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Phytochemical and aphrodisiac studies of ethanol root extract of *Rauwolfia vomitoria* Afzel (Apocynaceae)

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Abstract
The root of *Rauwolfia vomitoria* (Afzel) is known for its anti-hypertensive, psychoactive properties and aphrodisiac potentials. This research work was aimed at studying the phytochemical constituents and aphrodisiac activities of the crude ethanol extract and its fractions. The root material was macerated for 72 h and the dried ethanol extract (EE) partitioned successively to obtain dichloromethane (DCM), ethyl acetate (EA), butanol (BuOH) and aqueous (AQ) fractions. The LD_{50} was determined using Miller and Tainter method. The aphrodisiac potential of the crude extract and these factions were evaluated using adult albino mature male rats. The crude ethanol extract was subjected to phytochemical screening. The median lethal dose (LD_{50}) was determined to be 250±15.15mg/kg body weight. The result of phytochemical screening indicated the presence of alkaloids, saponins, cardiac glycosides, flavonoids, tannins and terpenoids. The extract and the fractions decreased mount latency, intromission latency, post ejaculation interval and increased ejaculation latency, mount frequency, erection frequency and penile erection. These effects were statistically significant ($p<0.05$, $p<0.01$, $p<0.001$) relative to control (normal saline). These findings authenticate the use of the root of *Rauwolfia vomitoria* as aphrodisiac agent.

Keywords: *Rauwolfia vomitoria;* Aphrodisiac; Sexual dysfunction; Intromission; Mount latency

INTRODUCTION

Erectile dysfunction (ED) or male impotence is a sexual dysfunction characterized by the inability to develop or maintain erection of the penis sufficient for normal satisfactory intercourse. There are various underlying causes, such as cardiovascular leakage and diabetes, many of which are medicinally treatable. The causes of erectile dysfunction may be psychological disorder such as anxiety, depression and stress, fear of previous sexual failure, neurological disorder like stroke, cerebral trauma, Alzheimer disease, and Parkinson disease. Others include hypertension, vascular insufficiency, atherosclerosis, penile disease, phinosis, peyronies, life style-chronic alcohol abuse, cigarette smoking, aging, decrease of hormone level with age, systematic disease-cardiac, hepatic, renal and pulmonary cancer [1].

Pharmacotherapy involves locally acting vasoactive drugs such as papaverine and alprostadin [2]. The first line oral therapy for erectile dysfunction (ED) includes phosphodiesterase type-5 (PDE-5) inhibitor such as sildenafil, verdenafil and tadanafil, which inhibit hydrolysis of second messenger

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cyclic guanosine mono phosphate whose production is promoted by nitric oxide (NO) release within penile smooth muscle cell. The available drugs have limited efficacy, unpleasant side effect and contra indication in certain disease conditions. Sildenafil citrate (Viagra) is a successful drug that modifies haemodynamics in the penis. The side effects reported with this drug are headache, flushing, dyspepsia, and nasal congestion. Indian medicine Ayurveda includes vajakarna therapy, which involves aphrodisiac for erectile dysfunction, causes of infertility, spermatogenesis, reproduction, method of correcting defective semen satisfaction [1].

Sexual dysfunction is a serious medical problem that adversely affects relationships. This occurs in both men and women but mostly in men between the ages of 40-70 years [3]. An estimation of 34.8% of men have moderate to complete erectile dysfunction. It was estimated in 1995 that more than 152 million men worldwide experienced erectile dysfunction and that the number will rise by 170 million to approximately 322 million by the year 2025 [3]. To reduce this, appropriate treatments must be put in place to reduce the rate of occurrence. Many plant extracts are traditionally employed among different cultures to manage sexual inadequacies [4]. Decoction of *Rauwolfia vomitoria* root is used locally here as an aphrodisiac. This plant is also recognized in natural medicine for its many therapeutic effects, among which are: treatment of diarrhea, malaria, hypertension, mental disorder, male infertility etc. [5].

The study was therefore aimed at ascertaining the authenticity of *Rauwolfia vomitoria* root in management of sexual dysfunction.

EXPERIMENTAL

Collection and identification of plant material. The root of *Rauwolfia vomitoria* used for this work was obtained from Itak village in Ikono Local Government Area of Akwa Ibom State and was identified by Mrs. Emmanuela G. Udoma, a taxonomist in the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo. A herbarium specimen with voucher number UUPH 6c was prepared and deposited in the Herbarium of the Department of Pharmacognosy and Natural Medicine, University of Uyo.

Preparation and extraction of plant material. The root of the plant, *Rauwolfia vomitoria* was chopped into small pieces and dried under shade for three (3) weeks, the dried root weighing 1.6kg (electric balance; Gerardt, England) was pulverized to coarse powder using mortar and pestle. The powdered root sample (1.6kg) was macerated for 72 h with 50% $^{v}/v$ ethanol at room temperature; the extract was filtered using Whatman filter paper no.4 and concentrated to dryness in a water-bath at a temperature of 40^{o}C. The extract (97.0 g) was stored in a desiccator until use.

Determination of median lethal dose (LD_{50}). The median lethal dose (LD_{50}) of the extract was determined using the method of Miller and Tainter [6].

Animals. Adult albino rats of both sexes weighing 150-250 g were obtained from the animal house of University of Calabar, Calabar. The male and female rats were kept separately and quarantined for two (2) weeks for acclimatization and maintained under standard conditions (12hours light/12hours dark cycle) at the animal house of the Department of Pharmacology and Toxicology, University of Uyo, Uyo. The animals were fed with grower marsh (Grand Bendel, Edo State) and water was given *ad-libitum*.

Preparation of female rats. Female receptivity was induced using the modified method of Padashettey *et al*. [7]. Forty-eight

(48) hours prior to the pairing in the cage, 17-β-estradiol was administered to the female subcutaneously (sc) to induce the estrous cycle, making the females receptive to the males. Forty (40) hours later, progesterone (500µg/kg) dissolved in corn oil was administered (sc) to the female rats to enhance the effect of the 17-β-estradiol. All this made the female rats to be highly receptive.

Characterization of male rats. The male rats were characterized into sexually active, sluggish and impotent. This was done by pairing the male rats with a sexually mature receptive female in a plexi-glass cage at 19.00hours, with a red bulb lamp and observing the following parameters: mounting latency, intromission latency, post ejaculatory latency and ejaculation latency.

Mount Latency (ML). Time from the introduction of the female to the first mount.

Intromission Latency (IL). Time from the introduction of the female to the first intromission (vagina penetration).

Post Ejaculatory Interval (PEI). Time from ejaculation to the first intromission of the second copulatory series.

Ejaculation Latency (EL). Time from the first intromission to ejaculation.

Only the results obtained from the last three pre-experimental tests were used. Rat achieving ejaculation in:

i. All the three tests were defined as sexually active.
ii. One or two of the three pre-experimental tests were considered as sexually sluggish.
iii. Those that failed to achieve ejaculation in all three tests were considered sexually impotent.

The sexually impotent males were discarded and the sexually active and sluggish males were used for the experiment [8].

Effect of extract on male sexual indices. Sexually, mature, active male rats were used for this experiment. Thirty-six (36) male rats were used to determine the aphrodisiac effect of the plant extract. These rats were divided into six groups of six rats per group. Group 1 was given normal saline (10 ml/kg, i.p) and served as the negative control. Groups 2, 3, 4 were given 25, 50, 75 mg/kg body weight (b.w.) intra-peritoneally (i.p) of ethanol extract of *Rauwolfia vomitoria* respectively. Group 5 was administered testosterone (1 mg/kg b.w) to serve as the reference group. Group 6 was given testosterone (1 mg/kg b.w (s.c) and 10 minutes later extract (50 mg/kg i.p) was administered. All agents were administered twice daily for 5 days. On the sixth (6) day, food was withdrawn 3 hours prior to the experiment. Each male rat was introduced into the plexiglass cage 10 minutes prior to the introduction of the female rat, for acclimatization. The rats were observed for 30 minutes. However, experiment was terminated if any of the following conditions occurred before 30 minutes, it showed that the animals were not responding to the extract and as such the extract were regarded as inactive:

(i) Immediately after first intromission, the post ejaculation interval occurred.
(ii) If the intromission did not occur within 15 minutes.
(iii) If in any case, the post-ejaculation interval exceeded 15 minutes.
(iv) If the ejaculation latency exceeded 30 minutes.

The tests were carried out seven times. Rats achieving ejaculation in the last three (3) times were defined as sexually active. The remaining rats who fail to achieve ejaculation in one, two or all three pre-tests were considered sexually sluggish or impotent.

Phytochemical screening. Phytochemical screening was carried out on the ethanol extract of the root of *Rauwolfia vomitoria*

according to standard methods to identify the constituent (s) compounds in the root extract.

RESULTS

Acute toxicity studies. The median lethal dose (LD_{50}) was determined to be 250±15.15 mg/kg body weight. The physical signs of toxicity observed were writhing, gasping palpitation, decreased respiratory rate and death.

Effect of extract on sexually active males. The effects of extract on sexually active male rat are shown in table 1. The extract decreased mount latency, intromission latency, and post ejaculation interval. However, the extract increased ejaculation latency, mount frequency, erection frequency and penile erection. These effects were statistically significant ($p<0.05$, $p<0.01$, $p<0.001$) relative to control (normal saline).

Effect of fractions on sexually active males. The extract were partitioned into dichloromethane, ethyl acetate, n-butanol and aqueous fraction respectively. The four fractions were tested on the male indices to identify the fraction with the highest aphrodisiac activity and dichloromethane fraction possessed the highest aphrodisiac activity as shown in table 2.

Table 1: Effects of ethanol extract of *Rauwolfia vomitoria* on sexual indices in rats.

Doses (mg/kg)	Mount latency (ML)	Mount frequency (MF)(s)	Intromission latency (IL)	Intromission frequency (IF)(s)	Ejaculation latency (EL)	Post ejaculation interval (PEI)	Erection frequency (EF)(s)	Penile erection (PE)
Normal saline (10ml/kg)	0.42±0.01	12.67±0.021	0.89±0.14	9.83±0.10	4.17±0.20	1.49±0.11	10.5±0.14	4.22±0.30
(25mg/kg)	0.34±0.02^{c}	11.67±0.05^{c}	0.49±0.03^{a}	13.2±0.01^{c}	5.56±0.20^{c}	0.64±0.10^{c}	17±1.03^{c}	2.85±0.20^{c}
(50mg/kg)	0.34±0.02^{c}	16.0±0.12^{c}	0.38±0.01^{b}	11.33±0.02^{c}	6.84±0.03^{c}	0.34±0.10^{c}	15.5±0.02^{c}	6.25±0.03^{c}
(75mg/kg)	0.19±0.01^{c}	15.67±0.14^{c}	0.24±0.11^{c}	12.2±0.05^{c}	11.35±0.20^{c}	0.56±0.08^{c}	16.8±0.04^{c}	6.11±0.10^{c}
Testosterone (1mg/kg)	0.43±0.03^{ns}	9.30±0.03^{c}	0.67±0.11^{c}	8.3±0.05^{c}	11.47±0.20^{c}	0.18±0.08^{c}	18.2±0.05^{c}	3.99±0.10^{ns}
Testosterone (1mg/kg) + (50mg/kg)	0.24±0.03^{c}	10.67±0.11^{c}	0.28±0.03^{c}	10±0.13^{b}	3.17±0.23^{c}	0.42±0.09^{c}	19±0.12^{c}	5.73±0.03^{c}

Values are Mean ± S.E.M; Significance relative to control: $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$ (n=6); ns=not significant

Table 2: Effect of the different fractions of extract on the sexual indices of rats.

Fractions	Mount latency	Mount frequency	Intromission latency	Intromission frequency	Ejaculation latency	Post ejaculation latency	Erection frequency	Penile erection
DCM (50mg/kg)	0.15±0.02^{c}	29.3±0.01^{c}	0.17±0.04^{c}	20.2±0.07^{c}	7.41±0.12^{c}	0.20±0.07^{c}	22.5±0.85^{c}	5.22±0.15^{c}
Aqueous (50mg/kg)	0.13±0.01^{b}	23.2±0.03^{c}	0.15±0.01^{c}	19.2±0.14^{c}	7.05±0.21^{c}	0.19±0.09^{c}	14.6±0.40^{c}	6.08±0.59^{c}
n-butanol (50mg/kg)	0.27±0.04^{a}	20.4±0.11^{c}	0.29±0.04^{c}	18.2±0.05^{c}	7.08±0.15^{c}	0.18±0.09^{c}	13.4±1.10^{c}	5.86±0.22^{c}
Ethyl acetate (50mg/kg)	1.01±0.01^{ns}	11.4±0.14^{b}	1.03±0.10^{c}	12.2±0.67^{c}	5.42±0.11^{a}	1.85±0.04^{b}	13.8±0.78^{c}	3.36±0.30^{b}

Values represent Mean ± S.E.M; Significance to: $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$ (n=6); ns=not significant

Table 3a: Effect of the ethanol extract on the weight of the organs and tissues in rats.

Doses	Organs			Tissues		
	Testes	Liver	Kidney	Seminal vesicle	Epididymis	Vas deferens
Control (Normal saline	2.26±0.09	5.3±0.49	1.09±0.09	0.73±0.12	1.09±0.09	0.17±0.02
25mg/kg	2.08±0.18	5.4±0.44	1.09±0.09	0.93±0.21	0.92±0.11	0.17±0.02
50mg/kg	2.19±0.07	6.7±0.30	1.15±0.04	0.47±0.08	0.95±0.05	0.17±0.01
75mg/kg	2.29±0.06	7.41±0.38	1.34±0.07	0.69±0.09	1.09±0.08	0.18±0.01
Testosterone (1mg/kg)	1.95±0.03	7.6±0.25	1.01±0.04	1.21±0.09	0.98±0.06	0.19±0.01
Testosterone+50mg/kg	2.19±2.08	6.9±0.23	1.06±0.05	0.46±0.08	0.09±0.05	0.19±0.01

Table 3b: Effect of the different fractions extract on the weight of the sexual indices in rats.

Fractions/Doses	Organs			Tissues		
	Testes	Liver	Kidney	Seminal vesicle	Epididymis	Vas deferens
DCM (50mg/kg)	2.12±0.13	1.18±0.08	6.12±0.32	Organ	2.58±0.19	0.27±0.02
Aqueous (50mg/kg)	2.06±0.14	1.18±0.11	5.58±0.38	0.42±0.06	2.88±0.24	0.22±0.04
N-butanol (50mg/kg)	2.06±0.13	1.10±0.03	6.0±0.42	0.46±0.13	2.6±0.19	0.36±0.13
Ethyl acetate (50mg/kg)	2.7±0.10	1.42±0.06	7.04±0.68	0.9±0.10	3.48±0.27	0.18±0.03

Effect of extract on body weight of animal and on the weight of sexual organ. Measurement of the body weight of the animals throughout the experimental period showed significant differences between the treated and control groups. The animals all gained weight. There were significant increases in the organ weight of extract treated rats compared to control as shown in tables 3a and 3b.

DISCUSSION

Aphrodisiac is any substance or chemical that is used to enhance sexual performance. It also refers to any substance(s) that is/are used to treat sexual dysfunction [9]. According to World Health Organization (WHO), a number of parameters are used to assess any substance or chemical that is said to possess aphrodisiac effect. These parameters include: mount latency (ML), mount frequency (MF), intromission frequency (IF), erection frequency (EF), and post ejaculatory interval (PEI) among others [10].

Preliminary phytochemical studies revealed the presence of alkaloids, saponins, tannins and flavonoids in *Rauwolfia vomitoria* root extract. Studies have shown that the flavonoid content of the plant may contribute to its sexual stimulating activity as flavonoids were shown to alter the androgen levels, which play an important role in sexual stimulation [11]. The phytochemistry of *Rauwolfia vomitoria* root extract further revealed the presence of saponins. Saponins are known to increase sexual drive or libido. Hence, plants with these secondary metabolites or ingredients enhance sexual activity when consumed by humans [12]. It has been reported that alkaloids possess good aphrodisiac potentials by increasing sexual desire or libido in male rat [13]. Compounds that increase libido do so by increasing the concentration of several anterior pituitary hormones and serum testosterone [14].

From the results of this work, it was observed that there were variations in the aphrodisiac activity of the different fractions based on the models used. Some fractions showed very high aphrodisiac activity in one or more groups but less in another model. Of the four fractions used in this study, dichloromethane possessed the highest aphrodisiac activity.

Post ejaculation interval is important for evaluating the effect of the extract on erectile function [15]. The extract increased penile erection of the male rats. An increase in PEI was observed in treated groups indicating the involvement of nitric oxide (NO) based intervention [9]. Sexual desire is

controlled and regulated by the central nervous system, which integrates the tactile, olfactory and mental stimuli. During sexual stimulation, the axons of parasympathetic nerves release nitric oxide gas. The gas diffuses into smooth muscle cells that line the arteries of the corpus carvenosum (spongy erectile tissue) and activates the enzyme guanylate cyclase (GC) which then converts the nucleotide guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP). The enzyme phosphodieseterase type-5-enzyme (PDE-5) which resides in penile tissue converts active cGMP into inactive GMP [16].

Conclusion. The study validated the effectiveness of *Rauwolfia vomitoria* roots in improving as well as protecting the functionality of sexual organ. It also substantiated the fact that the plant has aphrodisiac activity and may be helpful in improving the sexual behavior and performance.

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