtained from statistical analysis were shown as Mean $\pm$ Standard Error of Mean.

RESULTS

Effects of methanolic root extracts of Waltheria indica on sex hormones

Table 1 shows that the extract produced an increase in concentrations of serum Testosterone, Follicular stimulating hormone (FSH) and Luteinizing hormone (LH) when compared to the control at (P<0.05).

Table 1: Effect of Methanolic Root Extract of *Waltheria indica* on Sex Hormones in Male Wistar Rats after 40 Days of Daily administration

Treatment	Mean $\pm$ SEM		
	Testosterone nmol/L	FSH (mUI/mL)	LH (mUI/mL)
Normal saline	0.43±0.180	0.07±0.002	0.42±0.002
W. indica 50 mg/kg	5.43±0.440*	0.08±0.005	0.43±0.008
W. indica 100 mg/kg	7.90±2.437*	0.09±0.001*	0.44±0.003*
W. indica 200 mg/kg	3.04±0.534	0.08±0.005	0.43±0.001

n=6, *=P<0.05,when compared with control, nmol/L=nanomoles per liter, mUI/mL = milli-international units per milliliter

Effects of methanolic root extracts of Waltheria indica on sperm count

Methanolic root extract of *Waltheria indica* as showed in Figure 1 produced a significant increase in number sperm cell counts when compared to control (P<0.05),

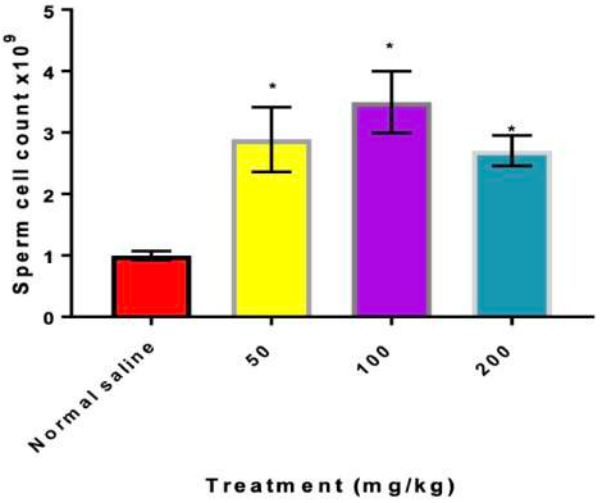

Fig. 1: Effect of Methanolic Root Extract of *Waltheria indica* on Sperm count in Male Wistar Rats after 40 Days of Daily administration

Effects of methanolic root extracts of Waltheria indica on sperm viability

The results in Table 2 show that the extract produced a significant (P<0.05) increase in sperm cells viability male in rats.

Table 2: Effect of Methanolic Root Extract of *Waltheria indica* on Sperm viability in Male Wistar Rats after 40 Days of Daily administration

Treatment	Mean±SEM	
	% Live	% Dead
Normal saline	82.10±3.789	17.9±3.789
W. indica 50 mg/kg	82.85±2.260	17.15±2.325
W. indica 100 mg/kg	85.80±1.349	14.2±1.533
W. indica 200 mg/kg	93.63±1.105*	6.37±1.105*

n=6, *=P<0.05, when compared with control

Effects of methanolic root extracts of Waltheria indica on sperm mobilit y progression

Results in Table 3 reveals that the extract increased the number of active sperms cell in relation to sperm mobile progression.

Table 3: Effect of Methanolic Root Extract of *Waltheria indica* on Sperm Progressive Motility in Male Wistar Rats after 40 Days of Daily administration

Treatments (mg/kg)	Active (%)	Sluggish (%)	Inactive (%)
Normal saline	58.00 ± 8.00	19.00 ± 4.00	23.00±4.36
50	46.00±4.85	29.00 ±1.00	25.00 ± 4.18
100	69.00±7.81	25.00 ± 4.18	15.00 ± 5.48
200	50.00 ± 6.33	21.00 ± 2.45	29.00 ±7 .48

n=5,* = P< 0.05 when compared with control

Effects of methanolic root extracts of Waltheria indica on Gonado-somatic index

Figure 2 shows an increase in Gonado-somatic index.

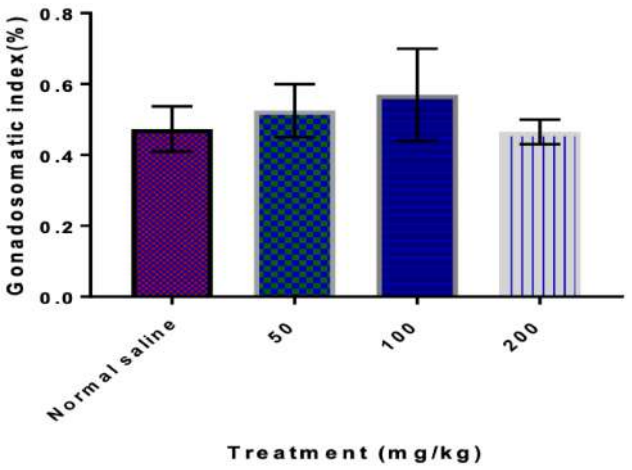

Fig. 2: Effect of Methanolic Root Extract of *Waltheria indica* on Gonadosomatic Index from Male Wistar Rats after 40 Days of Daily administration
n=6, *= P<0.05, when compared with control

Effects of methanolic root extract of Waltheria indica on body weight and gonadal sexual organ weights

Results in Table 4 reveals that the extract did not increase the body weight of animals and weight of sexual organ weights.

Effects of methanolic root extracts of Waltheria indica on testicular enzymes

Table 5 shows that the methanolic root extract of *Waltheria indica* produced an increase in concentration of Gamma glutamyltransferase (GGT), Alkaline phosphatase (ALP), and Acid phosphatase (ACP) which is significant when compared with the control (P<0.05).

Table 4: Effects of Methanolic Root Extract of *Waltheria indica* on Body and Sex Organs Weights of Male Wistar Rats after 40 Days of Daily administration

Treatment (mg/kg)	Final Body weight (g)	Testis (g)	Frontal Epidy-dimis (g)	Caudal Epidy-dimis (g)
Normal saline	295.40 ± 6.68	1.39 ± 0.11	0.29 ± 0.03	0.23 ± 0.01
50	257.00 ± 24.85	0.89 ± 0.27	0.18 ± 0.05	0.17 ± 0.05
100	258.60 ± 11.26	1.13 ± 0.11	0.25 ± 0.03	0.19 ± 0.03
200	330.70 ± 11.97	1.37 ± 0.07	0.28 ± 0.01	0.22 ± 0.02

n=6, *=P< 0.05 when compared with control

Table 5: Effects of Methanolic Root Extract of *Waltheria indica* on Some Testicular Enzymes in Male Wistar Rats after 40 Daysof Daily administration

Treatment (mg/kg)	GGT(U/L)	ALP (U/L)	ACP (U/L)
Normal saline	10.55±0.76	294.20±15.75	1.68±0.32
50	17.10±0.50	327.60 ±9.96	2.93±0.09*
100	24.27±4.90*	328.90±11.62	1.95±0.05
200	18.30 ±0.35	379.20±31.27*	2.32±0.05*

n=6, *=P< 0.05 when compared with control, U/L= unit per liter

Effects of methanolic root extracts of Waltheria indica on testicular protein and cholesterol

Results in Table 6 show that the methanolic root extract of *Waltheria indica* produced an increase in testicular cholesterol which was significant.

Table 6: Effects of Methanolic Root Extract of *Waltheria indica* on Some Biochemical Testicular Parameters in Male Wistar Rats after 40 Days of Daily Administration

Treatment (mg/kg)	Total Protein (g/dl)	Total Cholesterol (mg/dl)
Normal saline	0.50±0.03	0.12±0.01
50	0.50±0.04	0.20±0.01*
100	0.53±0.02	0.20±0.01*
200	0.38±0.02	0.19±0.02*

n=6, *=P< 0.05 when compared withcontrol

Effects of methanolic root extracts of Waltheria indica on histopathology of the testis

Histology of the testis from rats that received 50, 100 and 200 mg/kg of the methanolic root extract of *Waltheria indica*

reveals that the extract did not cause any morphological changes in the cellular integrity of the testis and spermatozoa cell (Plates 1, 2, 3 and 4).

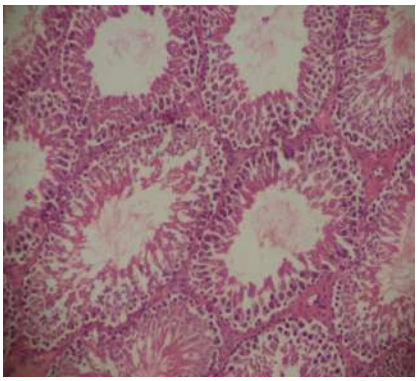

Plate 1: Photomicrograph of testis of male wistar rat administered normal saline, presenting seminiferous tubules with normal tissue architecture, with different cells of the spermotogenic series at different stages of maturation. Black arrows= Primary spermatocytes, White arrowheads= Spermatogonia, White arrows= Interstitial layer, white stars= seminiferous tubules H&E **A**: x 100 **B**: x 400

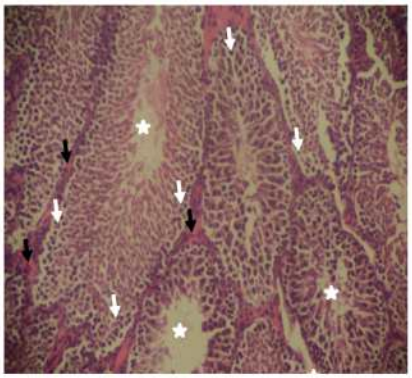

Plate 2: Photomicrograph of testis of male wistar rat administered 50 mg/kg body weight of methanolic root extract of *Waltheria indica* daily for 40 days, showing normal morphology. White arrows= Primary spermatocytes, Black arrows= interstitial cells, White stars= seminiferous tubules. H&E **A**: x 100

DISCUSSION

Enhancement of libido is due to increase in concentration of anterior pituitary hormones and serum testosterone. These hormones are believed to stimulate the synthesis of dopamine required for copulatory and sexual behavior.[28] Increase in testosterone concentration is said to produce an increase in sexual behavior.[29] Testosterone is responsible for penile tumescence and rigidity of accessory muscles that help

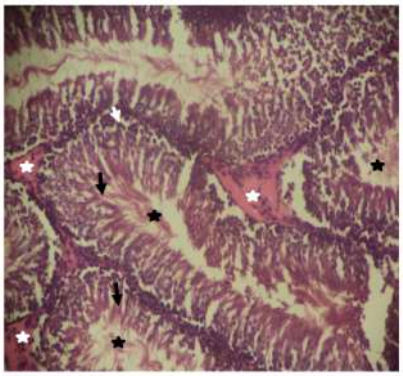

Plate 3: Photomicrograph of testis of male wistar rat administered 100 mg/kg body weight of methanolic root extract of *Waltheria indica* daily for 40 days, showing normal morphology. Black stars=seminiferous tubules, White arrow heads = Spermatogonia, Black arrows = spermatids, white stars=Interstitial cells. H&E **A**: x 100

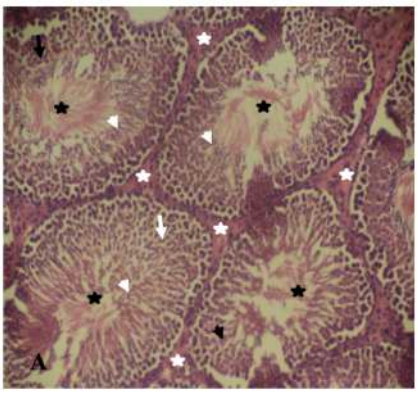

Plate 4: Photomicrograph of testis of male wistar rat administered 200 mg/kg body weight of methanolic root extract of *Waltheria indica* dailyfor 40 days, showing normal morphology. White arrow heads=spermatids, Black stars=seminiferous tubules, White stars=interstitial cells, white arrows=Spermatogonia. H&E **A**: x 100

in improving penile rigidity and ejaculation. Testosterone may enhance sexual behavior by increasing dopamine release in the medial preoptic area of the hypothalamus and potentiating nitric oxide neurotransmission.[30] The enhanced copulatory performance of male rats by the 50 and 100 mg/ kg body weight of methanolic root extract of *Waltheria indica* extract may be due to the increase in serum testosterone concentration. In the testes, luteinizing hormones binds to receptors on Leydig cells, stimulating synthesis and secretion of testosterone.[31]

In a similar trend, significant increase in the concentrations of luteinizing and follicular stimulating hormones at the 100 mg/kg doses investigated can said to be due to stimulation of the hypothalamic pituitary gonadal axis.

Increase in luteinizing hormones, follicular stimulating hormone, and testosterone levels also indicates an effect of methanolic root extract of *Waltheria indica* extract on gonadotropin release hormone (GnRH).

The total duration of spermatogenesis is approximately 4.5 spermatogenic cycles which ranges between 30 to 78 days in mammals. In humans, the entire spermatogenic process is very long and lasts more than 70 days.[32–34] Scientific studies reveal that increased sperm concentration is due to increase in testosterone and follicular stimulating hormone levels in testicular tissue.[35] The plant extract produced an increase in total sperm count, percentage of live sperm cell and decrease percentage of dead sperm cells. This effect may arise due to the fact that the plant extract is able to interfere with spermatogenetic processes. The observed increase in sperm counts further affirms spermatogenic nature of the extract and this may suggest a direct stimulatory effect on the testes resulting in an increase in the number of spermatozoa and concentration of testosterone production.[36] Proteins, sugars, ions and small organic molecule facilitate the journey of sperm from the site of production, subsequent release and fertilization process.[37] The observed improvement in sperm motility especially with the 100 mg/kg dose, may be as a result of the antioxidant properties of extract which can prevent the superfluous generation of oxidizing elements produced by sperm.[38] Gamma glutamyl transferase (GGT), a key androgenic enzyme foundin mammalian tissues, and testis, has its activity attributed to the Sertoli cell function, where it carries spermatozoa into the rete testes. GGT may also play a role in the synthesis of androgen-binding proteins/or the follicle-stimulating hormone (FSH)–inhibiting Sertoli cell factor.[21]

GGT plays a major physiological role in providing cysteine to cells for glutathione and protein synthesis, thereby playing a major role in antioxidant defense in the testis. Reduced glutathione can contribute to the redox potential and its content can be replenished by GGT activity. Significant rise in concentration of testicular GGT indicates potetial antioxidant activity of the plant extract.[39]

Alkaline phosphatase (ALP) and acid phosphatase (ACP) are widely distributed in the testis and both enzymes have been involved in the intercellular and intracellular transportation of metabolites during steroidogenesis and release of gonadotropin.[40,41] ACP facilitates the exchange of materials between the germinal and Sertoli cells and enhances the maturation of spermatocytes during spermatogenesis.[42] ALP is involved in mobilizing carbohydrates and lipid metabolites to be utilized by accessory sex structure or by the sperm cells in the seminal fluid.[43] The observed rise in concentration of testicular ALP activity may simply imply enhance utilization of carbohydrates and lipid metabolites by accessory sex structure. Increase in concentration of testicular ACP can be attributed to enhanced de novo synthesis of the enzyme in the testis, therefore, increase in the phosphatase activity may be an indicative of enhanced spermatogeniccharacteristic of the medicinal plant extract.[41,44]

Testicular proteins, which are androgen dependent, is among many substances required for maturation of spermatozoa.[45] There was no observed increase in levels of testicular protein in rats that received 50 and 100 mg/kg of the extract; however a decrease in testicular protein level was reported in rats that received 200 mg/kg of the medicinal plant.

Cholesterol a precursor for the synthesis of testosterone is required also for the development of germ cells during spermatogenesis.[46] Leydig cells can de novo synthesize cholesterol and use stored cholesterol ester to ensure steroidogenesis function.[47] The majority of nutrients, including lipids, needed for spermatogenesis are provided by supporting Sertoli cells which can synthesize cholesterol from acetate de novo.[47–49] The observed rise in concentrations of cholesterol in the testis may be due to stimulation of steroidogenesis via activation of either Leydig or Sertoli cells, thereby leading to increased androgen concentration.[50]

Histopathological slides provide a more in-depth study of any toxic effects or diseases by investigating its effects on tissues as the preparation process preserves the tissue architecture.[51] Normal histo-morphology of testis was observed after 40 days of oral administration graded doses of methanolic root extract of *Waltheria indica*.

CONCLUSION

Waltheria indica has demonstrated significant increase in androgens and spermatogenic indices and therefore, the claimed ethno-medicinal use for managing male sexual dysfunction has been scientifically substantiated.

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Conflict of interest

The authors declare that there are no conflicts of interest

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