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ORIGINAL ARTICLE

A Phytochemical Analysis and *in vivo* Effects of an Herbal Aphrodisiac *Newtonia hildebrandtii* on Male Wistar Rat Reproductive System

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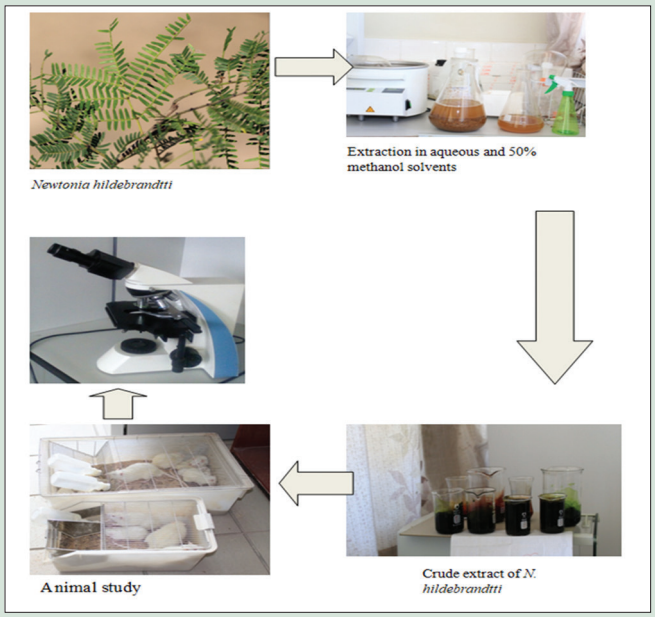
ABSTRACT

Background: The study was aimed at screening phytochemicals and evaluating the effects of aqueous and 50% (v/v) methanol stem bark extracts of *Newtonia hildebrandtii* on male rat reproductive system. **Materials and Methods:** Thirty male Wistar rats were divided into five groups and were given different treatments of *N. hildebrandtii* extracts at 200 and 400 mg/kg body weight. After 8 weeks, body and right testis weight, sperm concentration, hormonal levels, and histological structure of the testis were evaluated. Calorimetric method was used for quantitative analysis of phenolic compounds, whereas gas chromatography–mass spectrometry (GC-MS) was used for fingerprinting of chemical compounds. **Results:** Testosterone, luteinizing hormone, estradiol, and follicle-stimulating hormone levels were significantly increased, whereas prolactin levels were significantly decreased in the treated groups compared to the control. Sperm parameters were significantly increased compared to the control. Treated animals showed significant increases in body weight, seminiferous tubule diameter, and germinal epithelial height with the testes showing all the stages of spermatogenesis. Phytochemical screening of the methanolic extracts revealed the presence of alkaloids, carbohydrates, reducing sugars, steroids, anthraquinones, flavonoids, phenols, and saponins. GC-MS analysis revealed the presence of dimethylsilanediol; (R, R)-2,3-butanediol; hexamethylcyclotrisiloxane; succinimide; 1H-1,2,3-triazole-4-carboxylic acid, 1-(4-amino-1,2,5-oxadiazol-3-yl)-5-ethyl-, ethyl ester; l-(+)-ascorbic acid 2,6-dihexadecanoate; cis-13,16 docasadienoic acid, methyl ester; 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester; and 2-chloro-benzoic acid N'-[2-(3-oxo-3,4-dihydro-2H-benzo[1,4] thiazin-2-yl)-acetyl]-hydrazide. **Conclusion:** *N. hildebrandtii* appears to possess androgenic and anabolic effects. The aforementioned phytochemicals seem to be responsible for its aphrodisiac effects. This study supports the ethno-aphrodisiac claims that have been made against it.

Key words: Antioxidants, DPPH, estradiol, seminiferous tubule, sperm concentration, spermatogenesis, testosterone, total flavonoids, total phenols

SUMMARY

- *Newtonia hildebrandtii* can be used in the management of male infertility
- The crude extract of the stem bark contains phenolic compounds, saponins, triterpenes, and steroids
- The crude extracts promote male fertility through improved sex hormone levels, increased sperm concentration, and testicular histological structures.



Abbreviations Used: GC-MS: Gas chromatography–mass spectroscopy, GEH:Germinalepithelialheight;STD:Seminiferous tubule diameter; LH: luteinizing hormone; FSH: follicle-stimulating hormone.

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INTRODUCTION

Worldwide, herbal aphrodisiacs have been used to manage sexual disorders such as erectile dysfunction, premature ejaculation, lack of sexual desire, and infertility. Unfortunately, there is very little scientific evidence to support ethno-aphrodisiac claims against different herbs in reversing infertility and promoting erectile function. So far, studies that have been done on animal models and randomized clinical trials have demonstrated the ability of herbal aphrodisiacs to enhance sexual behavior, testosterone levels, erectile function, and spermatogenesis among others. Ethanol extracts of *Blepharis edulis* have been reported

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to enhance serum testosterone levels in male albino mice.[1] It has been reported that *Tribulus terrestris* improves libido and spermatogenesis in humans and animals.[2] Aqueous root extracts of *Anacyclus pyrethrum* at 50 and 100 mg/kg body weight have been reported to initiate anabolic and spermatogenic effects in Wistar albino rats.[2] Noteworthy is the ability of *Allium tuberosum* to promote corpus cavernosum smooth muscle relaxation and sexual arousal and enhance sexual behavior in male rats.[3]

Research-based data indicate that infertility affects 8%–12% of reproductive-aged couples worldwide, with an average global rate of 9%.[4] Male infertility can be reversed by provision of nutrient therapeutic agents such as carnitine, arginine, zinc, selenium, and Vitamin B12 and antioxidants such as Vitamin C, Vitamin E, glutathione, and coenzyme Q10[5] As regards erectile dysfunction, several plant-based pharmaceuticals have been developed to address erectile dysfunction. Drugs such as Viagra (sildenafil citrate)[6] tentex, phentolamine (Regitine), prostaglandin E1 (alprostadil) have been shown to correct erectile dysfunction.[7]

However, these management approaches are not only expensive for men with average income but also have side effects. Because of this, most men resort to herbal aphrodisiacs as they are cheap, easy to access, and with no side effects.[8]

From the southern part of Malawi in Nsanje district, claims have been made to the effect that *Newtonia hildebrandtii* promotes erectile function and fertility. To ascertain these claims, the stem bark of *N. hildebrandtii* was screened of its phytochemicals and evaluated its effects on the reproductive system of male Wistar rats.

N. hildebrandtii belongs to the family Fabaceae. It is used in the management of wounds and skin conditions and stomach ailments.[9] This tree is medium in size and can grow to a height of about 25 m.[10] Its leaves are bipinnate and up to 8 cm long. Each leaf can have 4–7 pairs of pinnae, and each pinna can have 6–19 pairs of leaflets. The inflorescence is a spike up to 8 cm long. The flowers are white or cream in color. These flowers give rise to flattened pods which can grow up to 30 cm long. The tree is mostly found growing on riverside forests and sandy areas with a high water table at an altitude of nearly 1,100 m above sea level.[11]

Phytochemical analysis of *N. hildebrandtii* leaves has shown the presence of phenols, flavonols, flavonoids, procyanidins, and tannins.[9] In this study, we hypothesized that *N. hildebrandtii* extracts have anabolic and androgenic effects on male rat reproductive system.

MATERIALS AND METHODS

Collection of plant material

Stem barks of *N. hildebrandtii* were collected from Bangula in Nsanje district, Malawi. Identification and authentication was done by a botanist from Malawi National Herbarium and Botanic Gardens and gave it voucher number MAL21374. The stem bark of the plant was washed with distilled water to remove any dirt and debris before drying in a shade. Mortar and pestle were used to prepare powder which was then stored in air-tight containers.

Extract preparation

The powdered material (100 g) was soaked in water (400 ml) and 50% (v/v) methanol (400 ml) for 48 h with intermittent stirring/shaking.[12] Thereafter, the sample was filtered through Whatman filter paper No. 1 (Whatman Ltd., England). The filtrate was then evaporated to dryness using a rotary evaporator (Buchi Rotavapor R-215 rotary evaporator, Switzerland) at 50°C. Thereafter, the extracts were further evaporated to dryness under ambient temperature for 3 weeks. The extracts were then used to prepare 200 and 400 mg/kg body weight.

Animals and treatment

The study was approved by the College of Medicine Research and Ethics Committee along with the Declaration of Helsinki on animal research. Thirty male albino Wistar rats weighing 160–280 g were housed in a temperature-controlled room under a 12-h-light/-dark period in cages (6 rats/cage) at College of Medicine, University of Malawi. They had access to food and water *ad libitum*.[13]

The rats were divided into five groups: Group A – control rats received pure tap water, Group B received aqueous extracts at 200 mg/kg body weight, Group C received aqueous extracts at 400 mg/kg body weight, Group D received 50% (v/v) methanol extracts at 200 mg/kg body weight, and Group E received 50% (v/v) methanol extract at 400 mg/kg body weight. The extracts were given orally for 56 days. Weight of individual rat was monitored every 10 days. Diet and water intake of each cage was monitored daily during the entire treatment period. At the end of treatment, both treated and control animals were sacrificed under phenobarbitone anesthesia by intraperitoneal injection at 0.4 mL/kg body weight.

Blood samples were collected through cardiac puncture into non-heparinized centrifuge tubes. Thereafter, it was centrifuged at 1500 rpm for 10 min and the serum was collected for hormonal analyses. Hormonal parameters assessed included testosterone, progesterone, follicle-stimulating hormones (FSHs), luteinizing hormone (LH), estradiol, and prolactin.

Right testes were harvested, cleaned off of excess fat, and weighed. Left caudal epididymis was excised in phosphate-buffered saline (PBS) solution to release sperms and incubated before determining sperm concentration, motility, and vitality.

Testicular histology

Histological studies were done according a procedure done by Mehranjani *et al*. 2009 with slight modifications.[14] In brief, following an incision on the abdomen, the testes were carefully dissected and fixed in 10% 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 ×40.

Sperm counts

Sperm count was done as described in Cheesbrough Laboratory Manual[15] with some modifications. The caudal epididymis was carefully cut from the testis and removed of any fat before homogenizing it in 2 ml of normal saline solution. The suspension was then diluted with PBS solution in the ratio 1–20. The improved Neubauer hemocytometer chamber was filled with the diluted sperm, and counting was done over 2 mm^2 of Neubauer hemocytometer chamber.

GC-MS analysis

GC-MS is a hyphenated analytical technique in which gas chromatography (GC) and mass spectrometry are coupled together. GC-MS analysis of the aqueous and 50% (v/v) methanol extract of *N. hildebrandtii* stem bark was done following a procedure a procedure as done elsewhere with slight modification.[16] A dedicated GC column (2010) coated with polymethyl silicon (0.25 nm × 50 m) was connected to a Shimadzu Japan GC QP2010 plus. The temperature was set between 80°C and 200°C. Initially, the temperature was held at 80°C for 1 min and allowed to change at the rate of 5°C/min for 24 min. Field ionization detector temperature was at 300°C, injection temperature was 220°C, and carrier gas nitrogen flow rate was 1 ml/min with a split ratio of 1:75.

GC-MS analysis was done using GCMS-QP 2010 plus Shimadzu Japan with injector temperature of 220°C and carrier gas pressure of 116.9 kpa. The column length was 30 m and a diameter of 0.25 mm and a flow rate of 50 ml/min. Elution through the mass spectrometer was done at 5 kv, and sampling was done every 0.2 s. The mass spectrum was then referred to a computer fed mass spectra data bank. The chemical constituents of the extracts were identified by matching the peaks with those in the National Institute of Standards and Technology GC-MS library.

Statistical analysis

Data were analyzed by one-way analysis of variance using RStudio statistical version 1.1.456 (Boston, MA). Data were expressed as mean ± standard deviation, and $P < 0.05$ was considered statistically significant.

RESULTS

Phytochemical screening

Qualitative phytochemical screening of both extracts showed the presence of flavonoids, phenols, reducing sugars, tannins, saponins, alkaloids, anthraquinones, steroids, and terpenes.

In reference to Table 1, quantitative analysis using 2,2‘-Diphenyl-1-picrylhydrazyl (DPPH) assay, aqueous *N. hildebrandtii* extract at 200 mg/kg body weight had a free radical scavenging capacity of 37.03 ± 2.3%. The 50% (v/v) methanol extract given at 400 mg/kg body weight had the radical inhibition capacity of 57.14 ± 0.86%.

Employing the 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) assay, aqueous extract administered at 200 and 400 mg/kg body weight had inhibition rates of 0.70 ± 0.42 and 3.77 ± 1.10%, respectively, whereas the 50% (v/v) methanol extracts had inhibition rates of 2.43 ± 1.04 and 4.54 ± 1.00%, respectively.

Using the Folin–Ciocalteu method, the total flavonoid content in aqueous *N. hildebrandtii* extracts when administered at 200 and 400 mg/kg body weight was 1327.80 ± 159.70 and 733.3 ± 59.90 µg QE/g dry weight, respectively, whereas the 50% (v/v) methanol extracts were 740.80 ± 59.06 and 1400.90 ± 92.40 µg QE/g dry weight, respectively.

Using the aluminum chloride method, the total phenol content in aqueous *N. hildebrandtii* extracts when administered at 200 and 400 mg/kg body weight was 265.32 ± 24.81 and 460.25 ± 28.90 µg GAE/g dry weight, respectively, whereas the 50% (v/v) methanol extracts were 235.75 ± 24.96 and 369.4 ± 23.93 µg GAE/g dry weight, respectively.

The ferric reducing antioxidant power of aqueous *N. hildebrandtii* extracts when administered at 200 and 400 mg/kg body weight body weight was 2.14 ± 0.51 and 1.06 ± 0.52 µM Trolox Eq. 100 mL^{-1}, respectively, whereas the 50% (v/v) methanol extracts were 1.40 ± 0.25 and 0.93 ± 0.15 µM Trolox Eq. 100 mL^{-1}, respectively.

GC and mass spectroscopy analysis of the stem bark extracts of *N. hildebrandtii* resulted in the identification of 5 and 9 compounds in aqueous and 50% (v/v) methanol, respectively.

Identified compounds are listed in Tables 2 and 3 with their retention time, chemical formula, molecular weight, and the peak area. The major compounds detected in aqueous extract were silanediol, dimethyl-; 2,3-butanediol, [R-(R*, R*)]-; cyclotrisiloxane, hexamethyl-; Succinimide; 1H-1,2,3-triazole-4-carboxylic acid, 1-(4-amino-1,2,5-oxadiazol-3-yl)-5-ethyl, ethyl ester; l-(+)-ascorbic acid 2,6-dihexadecanoate; cis-13,16-docasadienoic acid, methyl ester; 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester; and 2-chloro-benzoic acid N’-[2-(3-oxo-3,4-dihydro-2H-benzo[1,4] thiazin-2-yl)-acetyl]-hydrazide.

The major compounds identified in 50% (v/v) methanol extract included silanediol, dimethyl-; oxime-, methoxy-phenyl-; cyclotrisiloxane, hexamethyl-; tricyclo[4.2.1.0(2,5)]nonane, 3,3,4-trimethyl-; and 2,2,2-trichloroethyl 7-methoxycarbo nylamino-3-phenylthi omethyl-3-cephem-4-carbo xylate 1,1-dioxide [Table 3].

Effect of extracts of *Newtonia hildebrandtii* on the body and organ weight

The kidney and body weights of rats receiving aqueous extracts at 200 and 400 mg/kg body weight were comparable to those of the control. However, there was a dose-dependent drop in the weight of the right testis. While the 50% (v/v) methanol extract promoted significant increases ($P < 0.05$) in body and kidney weight at both doses, the right testis weight was comparable to that of the control [Table 4].

Effect of extract of *Newtonia hildebrandtii* on hormonal levels

In reference to Table 5, both aqueous and 50% methanol *N. hildebrandtii* extracts at 200 and 400 mg/kg body weight promoted significant increases ($P < 0.05$) in serum testosterone, leutinizing, and FSHs in the treated rats compared to the control. Interestingly, the 50% methanol extract at both doses promoted a significant increase ($P < 0.05$) in estradiol levels, whereas the aqueous extract treated animals showed estradiol levels that were comparable to the control.

Table 1: Antioxidant capacity, total phenol, and total flavonoid content of aqueous and 50% methanol *Newtonia hildebrandtii* extracts

Assay	Extract	Parameter	Dosage (mg/kg body weight)	
			200	400
DPPH	Aqueous	Inhibition (%)	37.03±2.30[b]	44.12±2.37[a]
		IC_{50} (µg/ml)	1357.76	1135.77
	50% methanol	Inhibition (%)	48.53±0.65[b]	57.14±0.86[a]
		IC_{50} (µg/ml)	1030.78	873.272
ABTS	Aqueous	Percentage inhibition	0.70±0.42[b]	3.77±1.10[a]
		IC_{50} (µg/ml)	14627.9	143046
	50% methanol	Percentage inhibition	2.43±1.04[b]	4.54±1.00[a]
		IC_{50} (µg/ml)	29604.7	119017.7
FRAP	50% methanol	µM Trolox equation 100/mL	2.14±0.51[a]	1.06±0.52[b]
	Aqueous	µM Trolox equation 100/mL	1.4±0.25[a]	0.93±0.15[b]
Flavonoid	Aqueous	mg QE g/dw	733.30±59.90[b]	1327.80±159.7[a]
	50% methanol	mg GE g/dw	740.80±59.06[b]	1400.90±92.40[a]
Phenol	Aqueous	mg GAE g/dw	265.32±24.81[b]	460.25±28.90[a]
	50% methanol	mg GAE g/dw	235.75±24.96[b]	369.40±23.93[a]

Data in the same raw with different letters are significantly different; QE=Quercetin equivalent; GAE=Gallic acid equivalent

Table 2: Chemical composition of aqueous *Newtonia hildebrandtii* extracts identified by gas chromatography-mass spectrometry analysis

Peak number	Retention time (min)	Compound identified	Height (%)	Area (%)	Molecular formula	Molecular mass (g/mol)
1	3.109	Dimethylsilanediol	11.98	6.89	$C_2H_8O_2Si$	92.17
2	4.068	2,3-Butanediol, [R-(R*, R*)]-	40.16	28.08	$C_4H_{10}O_2$	90.12
3	9.676	Hexamethylcyclotrisiloxane	15.39	16.96	$C_6H_{18}O_3Si_3$	222.46
4	11.344	Succinimide	6.29	8.66	$C_4H_5NO_2$	99.089
5	17.156	1H-1,2,3-Triazole-4-carboxylic acid, 1-(4-amino-1,2,5-oxadiazol-3-yl)-5-ethyl-, ethyl ester	2.55	7.19	$C_{15}H_{16}N_6O_4$	344.325
6	28.658	l-(+)-Ascorbic acid 2,6-dihexadecanoate	5.51	8.46	$C_{38}H_{68}O_8$	652.9
7	30.853	cis-13,16-docasadienoic acid, methyl ester	8.38	9.03	$C_{23}H_{42}O_2$	350.6
8	30.964	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	6.88	7.9	$C_{18}H_{30}O_2$	278.4
9	40.095	2-Chloro-benzoic acid N'-[2-(3-oxo-3,4-dihydro-2H-benzo[1,4] thiazin-2-yl)-acetyl]-hydrazide	2.86	6.83	$C_7H_5ClO_2$	156.56

Table 3: Chemical composition of 50% methanol *Newtonia hildebrandtii* extracts identified by gas chromatography-mass spectrometry analysis

Peak number	Retention time (min)	Compound identified	Height (%)	Area (%)	Molecular formula	Molecular mass (g/mol)
1	3.239	Dimethyl silanediol	75.39	61.72	$C_2H_8O_2Si$	92.17
2	5.786	Oxime-, methoxy-phenyl-	19.02	30.9	$C_8H_9NO_2$	151.16
3	9.673	Cyclotrisiloxane, hexamethyl-	2.88	4.02	$C_6H_{18}O_3Si_3$	222.46
4	30.835	Tricyclo[4.2.1.0(2,5)]nonane, 3,3,4-trimethyl-	1.41	2.06	$C_{12}H_{20}$	164.29

Table 4: Anabolic effects on organ and body weight of male Wistar rats treated with aqueous and 50% methanol *Newtonia hildebrandtii* extracts

Herb	Extract	Organ	Treatment (mg/kg body weight)		
			0	200	400
Newtonia hildebrandtii	Aqueous	Body (g)	153.30±10.10^a	140.70±20.20^a	181.31±5.20^a
		Right testis (g)	1.58±0.10^a	0.848±0.14^b	1.02±0.30^b
		Kidney (g)	0.55±0.12^a	0.513±0.11^a	0.57±0.20^a
	50% methanol	Body (g)	153.30±10.10^c	265.70±11.10^a	194.10±10.20^b
		Right testis (g)	1.58±0.10^a	1.80±0.50^a	1.47±0.41^a
		Kidney (g)	0.55±0.12^b	0.71±0.02^a	0.63±0.10^a

Values are represented as mean±SD from 6 rats. Means within the same row with different letters are significantly different (P<0.05). SD: Standard deviation

Table 5: Change in hormone levels of Wistar rats following treatment with *Newtonia hildebrandtii* for 8 weeks

Extract	Hormone parameter	Dosage (mg/kg)		
		0	200	400
Aqueous	FSH (IU/L)	0.77±0.05^a	0.9±0.8^b	1.4±0.1^a
	LH (IU/L)	0.1±0.01^c	0.4±0.04^b	0.8±0.1^a
	Prolactin (ng/ml)	0.6±0.9^a	0.0±0.0^b	0.0±0.0^b
	Estradiol (pg/ml)	37.7±12.9^a	35.03±1.0^a	36.9±10.5^a
	Testosterone (ng/ml)	0.7±0.12^b	1.0±0.87^a	0.93±0.38^a
50% methanol	FSH (IU/L)	0.77±0.05^b	1.3±0.06^a	1.5±0.5^b
	LH (IU/L)	0.1±0.01^c	0.97±0.12^a	0.7±0.4^b
	Prolactin (ng/ml)	0.6±0.9^a	0.0±0.0^b	0.0±0.0^b
	Estradiol (pg/ml)	37.7±12.9^b	46.8±3.25^a	50.4±11.95^a
	Testosterone (ng/ml)	0.7±0.12^b	0.9±0.8^a	1.4±0.95^a

Values are represented as mean±SD from 6 rats. Means within the same row with different letters are significantly different (P<0.05). SD: Standard deviation; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone

Sperm motility and concentration

Table 6 showed that treatment of male albino Wistar rats with aqueous and 50% methanolic stem bark extract of *N. hildebrandtii* at 200 and 400 mg/kg body weight promoted significant increases ($P < 0.05$) in the percentage of progressive motile, nonprogressive, and total motile cells.

The aqueous extract promoted a dose-dependent significant ($P < 0.05$) increase in sperm concentration in the treated animals compared with the control. The 50% (v/v) methanol extract promoted a significant ($P < 0.05$) nondose-dependent increase in sperm concentration in the treated animals compared with the control [Table 6].

Testis histology

Histoarchitecture of the testes revealed normal seminiferous tubules with increased spermatogenesis among the extract-treated Wistar rats compared to the control [Table 7]. There were also significant increases in seminiferous tubule diameter (STD) and germinal epithelium height with all stages of cell development. Microthin sections of the treated and untreated groups indicated normal cycle of spermatogenesis. The seminiferous tubules had well-presented Sertoli cells and tubular basement membrane. The interstitium between tubules and Leydig cells for the treated groups had an architecture similar to the control. The lumen of the treated groups appeared to contain more sperm than the control group [Figure 1].

DISCUSSION

The results of this study demonstrated that daily consumption of *N. hildebrandtii* extracts for 8 weeks caused increased spermatogenic cell density, caudal epididymal sperm concentration, and total sperm motility of male Wistar rats. It also caused significant increases in body and reproductive organ weights, STD, and germinal epithelial height (GEH). Testosterone, LH, estradiol, and FSH levels increased, whereas prolactin levels decreased in the treated groups compared with the control.

The significantly increased body weight of the treated animals showed that the extracts of *N. hildebrandtii* when administered at 200 and

Table 6: Change in sperm parameters of Wistar rats following treatment with *Newtonia hildebrandtii* for 8 weeks

Extract	Parameter	Dosage (mg/kg)		
		0	200	400
Aqueous	Percentage nonprogressive	15.38±11.5^{c}	47.8±9.8^{a}	33.3±3.8^{b}
	Nonmotile	69.1±15.9^{a}	21.5±7.04^{b}	27.9±3.8^{b}
	Percentage progressive	15.5±11.0^{b}	31.1±6.2^{a}	39.5±3.9^{a}
	Sperm concentration (×10^{6})	128.05.1±37.5^{c}	162.1±17.7^{b}	365.8±17.1^{a}
	Total motile	30.89±5.3^{b}	78.5±12.5^{a}	72.9±6.1^{a}
Methanol	Percentage nonprogressive	15.38±11.5^{a}	21.8±4.0^{a}	20.3±3.2^{a}
	Nonmotile	69.1±15.9^{a}	16.7±2.7^{b}	21.6±4.2^{b}
	Percentage progressive	15.5±11.0^{b}	61.5±3.7^{a}	58.1±6.9^{a}
	Sperm concentration (×10^{6})	128.05.1±37.5^{b}	182.9±11.3^{a}	159.9±23.4^{b}
	Total motile	30.89±5.3^{b}	81.8±4.9^{a}	78.0±9.0^{a}

Values are represented as mean±SD from 6 rats. Means within the same row with different letters are significantly different (P<0.05). SD: Standard deviation

Table 7: Seminiferous tubule diameter and germinal epithelial height of Wistar rats following 8 week treatment with *Newtonia hildebrandtii* extracts

Herb	Dosage (mg/kg)	Parameter	Treatment			*P*
			Control	Aqueous	50% methanol	
Newtonia hildebrandtii	200	STD (μm)	1366.0±80.70^{c}	1635.8±80.30^{b}	1804.7±78.70^{a}	0.00035
		GEH (μm)	343.4±25.30^{b}	436.4±51.38^{a}	361.2±13.80^{b}	0.0198
	400	STD (μm)	1366.03±80.70^{b}	1498.02±93.40^{a}	1440.6±52.8^{a}	0.0214
		GEH (μm)	343.4±25.30$^{a, b}$	392.9±59.80^{a}	323.0±17.80^{b}	0.0414

Values are represented as mean±SD from 6 rats. Means within the same row with different letters are significantly different (P<0.05). SD: Standard deviation; GEH: Germinal epithelial height; STD: Seminiferous tubule diameter;

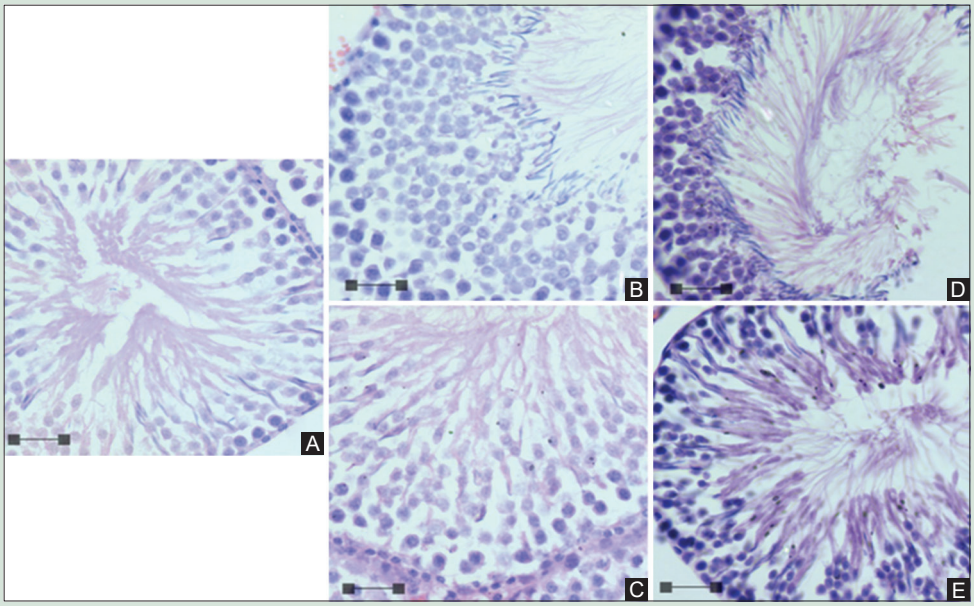

Figure 1: Effects of *Newtonia hildebrandtii* extracts on Wistar rats' seminiferous architecture. A = Control; B and C = Aqueous extract at 200 and 400 mg/kg body weight, respectively; D and E = 50% methanol extract at 200 and 400 mg/kg body weight, respectively. Magnification = ×40; Bar = 100 μm

400 mg/kg body weight promoted normal body growth suggesting the interpretation that all the metabolic processes were normal in the experimental animals. The increase in STD, right testis weight, and caudal epididymal sperm concentration is a manifestation of *N. hildebrandtii's* capacity to enhance male reproduction. Coincidentally, rats treated with aqueous extracts of vajikaran rasayana herbs at 200 mg/kg body weight promoted significantly increased body and reproductive organ weight.[17]

Preliminary phytochemical screening of the two extracts showed the presence of flavonoids, phenols, steroids, saponins, and alkaloids, and the GC-MS analysis showed the presence of ascorbic acids, linoleic acids, and omega-3 polyunsaturated fatty acids (PUFAs). The presence of sterols, flavonoids, and phenolic compounds are proof enough of the radical scavenging capacity of *N. hildebrandtii* and directly correlates with aphrodisiac and adaptogenic activities.[18]

Flavonoids have been described as phenolic substances with the capacity of suppressing reactive oxygen species (ROS) formation either by inhibition of enzymes or chelating trace elements involved in free radical production, scavenging ROS, and upregulating antioxidant defenses.[19] The improved sperm parameters observed in this study could be attributed to the antioxidant effects of flavonoids, which elsewhere have been reported to counteract the formation of ROS in infertile men[20] and protect sperms from lipid peroxidation and DNA mutation.[21]

Furthermore, the significantly increased spermatogenic cell density, caudal epididymal sperm concentration, and total sperm motility could be attributed to the antioxidant effects of ascorbic acid.[22] As a reducing agent, ascorbic acid protects DNA proteins from oxidation.[23] It also protects passive smokers and sperms from ROS, reduces sperm agglutination, enhances fertility in men, and improves sperm motility parameters and chromatin quality in animals.[22] In this regard, consumption of *N. hildebrandtii* extracts might have protected sperm from apoptosis hence the increase in sperm concentration. The GC-MS analysis also showed the presence of 9-octadecenoic acid (linoleic acid) in *N. hildebrandtii* extracts. Linoleic acid is one of the main omega-6 PUFAs, which promotes spermatogenesis through its ability to incorporate into spermatozoa cell membrane where it becomes part of the structural architecture.[24,25] Of particular significance, linoleic acid has higher antioxidant activity in human seminal fluid, and this enhances sperm count, sperm motility, and sperm morphology.[26]

The GC-MS analysis also showed the presence of docosahexaenoic acid (DHA; 22:6n-3) in *N. hildebrandtii* extracts. DHA is a long-chain omega-3 PUFA that is formed endogenously following desaturation of essential fatty acids such as linoleic acid (18:2n-6) and α–linolenic acid (18:3n-3).[27] Interestingly, asthenozoospermia is highly associated with low concentration of DHA.[28] Similarly, 9-octadecenoic acid and DHA present in the two extracts might have contributed to the increased sperm motility observed in this study. No wonder ethnomedicinal claims have been made against *N. hildebrandtii* in reversing infertility.

Exposure of Wistar rats to *N. hildebrandtii* extracts promoted significant increases in LH, FSH, testosterone levels, and sperm concentration. In addition to this, testicular histology showed a qualitative increase in Leydig cells. Studies have shown a direct correlation between an increase in the number of Leydig cells, testosterone levels, and spermatogenesis[29] and appear to agree with the present study. Further to this, testosterone promotes the maturation of round to elongated spermatids during spermiogenesis.[30] Qualitative studies of the testicular micrographs also showed a significantly increased number of Sertoli cells. This increased cell population appears to directly agree with the increased FSH levels also recorded in this study. In this regard, the increased caudal epididymal sperm concentration can be justified by the increases in LH and FSH since sperm production in the presence of LH is regulated by FSH and relies on normal Sertoli cell activity.[31]

It is noteworthy that there was a significant increase in serum levels of testosterone along with significant increases in STD and GEH in the treated animals compared with the control. Ideally, herbal aphrodisiacs are expected to upregulate serum testosterone levels.[32] From the GC-MS analysis, ascorbic acid was identified in *N. hildebrandtii* extracts. Studies have shown the capacity of ascorbic acid to stimulate testicular steroid dehydrogenase activity and increase plasma testosterone level.[33] In addition, studies have shown a direct correlation between increased testosterone production with growth and development of male reproductive organs,[34] and above all, in association with FSH, testosterone promotes increased spermatogenesis in the seminiferous tubules.[35] This suggests that the increased testosterone levels might have contributed to the increased STD and GEH.

However, in the present study, prolactin levels were significantly decreased in the treated animals compared with the control. Physiological levels of prolactin promote steroidogenesis through upregulation of LH receptors on Leydig cells.[36] Elsewhere, pathological increase in prolactin (male hyperprolactinemia) has been directly correlated with decreased libido and impotence.[36] High levels of prolactin can result in deregulated pulsatile release of LH, decreased serum testosterone secretion, and erectile dysfunction.[37] On the contrary, this study has observed an increase in LH, testosterone hormones, percentage total motility, and reduced prolactin levels, thereby suggesting enhanced libido and improved fertility in the treated animals.

Estradiol promotes sperm motility in asthenozoospermic patients' spermatozoa.[38] In this study, *N. hildebrandtii* extracts upregulated estradiol levels and promoted increased percentage of progressive and nonprogressive motile sperm cells in the treated animals. Similarly, estradiol promoted increased oxidative metabolism, intracellular ATP levels and enhanced sperm progressive velocity, linear motility, and longevity of both normal and asthenozoospermic patients.[39] Further to this, estradiol has a modulatory role in spermatogenesis and fluid resorption in the epididymis facilitating posttesticular sperm maturation.[33] The increased sperm parameters observed in this study could be of great significance to people visiting fertility clinics and further support the use of this herb as an aphrodisiac.

CONCLUSION

This study agrees with the ethno-aphrodisiac claims that have been made against *N. hildebrandtii* extracts. The phytochemicals present in this plant have antioxidant affects and modulate hormonal levels leading into cytoarchitectural changes in the testis of Wistar rats reflective of improved male reproductive functions. More studies need to be done to ascertain its mechanisms of action besides checking for its toxicity in man.

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Conflicts of interest

There are no conflicts of interest.

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