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Effect of Ethanolic Rind Extract of *Citrullus lanatus* on the Hpg Axis and Sexual Behavior of Male Wistar Rats

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ABSTRACT

Sexual dysfunction is very common among men of all ages, ethnicities, and cultural backgrounds. This work is aimed at evaluating the effect of Citrullus lanatus ethanolic rind extract on the sexual behavior and reproductive hormones of adult male wistar rats. ***Materials and Method:*** *Adult male wistar rats were randomized into four groups (A-D) of six rats each. Group A (negative control; 1ml of normal saline). Groups B and C (experimental groups; 500mg/kg and 1000mg/kg bw respectively). Group D (positive control; sildenafil Citrate 5mg/kg bw) 4hours before sexual behavioral test. Sexual behavioral tests were performed in a Plexiglas copulatory arena cage (60×50×40)cm and videotaped for proper scoring. Male sexual behavioral parameters: Frequencies of mount (MF), intromission (IF) and ejaculation (EF), latencies of mount (ML), intromission (IL), ejaculation (EL) and post ejaculatory intervals (PEI) were evaluated following the pairing of male rats (1:1) with experienced estrous female rats.* ***Results:*** *Rind extract of Citrullus lanatus stimulated mounting and mating behavior by increasing MF, IF and prolonged ejaculation latency. In addition, decrease in ML, IL and PEI were observed. Hormonal assay indicated an increase in serum sexual hormone levels like Testosterone, LH and FSH but only the rise in testosterone was statistically significant. These changes were collaborated by the histological findings of the testes and hypothalamic sections. In conclusion, the ethanolic rind extract of Citrullus lanatus increased sexual behavior in adult male wistar rats, and may be qualify to be used as a remedy for male sexual dysfunction.*

Key words: *Citrullus lanatus, Male sexual dysfunction, Sexual behavior.*

INTRODUCTION

Male sexual dysfunction is the difficulty experienced by an individual or a couple during any stage of a normal sexual activity. It is the persistent physical or emotional problem associated with sex, which include lack of sexual desire, difficulty becoming aroused, and difficulty having an orgasm or even pains during sex[1].

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For a normal sexual intercourse in males, the sexual organs and factors relating to erection and orgasm must function normally[2]. The use of herbal medicine has become increasingly popular worldwide especially in the African countries[3]. Some herbal plants have been acclaimed to possess sex enhancing potential. In Nigeria, one of the herbs is watermelon (*Citrullus lanatus)*. It is grown for its edible fruit, which has a smooth hard green rind and a juicy, sweet interior flesh, usually deep red to pink. The essence of marriage in humans is procreation and/or sexual fulfillment of both partners. Male sexual dysfunction can hinder a man from performing his sexual responsibility in the home. The repeated inability of couples to perform sexual activities effectively is one of the reasons some marriages fail. Although studies have established the aphrodisiac effect of *Citrullus lanatus* flesh extract on adult male wistar rats[4], there is lack of documented literature on the effect of the rind extract on sexual behaviors of adult male wistar rat.

MATERIAL AND METHODS

Plant material collection

The fruits of *Citrullus lanatus* (watermelon) were purchased from the local market, Ogbete main market Enugu. The freshly collected *Citrullus lanatus* was identified in the Department of plant Science and Biotechnology, University of Nigeria Nsukka.

Plant Extraction and Phytochemical Analysis

The watermelon fruits were cleaned; the flesh was isolated from the rind. Watermelon rind was shade dried to constant weight. Dried watermelon rind was ground into powder and a portion of the powder was set aside for phytochemical screening. Three hundred gram (300g) portion of the powder was of the powder was extracted with 70% (v/v) ethanol in a Soxhlet apparatus for 24 h. Using a vacuum rotary evaporator, the filtrate was concentrated to complete dryness and finally stored in a refrigerator at 4^0C for the experiment. The extract was later reconstituted in distilled water to give the required doses of 500 and 1000 mg/kg body weight used in this study.

Phytochemical Analysis was done at Brain- Phosphorylation Scientific Solution Service. Ogui Road Enugu, Enugu State. Qualitative tests were carried out to determine the composition of some pharmacological active secondary metabolites.

Animal Procurement

Twenty-four (24) number of male (200g-250g) and female (160g-200g) adult wister rats were used for the experiment. They were purchased from the National Institute of Research, Vom, Plateau State. The animals were housed in aluminum cages, placed in a well ventilated house with optimum condition (temperature 30^{0C} photoperiod; 12hours natural light and 12hours dark; humidity is 40-50%). The animals were fed growers mash manufactured by Top Feed Nigeria Limited and allowed water *ad libiitum.* They were allowed acclimatization period of 2 weeks and throughout the experimental period; the animals were handled according to the guidelines for animal research in National Institute of Health guidelines for care and use of laboratory animals. The study was carried out in accordance with the principles of laboratory animal care and standard experimental procedure.

Table 1: Animal Grouping/Administrative Schedule

The male rats were randomly assigned into 4 groups of six rats each. The extract was administered by oral galvage. The treatment was as follows:

Groups (n=6)	Treatment	Dosage/Kg body weight
A	Normal saline	1ml
B	Ethanolic rind extract	500mg
C	Ethanolic rind extract	1000mg
D (standard group)	Sildenafil citrate	5mg(orally, 1hrs before sexual behavioral test).

Preparation of Female Rats

The female rats were artificially brought into oestrus (heat) by the sequential administration of estradiol benzoate (10 mg/100 g body weight) and progesterone (0.5 mg/100 g body weight) through subcutaneous injections, 48 hours and 4 hours, respectively, before mating. After the confirmation of receptivity of the female rats, the experiment was started[5].

Sexual Behaviour for Male Rats

Single male rats were gently dropped into 60×50×40 cm plexiglass copulatory arena cage, and allowed to acclimatize for 5 min. Then, a receptive female was presented to the male and sexual behavior observed for 30min. The sexual parameters were recorded and calculated as follows[6]

a) Time from the introduction of the female into the male's cage to the first mount (Mounting Latency) (ML).
b) Time from the introduction of the female into the male's cage, to the first intromission (Intromission Latency) (IL).
c) Time from the first intromission to ejaculation (Ejaculatory Latency) (EL).
d) Time from the first ejaculation to the next intromission by the male or Post Ejaculatory Interval (PEI).
e) Number of mounts before ejaculation (Mounting Frequency) (MF).
f) Number of intromissions before ejaculation (Intromission Frequency) (IF).
g) Number of ejaculations in 30min (Ejaculatory Frequency) (EF)

Blood Collection and Hormonal Assay

On the last day of the experiment, about 1ml of blood was collected from each of the 24 rats. The blood sample was immediately placed in a centrifuge (Centurion Scientific Ltd., UK) and centrifuged for 5 minutes at 12,000 revolutions per minute. The serum was quickly decanted into a test tube and stored immediately in a chest freezer (Haier Electrical Appliances Inc., Philippines). The concentration of the serum testosterone, FSH and LH. were determined shortly after using ELISA Kit.

Histological Study

The animals were anaesthetized in a jar containing cotton wool soaked in Thiopental Sodium. The brain and gonads were carefully dissected out, fixed in 10% formol saline and processed for paraffin wax embedding. They were carefully sectioned using a rotary microtome, stained and the histological architecture examined. The stained tissues were micrographed and interpreted by a pathologist at the Enugu State University of Science and Technology Teaching Hospital (ESUTH).

Statistical analysis

Data are expressed as mean ± standard error of the mean. Significance difference was analyzed using one-way analysis of variance (ANOVA), followed by student's t-test. Differences were considered statistically significant at $P<0.05$.

RESULTS

RESULT OF PHYTOCHEMICAL ANALYSIS

The phytochemical analysis of the ethanolic rind extracts of *Citrullus lanatus* revealed high presence of citrulline and lycopene, slight presences of Saponins, phenols and flavanoid, trace amount of alkaloid and steroids but tannins, steroids, cardiac glycoside and anthraquinone were not detected. (Table 4).

Table 2: Qualitative analysis of the *Citrullus lanatus* rind extract

Phytochemical	Citrullus lanatus Rind
Sponin	++
Alkaloid	+
Flavonoid	++
Tanin	-
Phenol	++
Cardiac Glycoside	-
Steroid	+
Anthraquinone	-
Citrulline	+++
Lycopene	+++

Keys: - = Not detected ++ = Moderate concentration + = Low concentration +++ = High concentration

Result of the Effect of *Citullus Lanatus* Rind Extract on Body Weight of Male Wistar Rats

This showed that there was significant increase in the body weight of the wistar rats.

Table 3: Effect of *Citullus lanatus* rind extracts on the body weight of the male rats

	GROUP A (Normal saline)	GROUP B (Rind low dose)	GROUP C (Rind high dose)	GROUP D (standard drug; Sildenafil)
WEEK1	233.75± 2.1	233.89± 1.1	204.14± 1.6	213.80±3.3
WEEK2	239.50 ± 1.9	242.67± 3.3	212.17 ± 3.1	222.75 ± 3.5
WEEK3	248.00±3.6	249.83±4.0	227.00±2.2	232.50±3.8
Weight change	14.25	15.94	22.8	18.7
P.Value	0.000	0.000	0.000	0.005

Effect of rind extract of *citrullus lanatus* on male sexual behaviour:

The result of the sexual behavioral test on the male wistar rats are shown in (Table 4). There was increase mount, intromission and ejaculatory frequencies, increased ejaculatory latency, decreased mount and intromission latencies and decreased post ejaculatory interval in both week 1 and week 2.

Table 4: Effects of rind extract of *citrullus lanatus* on sexual behaviour of adult male rats

PARAMETER		GROUP A (Normal saline)	GROUP B (Rind low dose)	GROUP C (Rind high dose)	GROUP D (standard drug; Sildenafil)
Mount Freq.	**WK 1**	5.5 ±3.5	7.0 ± 2.3	10.0± 0.0	15.0± 12.7
	WK 2	9.0± 4.2	7.5± 2.1	16.0± 7.1	19.0± 7.0
Intro. Freq.	**WK 1**	12.5 ± 3.5	17.5± 6.3	19.0± .0	22.5± 2.1
	WK 2	19.5 ±7.1	19.0 ±1.4	27.5± 3.5	22.5± 7.7
Eja. Freq	**WK 1**	2.0 ± .00	2.0 ± 0.7	2.5± 0.7	2.5± 0.7
	WK 2	2.0± .01	2.5 ± 0.0	2.5± 0.7	2.5± 0.7
Mount Lat.	**WK 1**	115.5± 96.9	59.5± 44.6[a]	21.0 ± 28.3[a]	22.5± 36.1[a]
	WK 2	88.5 ± 18.3	41.5± 38.9	17.5± 17.7	13 ± 5.7
Intro Lat.	**WK 1**	84.0 ± 28.3	67.0± 8.5	52.5 ±13.4	16.5± 6.4
	WK 2	50.5± 62.9	55.5± 34.6[a]	40.0± 94.8	16.0± 7.0
Eja Latency	**WK 1**	135.5 ± 57.70	150.5± 12.0	198.8± 80.6	216.5± 10.6
	WK 2	145.0± 39.6	142.5± 28.4	207.0± 107.5[a]	276.0± 48.1
Post Eja Interval	**WK 1**	364.0 ± 60.8	321.5± 14.0	255.0± 7.0	168.5± 9.1
	WK 2	319.5± 40.7	300.5± 22.7	228.5± 89.8	126.0± 77.8

Values expressed as mean ±SD, n=6 [a]$p < 0.05$ vs negative control, [b]$p < 0.05$ vs positive control

Effect of rind extract of *Citrullus lanatus* on Sexual Hormones (FSH, LH and testosterone):

The levels of FSH, LH and testosterone in the serum of the animals treated with the rind extract were elevated at the end of the observatory period but only the rise in testosterone was statistically significant when compared with their respective control values (Table 4.3).

Table 5: Effects of ethanolic rind extract of *Citrullus lanatus* on sexual hormone levels of male rats

Parameter	A(Normal saline)	B(Rind low dose)	C(Rind high dose)	D(Standard drug; sil)
TESTOSTERONE (ng/ml)	0.3± 0.1[a]	2.9 ± 0.4[ab]	3.1± 0.1	5.0 ± 0.2[a]
LH miu/ml	3.2 ±1.0	4.6 ±0.4	4.6 ± 0.2	3.9 ± 0.1
FSH miu/ml	4.7 ± 0.8	6.8± 0.1	8.6± 1.2	9.8 ± 1.1

Values expressed as mean ±SD, n=6

[a]$p < 0.05$ vs negative control, [b]$p < 0.05$ vs positive control

Effect of Rind Extract of *Citrullus lanatus* on Histo-Architecture of Male Wistar Rats

After the tissue processing, the stained micrographed slides of hypothalamus and testes were examined and the following results were observed;

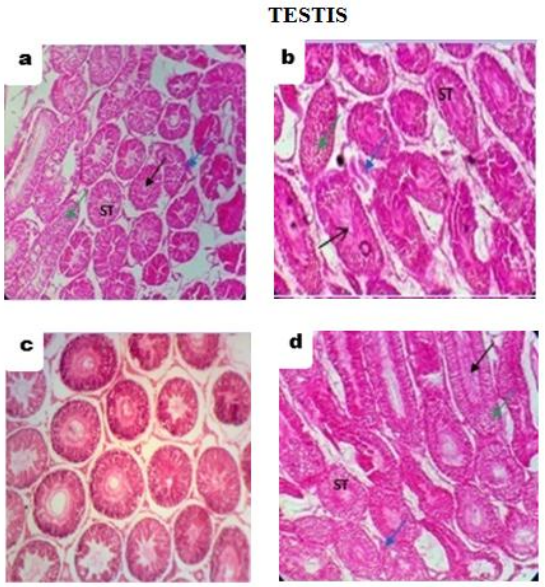

Fig. 1: Photomicrograph of coronal section through the testis of group (a) negative control rats, group (b) experimental rats (500mg/kg bw), group (c) experimental rats (1000mg/kg bw), and group (d) positive control rats (sildenafil citrate 5mg/kg bw), (ST; seminiferous tubule, green arrow; spermatogenic cells, black arrow; lumen of the tubule, blue arrow; interstitial/leydig cells).

The testicular sections of rats in all the groups show normal testicular features, consisting of seminiferous tubules (ST), lined by spermatogenic cells (green arrows) including the spermatogonia cells, spermatocytes, spermatids as well as the spermatozoa that fill the lumen of the tubules (black arrows). ST are held together by connective tissue interstitium consisting of interstitial cells and leydig cells (blue arrows).

In the experimental groups (**b)** and (**c),** administered with low and high doses of the rind extracts respectively, there were increased tubular size, more compact ST and increase number of spermatozoa in the lumen of the ST when compared to the negative control group (**a)**. These increases were most pronounced in the positive control group (**d**) that was administered with sildenafil citrate. **H&E x100**.

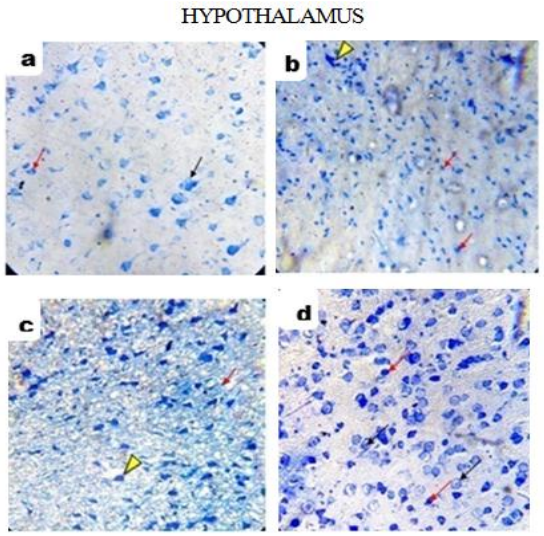

Fig. 2: Photomicrograph of coronal sections of neural tissue showing nissl staining of perikarya and neroglia cells (red arrows) in parts of the paraventricular nucleus of the hypothalamus of rats in group (a) negative control, group (b) experimental 500mg/kg bw, group (c)1000mg/kg bw and group (d) positive control rats; sildenafil citrate 5mg/kg bw.

Perikarya and neroglia cells (red arrows), hyperchromatic perikarya, without cytoplasmic vacuolation (yellow arrow heads), prominent nucleoli and cytoplasmic vacuolation (black arrows). There was slightly increase in the stain intensity which signifies increased neurosecretory activity in the experimental groups (**b**) and (**c**) when compared with the negative control group (**a**). This increased neurosecretory activity was observed to be more pronounced in the positive control group (**d**). **Methylene blue X100**

DISCUSSION

In this study, it was discovered that the major constituents found in watermelon rind are Citrulline, Lycopene, Saponins and Flavonoids. Previous studies have indicated that both Citrulline and Arginine were able to improve sexual function in patients with erectile dysfunction[7], as Arginine, which is produced from Citrulline is important in the production of Nitric Oxide (NO); a potent vasodialator that plays a key role in erection and sexual stimulation[8]. These could have contributed to the enhanced sexual property observed in this study. Other phytochemicals found in this extract have been implicated in enhancing sexual function in male rats; for example, saponins in *Fadogia agrestis* (Schweinf. Ex Hiern) and

Tribulus terrestris (Linn.) have been shown to be responsible for aphrodisiac activity[9,10]. These bioactive agents exhibit aphrodisiac activity either by increasing the biosynthesis and secretion of androgens or act directly on the central nervous system to modulate the action of neurotransmitters and gonadal tissues in animals. Specifically, saponins enhance androgen production[9].

The sexual behavioral observations in this study revealed that the extract has sex enhancing functions, evidenced by the increased MF, IF, EF, EL in male rats and decrease in ML, IL and PEI. Mount and intromission frequencies (MF and IF) which are indices of sexual arousability; MF is an indicator of libido while IF is an indicator of potency[11,12], mount and intromission latencies (ML and IL) are used to measure vigor[8]. The decrease in ML and IL and the increase in MF and IF produced by the Citrullus lanatus rind extract revealed that this plant may be a useful sexual stimulant. EL and PEI are important for evaluating prolonged duration of coitus and the rate of recovery from exhaustion after the first series of mating, respectively[11,12]. This finding was in agreement with that of Ratnasooriya and Dharmasiri who reported an increase in EF and EL; and a decrease in ML, IL and PEI in male rats administered with *Terminalia catappa* seed extract[13].

Result of hormonal assay in this worked showed significantly increased level of testosterone and non-significant increase in FSH and LH. The level of testosterone has been reported to be related to (gonadotropins) LH and FSH such that increased levels of the gonadotropins results in corresponding increase in testosterone as the pulsatile release of the GnRH from the hypothalamus through HPG axis, causes the secretion of the gonadotropins into the circulation which in turn stimulate the release of testosterone (in the case of LH) from the leydig cell[14]. Therefore, it is not surprising that in this study, the levels of the sex hormones were elevated, thou the elevation was not statistically significant except for testosterone; when compared with that of the normal control groups. Elevated level of testosterone has been associated with a moderate but significant increase in sexual desire and penile function[9]. Reports on testosterone also suggest that a slightly increased level of testosterone in adult males results in an enhanced sexual desire and arousability[15].

The histological observation of the testes revealed normal histo-architecture with increased mature sperm cells migrating towards the lumen of the tubules (figure 1). This could be as a result of increased FSH level which is directly involved in spermatogenesis. FSH acts on the sertoli cells to enhance spermatogenesis. Thou the study conducted by Kenjale et al., reported a contrary finding, where no evidence of spermatogenesis was found in the testicular tubules even with normal levels of the gonadotropins and testosterone[16]. This could be as a result of the ability of some of the leaf constituents crossing the blood testes barrier thereby hindering spermarogenesis[17]. Histological examination of the hypothalamus showed high intensity of nissil staining in the treatment and positive control groups, which suggest increased neurosecretory activity of the rind extract.

CONCLUSION

The ethanolic rind extract of *Citrullus lanatus* has aphrodisiac property, which might be due to the presence of Citrulline, Saponins or such compounds found in this plant, and as such may be used as a remedy for male sexual dysfunction.

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