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Full Length Research Paper

Effects of aqueous root bark extract of *Citropsis articulata* (Swingle & Kellerman) on sexual function in male rats

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About 80% of the world's population uses herbal medicine for the treatment of various health conditions. Erectile dysfunction is one of the conditions commonly treated using traditional herbs on large scale. In this study, our goal was to determine the safety and effects of the aqueous root bark extract of *Citropsis articulata* on sexual function in male Wistar rats. This study aimed to carry out phytochemical analysis of the aqueous root bark extract of *C. articulata*, conduct acute toxicity test to determine the safety of the aqueous root bark extract from *C. articulata,* determine the effect of the extract of *C. articulata* on inducing erection in male rats, and also to evaluate the effect of the extract of *C. articulata* on testosterone levels in male rats. Extraction was carried out by warm maceration, and phytochemical analysis done, following the methods of Trease and Evans, and acute toxicity studies were conducted following the Lorke's method. Efficacy was evaluated using non-contact and contact models. Testosterone analysis was performed using the AXSYM Testosterone reagent by Abbott AXSYM system. Results of phytochemical screening revealed the presence of saponins, proteins, free amino acids, arginine and phenolic compounds. The LD50 was estimated at 9486.833 mg/kg body weight. Extract did not induce erection, but had a significant effect on mounting (p-value = 0.013) and a significant effect on testosterone level (p-value = 0.02). Aqueous root bark extract of *C. articulata* increases mounting frequency and testosterone levels in male rats, is slightly toxic, and contains phytochemicals effective in the treatment of erectile dysfunction.

Key words: Acute toxicity, erectile dysfunction, *Citropsis articulata*, testosterone, mounting frequency.

INTRODUCTION

The use of herbal medicine for the treatment of various ailments is increasing worldwide (Daswani et al., 2006).

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Available reports indicate that “about 70 to 80% of the Ugandan population still relies on traditional healers for day-to-day health care, and that in some rural areas the percentage is around 90% compared to 80% reported world-wide” (Kakudidi et al., 2000; Kamatenesi-Mugisha 2005; Bukenya-Ziraba, 2000). The use of herbs to particularly solve the problem of erectile dysfunction among Ugandan men can only be substantiated by the number of herbs sold in Ugandan streets and those advertised in print Media.

Erectile dysfunction (ED), which is some time referred to as “impotence,” is the repeated inability to get or keep an erection firm enough for sexual intercourse (NIH, 2003, www.impotence.org). The estimated range of men worldwide suffering from ED is from 15 million to 30 million (NIH, 2003). According to the National Ambulatory Medical Care Survey (NAMCS), for every 1,000 men in the United States, 7.7 physician office visits were made for ED in 1985. By 1999, that rate had nearly tripled to 22.3.

According to a survey conducted in three countries of Nigeria, Egypt and Pakistan by Shaer et al. (2003), the age-adjusted prevalence rates of ED among men attending primary care clinics was 57.4% in Nigeria, 63.6% in Egypt, and 80.8% in Pakistan while in Uganda, the prevalence of erectile dysfunction is not known because there is no documented research done to determine it, coupled with the fact that many men do not openly declare their reproductive problems to the health workers but easily do so to traditional healers. This is a clear indication that there are many silent men, particularly couples affected by ED.

Of the many herbs used in the treatment of ED in South-Western Uganda, *Citropsis articulata* ranks number one, with the roots commonly used, a process that destroys the plant and now leading to its extinction (Kamatenesi-Mugisha and Oryem-Origa, 2005). There are several drugs currently available as treatment for ED with the most effective being sildenafil. These available modern medications like sildenafil for ED treatment in men has several side effects, and is very expensive for most of the rural people in Uganda and other developing countries (Magoha, 2000). Yet in traditional medicine, there are several medicinal plants like *C. articulata* that have been relied on for use in the treatment of ED. This ethnobotanical indigenous knowledge has not been earlier documented and scientifically validated for efficacy and safety, future drug discovery and development (Kamatenesi-Mugisha and Oryem-Origa, 2005). Available information indicate that the leaf extract of *C. articulata* improves testosterone levels and could be used to treat erectile dysfunction in men with low testosterone levels (Vudriko et al., 2014), but no data exist about the root extract which has been used for ages.

This study was conducted with the main aim of evaluating the effects of the aqueous extract of *C. articulate* as commonly used by the local people on sexual function in rats, and specifically to carry out phytochemical screening of the aqueous extract of *C. articulata*, determine the acute toxic effects and evaluate the effects of aqueous extract on mounting frequencies and testosterone levels in male Wistar rats.

MATERIALS AND METHODS

Study design

This was a short term (7 days) prospective experimental study conducted to determine the effects of administration of 3 dose levels of aqueous extract of the root bark of *C. articulata* on sexual function and testosterone levels in male Wistar rat.

Materials

Aqueous root bark extract of *C. articulata*, animal cages, observation chambers, oral cannulars, 5 and 2 ml syringes, 1 ml insulin syringe, distilled water, testosterone kits, sildenafil citrate, oestrogen and progesterone powder, 2 electronic balance, one for weighing animals and one for extract, testosterone and estrogen powder, animal feeds, watering cans, soft wood shavings, kitchen tissues to line the base of observation chambers, hand towels, disposable gloves and EDTA-containing vacutainers for blood samples were used in the study.

Plant collection, identification

The roots of *C. articulata* were collected in December, 2013 from Omukiyenje village, Masha sub-county, Isingiro district, South-Western Uganda. A herbarium specimen was prepared and taken for botanical identification at the Department of Science Laboratory Technology/Biology, Mbarara University of Science and Technology, was authenticated by a botanist, and a voucher specimen was deposited and assigned a herbarium number of OJ001.

Processing and drying of whole plant material

The plant material was washed under running water to remove soil and other dirt on the root bark, shade -dried at room temperature for 3 weeks; when the root bark dry, and easily breakable then it was crushed into powder form for extraction.

Extraction

About 500 g of the root bark powder was weighed and extracted using water by hot maceration since the local people normally boil it and take the decoction or chew the root bark. It was filtered using a piece of cloth and finally filtered using Whatman filter paper Number 1 to obtain a fine filtrate.

Separation of crude extract from extracting solvent

The final filtrate of the crude extract that was obtained was evaporated in an oven at 60°C for 4 days; the dried extract was crushed into powder form and stored in a fridge at ±2°C to prevent any enzymatic activity in the extract and temperature fluctuation that could affect the extract.

Phytochemical test

Phytochemical screening was conducted qualitatively according to

the methods described by Trease and Evans (2009) and Kokate et al. (2010) and Sakaguchi (1925).

Experimental animals

Young adult male Wistar rats, 4 to 5 month old were secured for use in the experiment. 30 for erectile function test and 10 for acute toxicity studies. They were kept at standard condition (free access to water and feeds ad libitum, cages lined with wood shavings and cleaned twice a week, temperature control using the animal house Air conditioners) following the National Institutes of Health (NIH) guidelines for animal handling in teaching and research (National Academy of Science., 1996).

Acute toxicity studies

Acute toxicity testing was conducted in two phases, Phase 1: Three groups consisting of three rats per group were given a geometrically increasing dose level of the extract (10, 100, and 1000 mg/kg) orally. The treated rats were observed for 4 h after administration of the extract for signs of toxicity, and after 24 h they were scored for mortality and general behavior. Phase 2: After 24 h, one rat was treated with 9,000 mg/kg of the extract and it survived. Another group of 3 animals were then treated with the same dose of 9,000 mg/kg to confirm the observation seen with one animal and all of them survived up to 14 days although signs of toxicity as indicated in Table 2 were noted. The observations were done as in phase 1. The lethal dose (LD50) was calculated as the geometric mean of the dose that caused 100% mortality (10,000mg/kg), and that which caused no mortality at all (9,000mg/kg) (Lorke, 1983).

Non-contact and contact erections

Non-contact erections were elicited by placing a male animal in the presence of a female animal in oestrus (Sachs et al., 1994). This was achieved by placing a male rat in one half of a glass cage or observation chamber, and in the other half was placed an ovariectomised female rat placed in behavioural oestrous by the administration of oestrogen and progesterone. The female animals were administered suspension of ethinyl estradiol orally at the dose of 100 µg/animal, 48 h prior to the pairing plus progesterone injected subcutaneously, at the dose of 1 mg/animal 6 h before the experiment. The receptivity of the female animals was confirmed before the test by exposing them to male animals, other than the control, test and standard animals. The most receptive females were selected for the study. A perforated dividing wall separated the two halves of the cage or observation chamber. The dividing wall allowed the passage of auditory, visual and pheromonal cues between the two animals.

The number of erections was monitored using video camera for one hour. As a non-contact evoked erection is dependent on visual, olfactory and auditory cues from the female and not (due to separation by a barrier) due to tactile reflexive mechanisms, the response is believed to be primarily under the influence of forebrain regions of the brain. Male rats with lesions to the medial Pre-optic area of the hypothalamus show normal non-contact erectile responses, and reduced copulatory behaviour (Liu et al., 1997). It has been postulated that the frequency of non-contact erections (alternatively called female-enhanced spontaneous erections) is an indicator of sexual arousal (Sach, 2000). Later on, the animals were paired and the number of mounting monitored for 1 h. The male rats were treated for 7 days with the extract at three dose levels of 500, 1000 and 1500 mg/kg respectively, and 1 h before the experiment with the standard drug sildenafil at a dose of10 mg/kg for the positive control group. The male rats were sacrificed at the end of the experiment; their blood samples removed and taken for testosterone levels analysis. Since it is known that the secretion of testosterone is influenced by circadian rhythm (Diatroptov., 2011), in designing this experiment, this was considered a constant factor as all the animals were kept under similar condition.

Ethical consideration

The proposal was submitted to the Mbarara University of Science and Technology faculty research and ethics committee (MUST-FREC) and Institutional Review Committee (IRC) for approval and permission to conduct the research using animals. The animals were obtained from Kampala International University Western Campus, Department of Pharmacology, they were kept in groups in cages cushioned with soft wood shavings, the room was maintained on a 12 h day light and 12 h darkness, they were fed on powdered feeds from NUVITA, and were allowed free access to water *ad libitum*. At the end of the experiment, the animals were anaesthetized using chloroform, laparatomy, and thoracotomy and, blood samples removed through cardiac puncture and taken for testosterone analysis. This was done following the guidelines published in Guide for care and use of Laboratory Animals 1996.

Testosterone analysis

Testosterone analysis was performed using the AXSYM Testosterone reagent by Abbott AXSYM system. The system is based on Microparticle Enzyme Immunoassay (MEIA) technology for the quantitative determination of testosterone in human serum and plasma. The AXSYM Testosterone assay displaces bound testosterone from the protein and measures total testosterone. Measurement of results by the AXSYM system is based on the Beer's Law. The unit of measurement that was used in this case was ng/ml (Abbott AxSYM system Operation Manual, 1996).

Standard drug

Sildenafil citrate (VIAGRA®) from Pfizer Pharmaceuticals (2008). Batch No. A030012 was purchased from Olive Pharmacy, Mbarara City Mall. Toxicological information obtained from the material safety data sheet from Pfizer Pharmaceutical company website that was revised on 18 November, 2010, Version 1.1, page 5/8 indicated that the Rat Oral LDmin (Minimum Lethal dose) was 300 to 500 mg/kg. From this information, the study took 1/10 of the lower limit, reduced by 10 mg since it was high, and used 20 mg/kg as the dose to use in this study.

Data analysis

Stata software Version 11 and Microsoft excel were utilized for the analysis of the data obtained and the data has been presented in tables and graphs and interpreted into usable information. The results were analyzed using the students't-test to compare the mounting frequency and testosterone levels in both the treated and non-treated animal groups. Results with a P-value <0.05 was considered statistically significant.

RESULTS

Phytochemical screening

Indicated the presence of saponins, proteins free amino

Table 1. Results of phytochemical screening.

Phytochemical	Results
Saponin	+
Protein	+
Free amino acid	+
Arginine	+
Terpenoid	+
Phenolic compounds	+
Tannins	+
Fats & oil	+
Glycosides	-
Anthraquinolines	-
Alkaloids	-

Key: + Positive, - Negative.

Table 2. Acute toxicity test results and observations.

Dose levels (mg/kg)	Number of animals	Observations
10,000	3	Weakness, diarrhea, poor feeding, 2 noted dead the following morning, last one died between 24 and 36 h
9000	1	Weakness, diarrhea, poor feeding, no death recorded after 14 days.
7,500	3	Weakness, mild diarrhea, no death recorded.
5,000	3	Lively feeding well, no abnormality noted.

Table 3. Mean number of mounts in 1 h against dose in experimental groups.

Groups	Mean mount in 1 h (SEM)	P-value	95% CI
Citro500	25.5 (5.5)	0.0310	11.33 - 39.67
Citro1000	23 (1.9)	0.015	18.08 - 27.92
Citro1500	0	0	0
Sildenafil10 mg	36 (0.97)	0.0002	33.52- 38.48
distilled water	10.33 (4.67)	-	1.66 - 22.34

This indicates that *C. articulata* at the dose of 1000 mg had a better effect on testosterone levels, followed by 500 mg/kg.

acids, Arginine, terpenoids, phenolic compounds, tannins, fats and oils in the aqueous extract of the root bark of *C. articulata* (as shown in Table 1).

Acute toxicity study

All the three animals treated with aqueous extract of *C. articulata* root bark at a dose of 10,000 mg/kg died, while at 9,000 mg/kg there was no death but signs of toxicity were noted as recorded in the table (as shown in Table 2). The probable LD50 was taken as the geometric mean of 10,000 and 9,000 mg/kg, and determined to be 9486.833 mg/kg.

Effects of erection

Neither the extracts nor the standard drug sildenafil was noted to induce erection in the non-contact model for investigating substance with erection inducing properties**.**

Effects on mounting frequency

It was noted that Sildenafil treated animals had the highest number of mounts, followed by Citro 500, Citro 1000 and distilled water, while the Citro1500 showed inhibition of mounting (as shown in Table 3). There was no statistical difference between the extract treated

Table 4. Mean testosterone levels against dose in experimental groups.

Groups	Mean testosterone levels ng/ml (SEM)	P-value	95% CI
Citro500	1.88 (0.34)	0.13	1.00 to 2.75
Citro1000	2.52 (0.46)	0.024*	1.34 to 3.70
Citro1500	2.28 (0.42)	0.043*	1.199 to 3.37
Sildenafil10 mg	1.49 (0.19)	0.396	1.01 to 1.98
Distilled water	1.43 (0.16)	-	1.02 to 1.83

Key: Citro = *Citropsis articulata* extract. This indicates that the extract of *C. articulata* indicated maximum effects on testosterone levels at the dose of 1000 mg/kg while the dose of 1500 mg/kg reduced it.

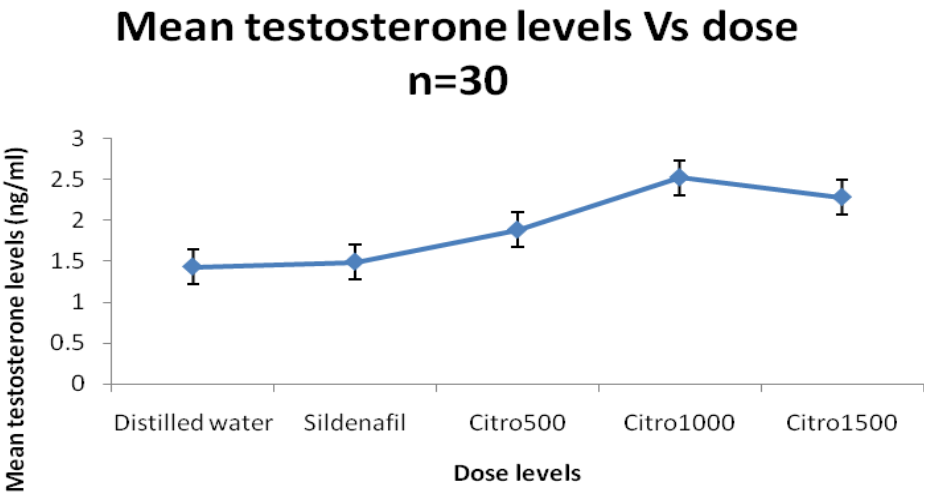

Figure 1. Curve showing mean testosterone levels against dose in experimental groups, Key: Citro = *Citropsis articulata* extract, sildenafil = sildenafil citrate 10 mg/kg.

groups.

Effects of extract on testosterone levels

Indicated that the extract did significantly increase testosterone levels at doses of 1000 mg/kg and slightly reduced it at a dose of 1500 mg/kg (Table 4), and a curve plot of the result indicated increase in testosterone level from 500 mg to 100 mg/kg, but with the highest does of 1500 mg/kg decreasing testosterone levels (Figure 1).

DISCUSSION

The present study was aimed at determining the acute toxicity and effects on sexual function considering three factors of: effects on erection, mounting frequency and testosterone levels of aqueous extract of *C. articulata* in male Wistar rats. Findings indicated that the extract is slightly toxic, increased mounting frequency and testosterone levels in a dose dependent manner.

Phytochemical screening indicated that the aqueous extract of *C. articulata* contains mainly phytochemicals that are known to be hydrophilic with the exception of fats and oils that were noted present. This study detected the presence of saponins, proteins, free amino acids, arginine, terpenoids, phenolic compounds, tannins and fats and oils. According to Lacroix et al. (2011), it was noted that alkaloids were present in the extract of *C. articulata* and that two known alkaloids (5-hydroxynoracronycine and 1,5-dihydroxy-2,3-dimethoxy-10-methyl-9-acridone) were isolated among other phytochemicals that were found present. According to Tanayen et al. (2010), on phytochemical screening and acute toxicity of *Koseteletzyskya begoniifolia,* they also noted the presence of arginine in the extract and proposed that the arginine may be responsible for the effects of the plants extract on erectile dysfunction. It may also be possible that because of the limited test we were able to conduct, there might have been some other phytochemicals that may have been responsible for the activity noted.

All the three animals treated at 10000 mg/kg died while at 9000 mg/kg, there was no death. By this, the study cannot conclusively state that the LD50 is 9486.833

mg/kg which is the geometric mean of those two doses. However, the study state that the LD50 is in between the two dose levels. According to Gosselin et al. (1984), a substance with acute toxic dose lying between 5 to 15 g/kg is considered slightly toxic. This therefore, implies that the aqueous extract of *C. articulata* is slightly toxic, and therefore should be used with caution. During the toxicity study, one of the signs expected to have been peculiar to this extract (penile erection or priapism) was not noted. This further indicated that the extract does not induce erection on its own.

There was no penile erection observed on the treated animals before pairing. However, the male animals in the citro500, citro 1000, sildenafil and the distilled water group (negative control) showed active sexual behaviours like orientation towards the female side of the cages, sniffing and roaring. This therefore indicated that the aqueous extract of *C. articulata* does not induce erection.

When pairing was done, the animals in the sildenafil treated group and citro1000 showed maximum sexual activity. This was because all the animals in those two groups were noted sexually active. This was followed by the citro500 and distilled water group (negative control). The observation with the citro1000 and sildenafil treated group does not however mean the strength of activity were the same in the two groups. It was noted that, the sildenafil treated group was much more active than the citro1000 treated group. This is probably because sildenafil is a known inhibitor of Phosphodiesterase type-5 (PDE-5), and thus the animals in the group had sustained erection leading to increased mounting frequency than the extract treated groups. There was a significant increase in mounting from citro500 to citro1000, P-Value = 0.0310 (11.33 to 39.67), 0.0154 (18.08 to 27.92) respectively while citro1500 inhibited mounting completely.

This result is similar to that of Wawata et al. (2010) in which they found 6.60, 8.40, 3.80, and 0.20 mount in 3 h with dose levels of Sildenafil 5 mg/kg, extracts at 150, 300 and 600 mg/kg respectively. The number of mounting noted with the treatment groups for extract of *C. articulata* were however much higher than that reported for *Acacia polycantha* ethanolic stem bark extract (Wawata et al., 2010). This is probably an indication that *C. articulata* extract is much more potent, and could be used to treat certain types of erectile dysfunction, especially those associated with low testosterone level. This is in agreement with studies conducted on the leaf extract of the same plant by Vudiriko et al. (2014) which showed similar result. The increase in activity from 500 to 1000 mg/kg and the lack of activity at 1500 mg/kg could mean that the extract is much potent at doses around 1000 mg/kg and that there was some toxicity that went unnoticed at the dose of 1500 mg/kg. Also, because the animals were treated with the extract for 3 days before the test was conducted, it could also be possible that some desensitization might have occurred at higher dose if the mechanism of action of the active ingredient(s) in the extract is/are receptor based.

In comparison to the no treatment group and sildenafil treated group, there was a significant increase in testosterone levels from citro500, citro1000 with P-values 0.1 to 0.02 respectively and a slight reduction with citro1500, P-value 0.04. The decrease in testosterone level at this dose level corresponds to the lack of mounting activity at the same dose level which provides some support to the study proposition that there could have been some toxicity occurring at that dose level.

The increased testosterone levels noted with the dose at 1000 mg/kg and the significant effects on mounting noted at the same dose could probably be indicating that, the aqueous extract of *C. articulata* improves sexual function by increasing testosterone levels, similar to the result found by Vudriko et al. (2014) on the leaf extract of *C. articulata* on male rats. The testosterone levels noted in this case were however, lower than the results obtained from the use of *A. polycantha* ethanolic stem bark extract by Wawata et al. *(*2010), but were much higher than that reported by El-Tantawy et al. (2007) on the effects of *Tribulus alatus* extracts on free serum testosterone levels.

Conclusion

This study has shown that the aqueous extract of *C. articulata* is slightly toxic, indicated positive effects on sexual activity in Albino Wistar rats in terms of the mounting frequency, increased the levels of testosterone in Albino Wistar rats, but did not induce erection. This study thus, provides a missing knowledge about the effects of aqueous extract of root bark on sexual function, and provides support for its traditional use in the treatment of male sexual dysfunction by local communities in south-Western Uganda.

Conflict of Interest

The authors have not declared any conflict of interest.

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