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Original Research Article

Aphrodisiac Potential of Methanol Root Extract of *Hunteria umbellata* in Male Wistar Rats

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ABSTRACT

Hunteria umbellata (*H. umbellata*) have been used locally for the treatment of low libido, obesity, diabetes mellitus, infertility, pyresia, pain, hypertension and as immune booster. This study was aim to evaluate the aphrodisiac activity of the methanol root extract of *H. umbellata* in male Wistar rats. Acute toxicity of the extract was evaluated using standard protocol. The extract at graded doses (25, 50 and 100 mg/kg) and reference drug (10 mg/kg sildenafil citrate) were orally administered for 14 days to investigate aphrodisiac property. The female animals were made receptive via artificial hormonal stimulation. The effects of *H. umbellata* on body and reproductive organ mass indices were determined. The aphrodisiac behaviour monitored were; Mounting Frequency (MF), Intromission Frequency (IF), Ejaculation Frequency (EF), Mounting Latency (ML), Intromission Latency (IL) and Ejaculation latency (EL). The obtained result showed no mortality or side effects from the acute toxicity study. There was a significant increase in MF, IF, EF, ML, IL and EL as well as a significant increase in serum testosterone level across the treated groups when compared with the control. In conclusion, the result of this study indicates that *H. umbellata* has aphrodisiac capacity that may be of great ethnomedicinal benefit since there are no adverse toxicity reports.

***Keywords*:** *Hunteria umbellata*, Aphrodisiac, Male rat, Reproductive properties.

Introduction

Most natural products gotten from plant materials have been useful to man's survival for a healthy living since ancient time of herbal practice. These active constituents present in plant attract scientist interest to understudy the active ingredients with therapeutic properties.[1] Herbal medicine derived from plant substances aid in the treatment of several clinical diseases, including poor libido or infertility.[2]

Hunteria umbellata (K. Schum) Hallier belongs to Apocynaceae family. It is commonly called Abeere in Nigeria. In African ethnomedicine, several portions are extremely valued in human and countless veterinary diseases treatment.[3] *H. umbellata* is used in the local treatment of infertility, obesity, diabetes mellitus, fever, pains, swellings, abdominal colic, stomachache, immune booster and hypertension.[4]

An aphrodisiac is a distinct agent (nutraceutical or therapeutic) that stimulates sexual zeal. In ethnomedicine, several plants serve as sex enhancers.[5] For decades, the Arabs have utilized medicinal drugs to increase sexual recital and upsurge libido.[6] Male erectile dysfunction or impotence is a substantial factor that promotes infertility.[7] Globally, an upsurge in the occurrence of erectile dysfunction, perhaps can be associated with aging people and some other factors including; chronic diseases (diabetes mellitus and heart disease), stress, alcohol, smoking, drug misuse and deskbound lifestyles.

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These factors and diseases lead to erectile dysfunction and low libido.[8] The possible treatments required include psychotherapy, surgery, vacuum devices, drugs and penile implant. Health Organization evaluations indicates that about 80 percent of individuals depend solely on herbal therapies.[9] In regards to several adverse effect, cost implications and unavailability of orthodox medicine, this study therefore can promote novel ingredients found in medicinal plants to correct the inconsistency associated with orthodox medicine. This study was aim to evaluate the aphrodisiac activity of the methanol root extract of *Hunteria umbellata* in male Wistar rats.

Materials and Methods

Collection of plant material

Hunteria umbellata were collected from Ekosodin in Ovia North East Local Government Area, Benin City, Edo State, Nigeria in the months of July 2018. It was identified and authenticated by Dr. O. Timothy in the Herbarium Unit of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Edo State, Nigeria. Herbarium specimen was deposited and voucher number (GE: W852) was obtained.

Preparation of plant material

The roots were washed, cleaned and chopped into pieces and shade-dried for four (4) weeks. The dried roots were pulverized with the aid of a British milling machine and 1000 g of the powdered sample was obtained. The sample was macerated in methanol with constant agitation. It was concentrated in water bath into a semi-solid extract. It was then stored in a refrigerator at 4 ^{0}C.

Experimental animals

Adult male Wistar rats weighing 185-210 g were gotten from the animal house in the Department of Pharmacology and Toxicology, University of Benin, Benin City. The animals were housed in conducive woody cages and allowed free access to grower rat chow and clean water. They were acclimatized for 14 days. The animals were properly handled with a standard technique of the use of Laboratory animals approved and permitted by the ethical committee

of the Faculty of Life Sciences, Faculty of Life Sciences, University of Benin with ethical number LS20103.

Acute toxicity study
Healthy male Swiss mice were denied food for 4 hr. and exposed using acute toxicity doses recommended by the Organization of Economic Co-operation and Development (OECD) procedures No: 423.[10] Animals were randomLy selected into 4 groups (n=5) and kept separately in cages throughout the experiment. Group 1 served as untreated control (5 mL/kg of distilled water orally). Groups 2- 4 received graded doses of the extract of methanol root of *Hunteria umbellata* orally at (1600, 2900 and 5000 mg/kg) all through 7 days. Swiss mice were continuously observed for 2 hours for any change in behavioural, autonomic and neurological indexes for 24 and 72 hours against lethality.

Mating behaviour test
Treated groups were experimented using a method described by Sengupta *et al*.;[11] Azantee *et al.*[12] and adapted by Amin *et al.*[13] Sexually experienced healthy male Wistar rats weighed (185-210 g) that showed abrupt sexual effect were designated for this study. They were randomLy divided into 5 groups (n=5) and kept separately during the experiment. Group 1 serves as the control (0.5 mL/kg DW orally. Groups 2–4 were administered with *Hunteria umbellata* methanol root extract at graded doses (25, 50 and 100 mg/kg), respectively for 14 days. Group 5 as the standard group received (10 mg/kg sildenafil citrate o.p). An hour prior to initiate the experiment, male Wistar rats were introduced to indistinct light at specified time of 6 days. Female Wistar rats were made receptive in oestrus[14] via Azantee *et al.*[12] procedure. They were pre-exposed to 100 µg/kg of ethinyl oestradiol orally, 48 hours prior to mating, 1 mg/kg progesterone was injected subcutaneously 6 h earlier to the research. The female animals were made receptive and exposed to male Wistar rats. The receptive female Wistar rats were randomly grouped. The test was carried out on day 14 of instigation across the treatment of the male Wistar rats. The experiment conducted in the dark hour at night. The receptive female animals were paired with male animals in the cage via ratio 1:1 of female to male. Evaluations for mating performance was instantaneously instigated and sustained for the first two (2) successions of mating. The experiment was completed for an hour. The females that failed to display receptiveness were immediately replaced. The existence of actions and stages of mating were documented on video camera. The following parameters; mounting, mounting frequency (MF), mounting latency (ML), intromission, intromission frequency (IF), intromission latency (IL), ejaculation, first mount or intromission of series ejaculation or ejaculatory latency (EL), ejaculation, first intromission subsequent sequences, post-ejaculatory interlude were examined.

Effect on weight of sexual and vital organs
After the copulating behavioural investigation, the control, reference and test groups were assessed against their body and organ weight. The rats were introduced into mild anaesthetic chloroform, and then sacrificed; the testis and epididymis were cautiously aloof and weighed with a digital balance. Relative organ weight was ascertained.[15-17]

Histology
Testes across the groups were isolated and fixed using Bouin's fluid. Organs were sectioned in 10 % formalin before histological investigation. Slides preparation was performed by a pathologist. Slides were properly stained in eosin and hematoxylin, it was viewed under light microscopy. Micrograph of slide preparation was observed.

Statistical analysis
Data were reported as Mean ± SEM standard error of mean. The means across the groups were compared using ANOVA using Graph pad prism version 6 computer software packages. The $p \leq 0.05$ (95% confidence interval) was considered significant.

Results and Discussion

Pharmacologic screening
Acute Toxicity Test
No mortality was observed at 5000 mg/kg after fourteen days of the study. No significant toxic sign was recorded within the fourteen days period (Table 1).

Results in Table 1 showed the acute toxicological effect with less or no adverse effect such as; respiratory distress, change in hair advent, stooling, maternal mortality, salivation, coma and death. This showed that acute toxicity is apparently safe with no trace adverse effects. This is in conformity with a similar study by Tajuddin *et al*.[18] on ethanolic extract of *Myristica fragrans* with absent mortality and adverse effects on the animals used.
Studies have shown that certain constituents of several plants possess libido enhancing effect, improve fertility and aid in the management of impotence.[19] The Methanol root extracts showed an aphrodisiac property as shown in Table 3.[20] The study groups that received doses of the extract promote increase testosterone which further triggered libido promoting activity as shown in Table 2.[21] *H. umbellata* root extract, also can be associated with the function of varying androgen concentration which could be liable in the enhancement of male sexual performance as shown in this study.[22] Certain phytoconstituents stimulate sexual urge which promote sexual desire.[23] Enhancement in sexual role validated in this research could be as a result of bioactive constituents present in *H. umbellata* root extract.
The latency of intromission and mount serves as a vital display for sexual stimulus, while ejaculation and intromission are considerable behavioural pattern for sexual recital and enhancement.[24] Subsequent to treatment with graded doses (25, 50 and 100 mg/kg) of the root extract of *H. umbellata* with significant increase in mount and intromission latency, serves as an indicator of sexual stimulation after 14 days (Table 3). Also, increase in ejaculation frequency and ejaculation latency elicited to trigger sexual enactment. The extract displayed distinctive property in sexual behavioural pattern with increase in the level of significance; in mounting frequency (MF), Intromission frequency (IF) and ejaculation frequency (EF) when compared with the control. The MF, IF and EF are known as a measuring factor to determine libido and potency effect. An increase in the significant level of Mounting Latency, Intromission Latency and Ejaculation latency (EL) proposed that the extract and reference drug showed prolonged interval of coitus, acting as a pointer to sexual impulse.[25] Decrease in inter-copulatory efficacies are signals of constant upsurge in sexual action and aphrodisiac effect intrinsic to these reports. [26]From this present study, the methanol root extract of *H. umbellata* elicited an increase in sexual ability. This study is in line with the report of Tajuddin *et al*., [27] on aphrodisiac property of the seeds extract of *Allium tuberosum* with a significant decrease in male animals for 21 days across the following indexes: IL, PEI and ML with an increase in IF, EL and MF. [28]
The semen analysis serves as onset of selection for fertility evaluation and is frequently utilized in the definition of semen quality (morphology, viability and sperm motility) and quantity (sperm count).[29] The findings showed rise in percentage increase in motility and sperm count, reduction in percentage abnormal sperm cells and viability and morphology of the extract-treated rats when compared with the control. Hence, it is designated that *Hunteria umbellata* elicited fertility improving effect.
The graded dose level of 25, 50 and 100 mg/kg body weight of the root extract ensued weight increase across treated group (Table 5). The organ mass index of the testes and epididymis significantly increase. Origin of steroids serves as a major bases to the body and testes organ weight increased, which serving as a stared natural indicator to the efficiency of *H. umbellata* to further stimulate steroidal hormones.[16] Meanwhile, androgenic property known to be linked with serum testosterone concentration[17], which could be owing to the extract function in testosterone discharge consenting with improved hormone in gonads.

Table 1: Acute toxicity of methanol root extract of *Hunteria umbellata* in rats

Groups	Doses (mg/kg)	No of lethality	% mortality	Adverse Effect
Control	DW	0/5	0	Absent
H. umbellate	1600	0/5	0	Absent
H. umbellate	2900	0/5	0	Absent
H. umbellate	5000	0/5	0	Absent

Supplementation of Testosterone has formerly been elicited to increase sexual libido and role[29], which strengthen ejaculations and orgasm to improve.[5] This is similar to the study by Watcho *et al*.[30] on *Mondia whitei* hexane extract on male sexual reproductive in rats.
Natural medicine in African involved extracts preparation across the various plant parts are utilized in managing diverse human disorders including yaws, pains, swellings, stomach ulcers, *diabetes mellitus*, low lipido, dysmenorrhoea and induces labour.[4]
Histopathological study of the testes revealed standard structural architecture when compared with the control as shown in plate 1.

Treated organs at 25, 50 and 100 mg/kg extracts showed to enhanced architecture. However, across the treated groups, there was an increase in spermatogenic action in the lumen of seminiferous tubule. This stimulated cellular activity occurrence from cellar membrane all through to the lumen in seminiferous tubules found in the testes. Increase in primary spermatogonia is a proof in these discoveries.[30] this acclaims that, the extract stimulates functional testes architecture in disparity with biochemical parameters with significant upsurge ($P < 0.05$). These observations could be associated with the possible antioxidant phytoconstituents.

Table 2: Effect of methanol root extracts of *Hunteria umbellata* on sex hormones in rats

Parameters	Control	10 mg/kg Sildenafil	25 mg/kg *H. umbellats*	50 mg/kg *H. umbellats*	100 mg/kg *H. umbellats*
Testosterone (pg/mL)	1.61 ± 0.68^{a}	2.63 ± 0.93^{b}	2.67 ± 1.01^{b}	2.87 ± 0.75^{b}	2.79 ± 1.10^{b}
FSH (mIU/mL)	1.38 ± 0.21^{a}	1.92 ± 0.59^{b}	2.23 ± 1.00^{b}	2.46 ± 0.74^{b}	1.99 ± 0.81^{b}
LH (mIU/mL)	0.26 ± 0.15^{a}	0.37 ± 0.08^{b}	0.45 ± 0.10^{b}	0.48 ± 0.06^{b}	0.39 ± 0.05^{b}
Progesterone (ng/mL)	0.12 ± 0.03^{a}	0.31 ± 0.08^{b}	0.36 ± 0.11^{b}	0.37 ± 0.15^{b}	0.30 ± 0.09^{b}

P<0.05 showed significant difference, Superscript [a]--p>0.05, b-- p<0.05 FSH- Follicle stimulating hormone, LH -Luteinizing hormone

Table 3: Effect of methanol root extract of *H. umbellata* on male rat sexual parameters

	Dose mg/kg	MF	IF	EF	ML (sec)	IL (sec)	EL (sec)
Control	0.5mL	10.00 ± 0.22^{a}	2.05 ± 1.01^{a}	0.00 ± 0.00^{a}	108.290 ± 8.12^{a}	58.81 ± 3.09^{a}	0.00 ± 0.00^{a}
Sildenafil	10	31.60 ± 3.11^{c}	19.16 ± 4.04^{c}	5.94 ± 1.62^{c}	265.18 ± 8.42^{c}	129.00 ± 8.07^{c}	51.24 ± 2.95^{c}
H. umbellata	25	15.03 ± 2.06^{b}	7.92 ± 4.24^{b}	0.69 ± 0.41^{b}	211.92 ± 6.03^{c}	80.35 ± 6.02^{b}	21.94 ± 0.75^{b}
H. umbellata	50	18.27 ± 3.14^{b}	10.25 ± 3.00^{b}	1.89 ± 0.40^{b}	253.04 ± 7.79^{c}	93.70 ± 6.29^{b}	38.23 ± 3.06^{b}
H. umbellata	100	28.91 ± 1.97^{c}	16.09 ± 2.93^{c}	4.61 ± 0.53^{c}	283.50 ± 10.12^{c}	101.05 ± 8.28^{c}	46.00 ± 3.11^{c}

Results are expressed as Mean ± SEM; n=5; [a] >0.05 [b, c] < 0.05 vs control; ML= mounting latency; IL= intromission latency, EL= ejaculatory latency; MF = mounting frequency; IF = intromission frequency; EF = ejaculatory frequency.

Table 4: Effect of methanol root extracts of *Hunteria umbellata* on rat sperm parameters

Groups	Dose (mg/kg)	Total Sperm Cell Count	Progressive Motility	Non-Progressive Motility	Immotile	Normal Morphology	Abnormal Morphology
Control DW	0.5 mL	193.33 ± 48.07^{a}	60.00 ± 0.00^{a}	20.00 ± 0.00^{a}	46.67 ± 26.67^{a}	90.00 ± 0.00^{a}	10.00 ± 0.00^{b}
Sildenafil	10	353.33 ± 12.02^{c}	73.33 ± 3.33^{b}	10.00 ± 0.00^{b}	16.67 ± 3.33^{c}	70.00 ± 5.77^{b}	30.00 ± 5.77^{b}
H. umbellata	25	376.67 ± 29.63^{c}	56.67 ± 23.33^{a}	13.33 ± 3.33^{b}	30.00 ± 20.00^{b}	70.00 ± 0.00^{b}	30.00 ± 0.00^{b}
H. umbellata	50	256.67 ± 20.28^{b}	73.33 ± 6.67^{b}	13.33 ± 3.33^{b}	13.33 ± 3.33^{c}	70.00 ± 0.00^{b}	30.00 ± 0.00^{b}
H. umbellata	100	330.00 ± 20.82^{c}	73.33 ± 3.33^{b}	13.33 ± 3.33^{b}	13.33 ± 3.33^{c}	76.67 ± 3.33^{b}	23.33 ± 3.33^{b}

Superscript [b, c] showed the level of significance *p<0.05* and [a] > 0.05 with respect to control. Values expressed in mean ± SEM; n=5.

Table 5: Effect of methanol root extracts of *Hunteria umbellata* on body and reproductive organ weights changes

Groups	Doses mg/Kg	Body weight (g)	Testis	Epididymis
Control DW	0.5 mLs	19.6 ± 2.5	1.16 ± 0.12	0.40 ± 0.03
Sildenafil	10	24.72 ± 6.02	1.19 ± 0.11	0.58 ± 0.11
H. umbellata	25	23.12 ± 3.31	1.13 ± 0.09	0.49 ± 0.07
H. umbellata	50	22.69 ± 3.35	1.18 ± 0.10	0.54 ± 0.10
H. umbellata	100	26.94 ± 2.68	1.20 ± 0.15	0.60 ± 0.12

Superscript [b, c] showed the level of significance *p<0.05* and [a] > 0.05 with respect to control. Values are Mean±SEM; n=5.

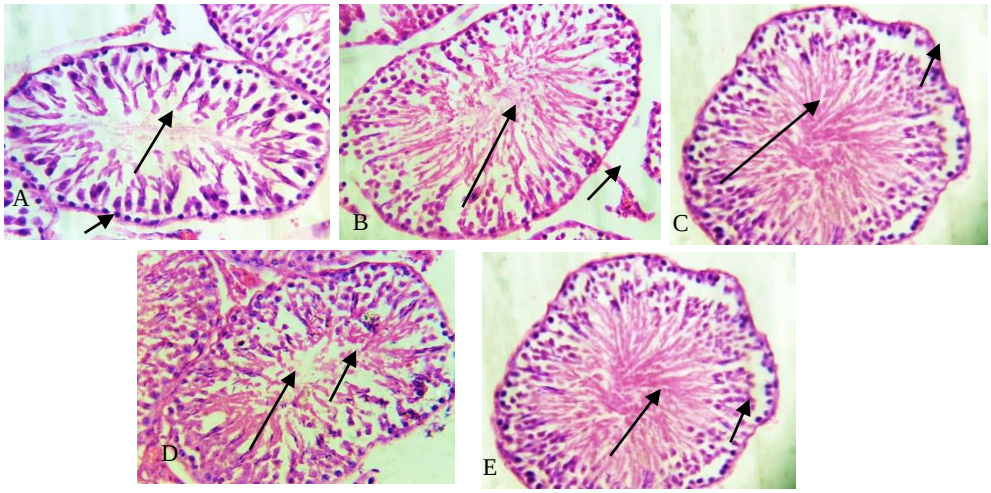

Plate 1: Aphrodisiac effect of *Hunteria umbellata* extract on the Testes

A. CONTROL TESTIS: Elicited impartially circular Seminoferous tubules [long arrow] comprising of visible spermatogonia, spermatids and sertoli cells [short arrow]. **B.** Sildenafil TESTIS: Showed fairly circular Seminoferous tubules [long arrow] with visible spermatogonia, spermatids and sertoli cells [short arrow]. **C.** 25 mg/kg *Hunteria umbellata* TESTIS: Exhibits impartially circular Seminoferous tubules [long arrow] with detectible spermatogonia, spermatids and sertoli cells [short arrow]. **D.** 50 mg/kg *Hunteria umbellata* TESTIS: exposes fairly circular Seminoferous tubules [long arrow] comprising perceptible spermatogonia, spermatids and sertoli cells [short arrow]. **E.** 100 mg/kg *Hunteria umbellata* TESTIS: reveals equally circular Seminoferous tubules [long arrow] enclosing discernible spermatogonia, spermatids and sertoli cells [short arrow].

Conclusion

Hunteria umbellata elicited an effective aphrodisiac activity with little or no adverse toxicological effect. The therapeutic property observed in this study concurred with its ethnomedicinal reports. Further studies geared towards the isolation and structure elucidation of bioactive compounds from the plant is therefore encouraged.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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