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Effects of Crude Aqueous Leaf Extracts of *Citropsis Articulata* and *Mystroxylon Aethiopicum* on Sex Hormone Levels In Male Albino Rats

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ABSTRACT: *The study evaluated effect of aqueous leaf extracts of C. articulata and M. aethiopicum on serum levels of testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin in male albino rats as well as on organ weights and histological structure of the testis. The phytochemical composition of the extracts was also qualitatively determined. This was an experimental study. Shade dried C. articulata and M. aethiopicum leaves were pounded and crude extracts were prepared using distilled water; then boiled at 100°C for 20 minutes. Adult male rats were randomly divided into eight groups (n=6) and kept in separate cages. The rats from group i-vii were orally dosed daily with 5mg/kg of flutamide to induce erectile dysfunction one hour prior to the administration of the extracts at doses of 150, 300 and 450 mg/kg body weight respectively of each extract. Group vii rats received testosterone, 100µg/kg intraperitoneally while those in group 8 were administered with 10mg/kg of normal saline (negative control). On days 0, 7, 14 and 21 the males from different groups were subjected to estrus induced females in separate cages. The sexual behavior of the rats i.e. mounting and intromission frequencies were recorded. Serum levels of testosterone, LH, FSH and prolactin; organ weights and the histological structure of the testis were determined on day 22 post last treatment. The extracts were also screened for phytochemical composition.Rats treated with 450mg/kg of C. articulata and M. aethiopicum had a significant increase in level of serum testosterone level ($p<0.01$ and $p<0.001$ respectively) when compared with both negative and positive control. There was also a significant increase ($p<0.01$) in LH levels in rats treated with 450mg/kg of M. aethiopicum. There was a dose dependent increase in mounting and intromission frequencies, testis and heart weight for both aqueous extracts for all doses. Statistically significant ($p<0.001$) increase in testis weight was witnessed in rats treated with 450mg/kg of C. articulata. The phytochemical screening revealed presence of high concentrations of catechol tannins in the aqueous extracts of both plants. The results also revealed high concentrations of saponins, flavonoids, steroid glycosides, anthrasenosides (aglycones) and triterpenes in the aqueous extract of C. articulata. Histological examination of the testes revealed increased series of spermatogenesis and Leydig cell proliferation with higher doses of both extracts.This study demonstrated the androgenic effects of the aqueous leaf extracts of both plants i.e. increased mounting, intromission, testes weight, spermatogenesis and Leydig cell proliferation. These effects are probably explained by the increase in serum testosterone and LH levels. The results of this study may explain the use of these plants in the management of erectile dysfunction attributed to hypogonadism in local communities of Uganda.*

KEYWORDS: *Erectile dysfunction; Flutamide; leydig cells; Luteinizing hormone; Follicle stimulating hormone.*

I. INTRODUCTION

Erectile dysfunction is the inability of the male to attain or maintain an erection sufficient for satisfactory sexual intercourse (NIH, 1993). Erectile dysfunction is a common and widespread health problem that affects approximately 30 million men in US (Malviya *et al*., 2011; Feldmann *et al*., 1994) and in 1995 there was an estimated projection of 152 million men worldwide who experienced ED. This is estimated to rise to about 322 million men by the year 2025 (Ayta *et al.,* 1999). Erectile dysfunction is a common medical condition that affects the sexual life of millions of men worldwide (Montorsi *et al*, 2003; Shabsigh and Anastasiadis, 2003). Although ED

is tied closely as it is to cultural notions of potency, success and masculinity it can have severe psychological consequences. There is a strong culture of silence and inability to discuss the matter. In reality, it has been estimated that around 1 in 10 men will experience recurring impotence problems at some point in their lives (Anonymus, 2009). Erectile dysfunction may develop due to hormonal deficiency, disorders of the neural system, lack of adequate penile blood supply or psychological problems. In some diabetic patients it could be due to apoptosis owing to loss of BCT-2 expression in smooth muscle and increase connective tissue synthesis, due to TGF_{β}. This results in decreased compliance of cavernosal tissue (Acici *et al*., 2000, Dahiya *et al*., 1999). These changes reduce or interfere with the gap junctions and K channels in cavernosal smooth muscle that are necessary for coordinated relaxation of cavernosal tissue (Vickers *et al*., 2002). Testosterone, the primary sex hormone produced by the testes plays an essential role in healthy men. As men grow older, testosterone levels fall, with a steeper decline in unbound or free testosterone; the biologically active portion (Guyton and Hall, 2006). Low testosterone levels have been associated with poorer cognitive function, higher body mass index, increased body fat, declining muscle mass and strength and impaired general and sexual health in aging men. Low testosterone levels have also been linked to some chronic diseases such as type II diabetes, cardiovascular disease, prostate cancer etc. and have been shown to predict higher overall mortality. Interestingly, average male testosterone levels have dropped over the last 15 years (Guyton and Hall, 2006). The increasing number of men seeking help for ED has expanded basic physiological and pharmacological research on sexual performance (Cicero *et al*, 2001).

In developing countries, the inability to afford modern medical healthcare has forced patients to seek traditional medical attention. The use of herbs in the treatment of ailments in Africa is an age-old practice. Man's continuous reliance on herbs for therapeutic and nutritional benefits cannot be overemphasized. Many plant extracts are traditionally used to improve sexual performances (Kamtchouing *et al.,* 2002; Carro-Juarez *et al.,* 2004, Kamatenesi *et al.,* 2004). In Uganda, several plants such as *Ekebergia capensis, Mondia whitie* and *Cola acuminate* are claimed to possess aphrodisiac potential (Ndukui *et al*., 2012; Kamatenesi *et al*., 2002). *C. articulata* (Rutaceae) is widely known as Africa cherry orange or Uganda cherry orange (locally as omuboro) is a small citrus fruit about the size of a tangerine. The plant is endemic to Central and Western Africa, and is used primarily as food and as a medicinal herb. An infusion made of the grounded root of the omuboro, drunk once a day for three days is considered to be a powerful aphrodisiac for men only.

Mystroxylon aethiopicum which belongs to the Celastraceae family, commonly known as the spike-thorn family is an attractive evergreen tree that is useful as an ornamental garden tree. Traditionally leaves of the plant are used to manage helminthosis and Black water in sheep and as a magic potion to keep the community together. However, documented experimental and clinical data on many of these plants are lacking. This study sought to determine the effect of oral administration of the aqueous leaf extracts of *C. articulata* and *M. aethiopicum* on the sexual performance and androgens effects on flutamide treated male albino rats in order to evaluate the traditional claims on the therapeutic efficacy of these plants in management of ED.

II. MATERIALS AND METHODS

Collection, identification and extraction of the plant materials

Plant samples were collected from Kibale forest (western Uganda) and taken to Makerere Herbarium for identification; authentication and a voucher specimen number were deposited (NJ2 and NJ5) respectively by Mr. Protase Rwaburindore (Botanist). The leaves of *C. articulata* and *M. aethiopicum* were air dried under a shade for 1 week to avoid loss of volatile bioactive compounds. The dried samples were pulverized to powder using an electrical grinder and finely sieved. The powder (250g for each plant) was weighed using a balance (Mettler PJ3000) and boiled in 2 litres of distilled water for 20 minutes, after which filtration was done using Whatman filter paper No. 2 (Whatman International Ltd, England). The filtrate was freeze dried at pressure 32pa, with original temperature set at -47^{o}C and then maintained at 0^{o}C for 36hrs. The solid extracts were used to determine the percent yield before storage at 4^{o}C. Fresh aliquot portions of the extract were weighed and dissolved in distilled water (at room temperature) for use on each day of the experiment.

Percentage yield

Percentage yield = W_2/W_1*100, whereby, W_1 is the weight of the powdered sample before extraction, while W_2 is the weight of the semi-solid aqueous extracts. Therefore percentage yield of *C.articulata* was = 30.21 /250x100; Percentage yield= 12.08% w/w

Percentage yield of *M. aethiopicum* was; 20.16 /250x100 = 8.06%

Phytochemical screening

This process involved use of qualitative chemical analysis (Kokate, 1994, Harbourne, 1973 and Marinova. *et al.,* 2005) whereby phytoagents with claimed androgenic effects were determined by adopting the procedures described by Stephen (1970) and Parekh and Chanda (2007) and was done by use of color intensity.

Experimental animals

Fifty six disease-free Wistar albino rats (48 male: 8 females), weighing about 150-200g and 24 Swiss mice (acute toxicity) of 8 weeks old were used in this study. The study animals were obtained from the Division of Pharmacology and Toxicology, College of Veterinary Medicine, Animal Resources and Biosecurity animal house. The male rats were randomly divided into different groups of six male rats per cage during the course of the experiment and kept at room temperature (24± 2°C) with a 12 : 12h light/dark cycle and 60-65% relative humidity. The animals were given two weeks of acclimatization prior to the study and were fed on standard pellet diet (Unga limited, Kampala). Water was provided *ad libitum.*

Administration of the extract

The extract doses were determined from 1/11, 1/16 and 1/33 of limit dose test of 5,000mg/kg from the preliminary LD_{50} determination. The anti-androgen drug flutamide (5mg/kg) was used to suppress the effects of testosterone on the Leydig cells in the extracts and testosterone treated groups. Forty eight disease free male wistar rats were randomized into 8 (i-viii) groups (n=6). The study animals from I-VII groups were treated with flutamide (5mg/kg) 1 hour prior to extracts and testosterone treatment respectively. Group Viii: rats received 10ml/kg of distilled water daily for 21 days as shown in Table 1.

Table 1: Treatment groups

Group	Treatment
I	*C. articulata* crude extract (150mg/kg) + flutamide (5mg/kg)
II	*C. articulata* crude extract (300mg/kg) + flutamide (5mg/kg)
II	*C. articulata* crude extract (450mg/kg) + flutamide (5mg/kg)
IV	*M. aethiopicum* crude extract (150mg/kg) + flutamide (5mg/kg)
V	*M. aethiopicum* crude extract (300mg/kg) + flutamide (5mg/kg)
VI	*M. aethiopicum* crude extract (450mg/kg) + flutamide (5mg/kg)
VII	Testosterone 100µg/Kg (positive control) + flutamide (5mg/kg)
VIII	10 ml/kg of distilled water only (negative control)

Induction of estrous to female rats

Eight female rats were administered with estradiol benzoate (100µg/kg) and progesterone (5mg/kg) subcutaneously to induce estrus (Ndukui *et al*., 2012; Gundidza *et al*., 2009; Yakubu *et al*., 2005) 48hrs before experimentation and 4hrs prior to the exposure to males.

Body and organ weight

The body weight of the rats was recorded on day 1, 14 and 21 of the study. At the end of the 21 day treatment period, 3 animals each from all groups were sacrificed by euthanasia 18 hours post treatment. The relative organ weight (ROW) of the testis, liver, kidney, and heart were determined using weighing balance (NVT1601/1, OHAUS Corporation, USA) and recorded as described by Thakur and Dixit (2006). Two organs were collected from two animals of different groups and fixed in 10% buffered formalin. The organs were later subjected to automated tissue processing machine where they were dehydrated and rehydrated by varying percentage of ethanol. Sections of 5 µm thickness of each tissue were fixed on glass slides and stained with hematoxylin and eosin. Microscopic evaluation of the thin section was undertaken and variations in histoarchitecture were recorded (Chauhan and Dixit, 2008).

Determination of hormone levels

After 18 hours following the administration of the last dose, the animals were sacrificed and whole blood collected by cardiac puncture and kept in non-heparinised vacutainer which was span at 2500rpm for 10min using a

bio-centrifuge (MSE, O-5122A, Germany). The level of free serum testosterone, LH, FSH and prolactin in serum was measured with ECOBAS-6000 hormone analyzing machine as described by Atlas *et al*., (1995).

Mounting and Intromission Frequency determination

These variables were determined on day 1, 14 and 21 of the study. Each male rat from the various treatment groups was individually subjected to estrous female rat in a separate cage for 30 minutes with the number of mounts and intromission recorded separately.

Statistical Analysis

Qualitative data on phytochemical screening was presented in form of tables. However quantitative data on serum testosterone, LH, FSH and prolactin levels, mounting and intromission frequency, body and organs weight was expressed as mean ± SEM with 95% confidence interval and p-value of ($p<0.05$). The treated groups were statistically compared to both normal and positive controls with ANOVA and post hoc Dunnet's test, using Graph Pad Prism version 5.01 inc U.S.A.

Ethical considerations

The experimental animals were handled in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines for testing chemicals. Proper handling of the study animals was provided by international guidelines on care and use of lab animals (PHS, 1986). The research protocol was approved by School of Biomedical Sciences Research and Ethical Committee, Department of Pharmacology and Therapeutics of the College of Health Sciences and an ethical clearance number (SBS 115) was given. In addition, the Uganda Council of Science and Technology approved the study.

III. RESULTS

Phytochemical screening

Both extracts showed strong presence of catechol tannins with absence of reducing sugars in the aqueous extract of *C. articulata*. The saponins, flavonoids, steroid glycosides, aglycones of anthrasenosides and triterpenes were strongly present in *C.articulata.* Most of the phytochemicals in *M. aethiopicum* were weakly present with steroid glycosides and triterpenes (phytosterols) moderately present (Table 1).

Table 1: Phytochemical present in aqueous extracts of *C. articulata* and *M. aethiopicum*

Phytochemical agent screened for	Results		Inferences
	C. articulata	***M. aethiopicum***	
Saponins	+++	+	Froth (1cm long)
Catechol tannins	+++	+++	Blackish green
Basic alkaloids	++	+	White yellowish ppt
Steroid glycosides	+++	++	Intense red coloration
Flavonoids	+++	+	Intense red coloration
Aglycones of anthrasenosides	+++	+	Red coloration
Reducing sugars	-	+	Faint red ppt
Triterpenes (phytosterols)	+++	++	Violet coloration
Proteins	++	+	Yellow coloration

Key: + weakly present; ++ moderately present; +++strongly present; -Absent; ppt- precipitate

Effects of the plant extracts on hormonal level in male albino rats

Rats treated with dose of 450mg/kg body weight of *C. articulata* and *M. aethiopicum* had a significant increase in level of serum testosterone level $P<0.01$ and $p<0.001$ respectively while rats treated with 300mg/kg bodyweight of *M. aethiopicum* showed a significant increase in serum testosterone level ($p<0.01$) when compared to both negative and positive control. The dose of 450mg/kg of *M. aethiopicum* caused a significant increase in LH ($p<0.01$) as compared to both normal and positive control groups. There was no significant increase in FSH and prolactin level for both extracts when compared with the negative control ($p>0.05$) as shown in Table 2.

Table 2: Effects on hormones level of rats treated with the aqueous extracts of C. articulata and M. aethiopicum for 21 days

Hormone	Controls		*Citropsis articulata*			*Mystroxylon aethiopicum*		
	Negative	Positive	150mg/kg	300mg/kg	450mg/kg	150mg/kg	300mg/kg	450mg/kg
TESTO (ng/ml)	0.27±0.12	1.55±0.46	0.71±0.20	1.05±0.41	2.59±0.49**Aa	1.73±0.56	4.43±1.04**Aa	6.49±1.56***Aa
FSH (miU/ml)	0.10±0.0	0.10±0.0	0.10±0.0	0.10±0.0	0.11±0.00	0.10±0.0	0.10±0.00	0.10±0.00
LH (miU/ml)	0.10±0.0	0.10±0.0	0.10±0.0	0.10±0.0	0.11±0.00	0.10±0.0	0.11±0.01	0.13±0.01**Aa
PRL (uIU/ML)	1.00±0.0	1.00±0.0	1.00±0.0	1.00±0.0	1.00±0.0	1.00±0.0	1.00±0.0	1.00±0.0

Values expressed as mean±SEM, n=6, p<0.05, *p<0.05, **p<0.01, ***p<0.001,
A-significant when compared with negative control
a- significant when compared with positive control

Mounting frequency

The aqueous extracts of both plants showed a dose dependent increase in mounting frequency among the treated groups as compared to negative and positive control groups. The aqueous extract of *C. articulata* at a dose of 450mg/kg showed a statistically significance increase (p<0.001) in mounting frequency on days 7, 14 and 21. The dose of 300mg/kg showed a statistical significance increase (p<0.001) when compared with both negative and positive control groups on days 14 and 21. At day 7 the dose of 150 and 300mg/kg of *C. articulata* showed a statistical significance (p<0.01 when compared to both controls. All doses of aqueous extracts of *M. aethiopicum* showed a significant increase (p<0.001) on mounting frequency on days 7, 14 and 21 respectively except that the dose of 150mg/kg at day 14 (p<0.05) and 21 (p<0.01) was statistically significant when compared with the negative control group only while others were compared with both controls groups (Table 3 and Figure 1).

Table 3: Effects on mounting frequency of male rats treated with aqueous extracts of C. articulata and M. aethiopicum

Treatment Groups	Day 0	Day7	Day14	Day21
Normal control	2.33±0.21	3.50±0.22	5.17±0.40	5.00±0.37
Positive control	2.67±0.21	5.00±0.26*A	6.83±0.30	6.33±0.33
C. articulata				
150mg/kg	2.83±0.17	5.17±0.31*A	5.50±0.50	6.33±0.21
300mg/kg	2.67±0.21	7.33±0.33*Aa	9.83±0.54***Aa	10.67±0.67***Aa
450mg/kg	2.83±0.31	8.50±0.43***Aa	12.33±0.42***Aa	16.67±0.88***Aa
M. aethiopicum				
150mg/kg	2.67±0.21	6.83±0.31***Aa	7.67±0.33*A	9.33±0.88**A
300mg/kg	2.67±0.21	8.83±0.30***Aa	12.00±1.03***Aa	13.33±1.12***Aa
450mg/kg	2.83±0.17	9.67±0.56***Aa	12.33±0.95***Aa	15.83±1.47***Aa

Values expressed as mean±SEM, n=6, p<0.05, *p<0.05, **p<0.01, ***p<0.001
A-Significant when compared with Negative control
a- significant when compared with Positive control groups

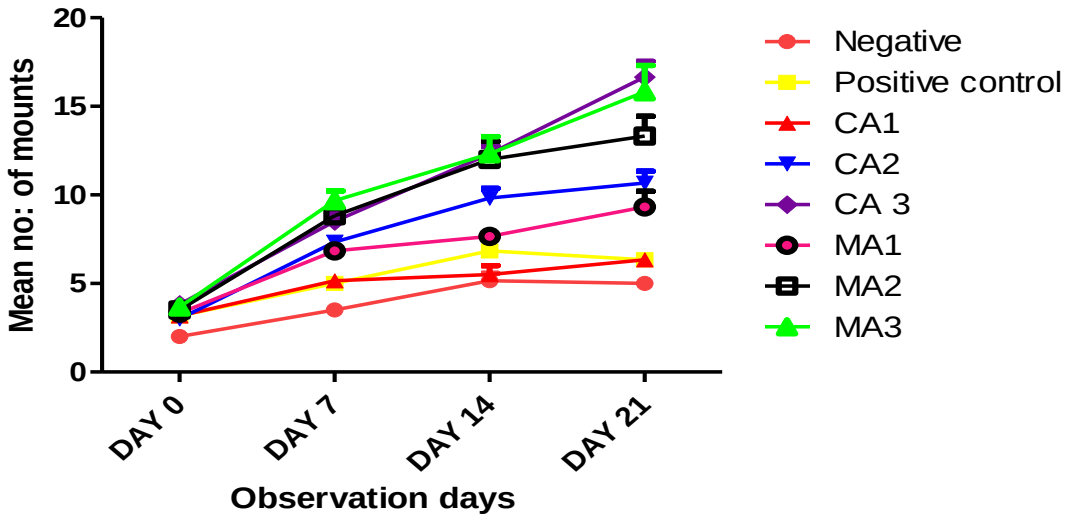

Figure 1: Effects of aqueous extracts on mounting frequencies male rats treated with *C. articulata* and *M. aethiopicum* on given days. CA= *C. articulata* MA= *M. aethiopicum*
CA1/MA1 = 150mg/kg; CA2/MA2 = 300mg/kg; CA3/MA3 = 450 mg/kg

Intromission frequency

Both extracts showed a dose –dependent increase in intromission frequency with the number of days when compared to both normal (normal saline) and positive (testosterone) control group. The dose of 450mg/kg of both extracts on days 7,14 and 21 displayed as statistically significant increase in intromission frequency ($p<0.001$) when compared to both normal and positive control. On days 7, 14 and 21 the 300mg/kg dose of the aqueous extract of *M. aethiopicum* had a statistical significance increase ($p<0.001$) on intromission frequency (<0.01 and $p<0.001$) respectively when compared to both control groups. However *M. aethiopicum* showed a statistically significant increase in levels of intromission frequency as with *C. articulata* when compared to both control group (Table 4 and Figure 2).

Table4: Effects on intromission frequency of male rats treated with aqueous extracts of *C. articulata* and *M.aethiopicum*

Treatment Groups	Day 0	Day7	Day14	Day21
Normal control	1.83±0.31	1.83±0.31	2.50±0.22	3.17±0.31
Positive control	2.33±0.33	2.50±0.34	2.33±0.21	4.33±0.33
C. articulata				
150mg/kg	2.17±0.31	2.33±0.33	2.67±0.33	5.33±0.42**A
300mg/kg	2.50±0.34	3.33±0.21**^{A}	3.83±0.31*^{Aa}	7.17±0.31***Aa
450mg/kg	2.50±0.22	3.83±0.17***^{Aa}	4.67±0.21***^{Aa}	10.83±0.60***^{Aa}
M. aethiopicum				
150mg/kg	2.50±0.22	3.00±0.37*A	3.67±0.33*a	7.00±0.26***Aa
300mg/kg	2.50±0.22	3.67±0.21**Aa	4.50±0.22***Aa	8.67±0.33***Aa
450mg/kg	2.83±0.31	4.17±0.31***Aa	6.17±0.48***Aa	12.83±0.48***Aa

Values expressed as mean±SEM, n=6, $p<0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$
A-Significant when compared with Negative control
a- significant when compared with Positive control groups

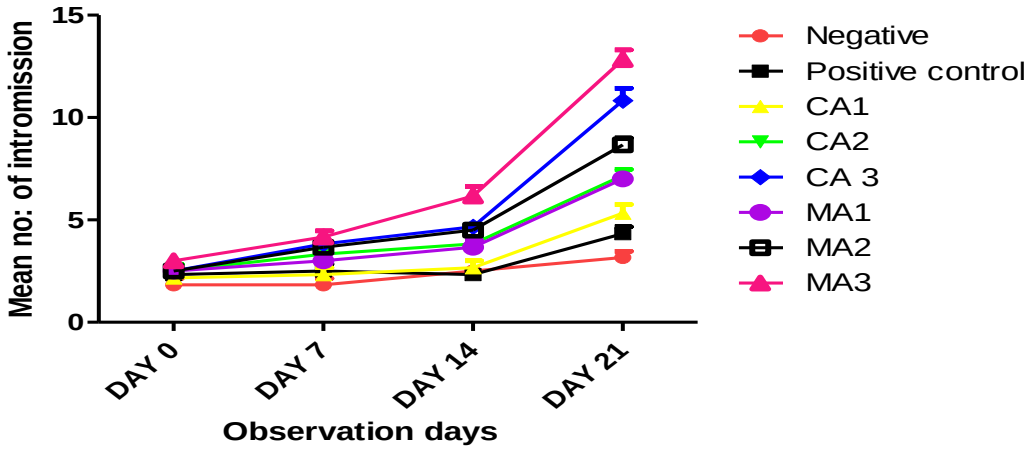

Figure 2: Effects of aqueous extracts on intromission frequencies of male rats treated with *C. articulata* and *M. aethiopicum* on given days. CA= *C. articulata* MA= *M. aethiopicum*
CA1/MA1 = 150mg/kg; CA2/MA2 = 300mg/kg; CA3/MA3 = 450 mg/kg

Changes in organs weight

The effects of organ weight with aqueous extract treated group showed a dose- dependent increase in weight. There was percentage statistical increase in testicles weight ($p<0.05$ and $P<0.01$), 31.83% and 53.82% in dose of 300mg/kg and 450mg/kg of aqueous extract of *C. articulata* when compared with both negative and positive control groups. There was also a dose dependent statistical significant increase ($P<0.05$,) on the heart weight on groups treated with doses of 150 and 450mg/kg of *C. articulata* with the doses of 450mg/kg of *M. aethiopicum* having 66% increase with $p<0.001$ (Table 5).

Table 5: Anabolic effects on organ weights of male rats treated with aqueous extracts of C. articulata and M. aethiopicum

Organs Weighed	Controls		*Citropsis articulata*			*Mystroxylon aethiopicum*		
	Negative	**Positive**	**150mg/kg**	**300mg/kg**	**450mg/kg**	**150mg/kg**	**300mg/kg**	**450mg/kg**
Testicles	2.20±0.06	2.37±0.20 (7.72%)	2.80±0.21 (27.27%)	2.90 ± 0.29*Aa (31.82%)	3.37 ± 0.19***Aa (53.18%)	2.60 ± 0.10 (18.18%)	2.27 ± 0.23 (3.18%)	2.67 ± 0.09 (21.36%)
Heart	0.50±0.0	0.57±0.03 (14%)	0.73 ± 0.09**Aa (46%)	0.57 ± 0.03 (14%)	0.70 ± 0.00*Aa (40%)	0.60 ± 0.0 (20%)	0.67 ± 0.07 (34%)	0.83 ± 0.03***Aa (66%)

Values expressed as mean±SEM, n=3, $p<0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$,
A-Significant when compared with Negative control
a- significant when compared with Positive control groups and %- percentage

Body weight changes

The aqueous dose of 450mg/kg of *C.articulata* and *M.aethiopicum* demonstrated a percentage increase in body weight as 53.49%,50.01%,65.55%,41.91%, 50.05% and 58.70% with $p<0.001$ when compared with both negative and positive control on days 7, 14 and 21 respectively. The dose of 150mg/kg of *C. articulata* on day 7 had a 37.91% increase on bodyweight with $p<0.01$ when compared with both control groups while that of 300mg/kg had 34.75% and 23.84% on day 7 and 14 with $p<0.05$ when compared with positive control group only. The dose of 150mg/kg of M.aethiopicum had a 47.13% ($p<0.05$ compared to both controls) and 53.39% ($p<0.05$, compared only to positive control) increase on body weight on days 14 and 21 concurrently. On day 14 the dose of 300mg/kg of *M. aethiopicum* had an increase on bodyweight with 30.77% ($p<0.05$, when compared to positive control only) (Table 6).

Table 6: Effects on body weight on rats treated with aqueous extracts of *C. articulata* and *M. aethiopicum* on given days of the study

Treatment Groups	Day 0	Day 7	Day 14	Day 21
Normal control	89.30±6.87	97.67±6.69 (9.37%)	108.3±7.57 (21.27%)	123.7±7.01 (38.52%)
Positive control	90.79±4.13	105.0±3.69 (15.65%)	92.81±4.07 (2.22%)	113.3±2.84 (24.79%)
C. articulata				
150mg/kg	95.85±2.61	131.5±5.12 (37.19%)**^{Aa}	121.6±3.30 (26.86%)	122.2±3.19 (27.49%)
300mg/kg	92.54±7.50	124.7±7.37 (34.75%)*^{a}	114.6±5.13 (23.84%)*^{a}	112.4±5.04 (21.46%)
450mg/kg	94.41±3.34	144.9±6.17 (53.49%)***^{Aa}	145.4±6.18 (54.01%)***^{Aa}	156.3±6.86 (65.55%)***^{Aa}
M. aethiopicum				
150mg/kg	89.51±4.99	125.0±4.76 (39.65%)^{A}	131.7±4.66 (47.13%)*^{Aa}	137.3±4.39 (53.39%)*^{a}
300mg/kg	91.23±9.48	115.4±5.75 (26.49%)	119.3±5.82 (30.77%)*^{a}	123.6±5.19 (35.48%)
450mg/kg	104.5±7.17	148.3±8.14 (41.91%)***^{Aa}	156.8±6.41 (50.05%)***^{Aa}	158.7±6.07 (58.70%)***^{Aa}

Values expressed as mean±SEM, n=6, p<0.05, *p<0.05, **p<0.01, ***p<0.001
A-Significant when compared with Negative control
a- significant when compared with Positive control groups and %- percentage.

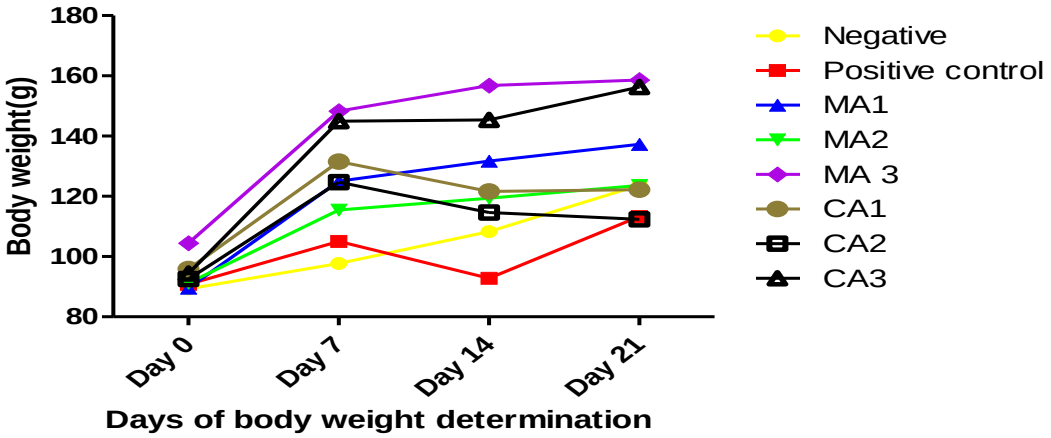

Figure 3: **Effects of aqueous extracts on body weights of male rats treated with *C.articulata* and *M.aethiopicum* for given days. CA= *C. articulata* MA= *M. aethiopicum* CA1/MA1 = 150mg/kg; CA2/MA2 = 300mg/kg; CA3/MA3 = 450 mg/kg**

Histological examination of testis treated with extracts of *C.articulata* and *M. aethiopicum* in comparison with both normal and positive control groups

When the histological section of the negative and positive controls was compared with groups treated with 450mg/kg aqueous extract of *C. articulata* and *M. aethiopicum* showed observable differences in the various stages of spermatogenesis. All stages of spermatogenesis were clearly observed and distinct in the various treatment groups inclusive of; primary spermatocytes, secondary spermatocytes, spermatogonia, spermatid and spermatozoa,

basement membrane and leydig's cells were. The proliferation and increase of leygid's cells was more pronounced in *M. aethipicum* and *C. articulata* (450mg/kg) aqueous extracts as compared to both control groups (Fig. 2 and 3). In the extracts treated groups the primary spermatogenic cells , the spermatogonia are in their first stage of repetitive and multiplicative cell division, moreover the lumen size is decreased and with an observable vascularisation, whereas the effect is slightly restrained in the testosterone treated group and is very minor in normal control group as in figure 2, 3, 1 and 4. There is a distinct difference in the layout, hypertrophy of leydig cells and spermatid differentiation in aqueous extracts treated groups as compared to testosterone treated group which further support their androgenic activity. The aqueous extract of *M. aethiopicum* showed higher number of spermatozoa in seminiferous tubules as compared to *C. articulata* and both control treated groups, which further confirmed the increased spermatogenesis, which is highly elaborated by an increase in spermatogenic elements as depicted figure 2.

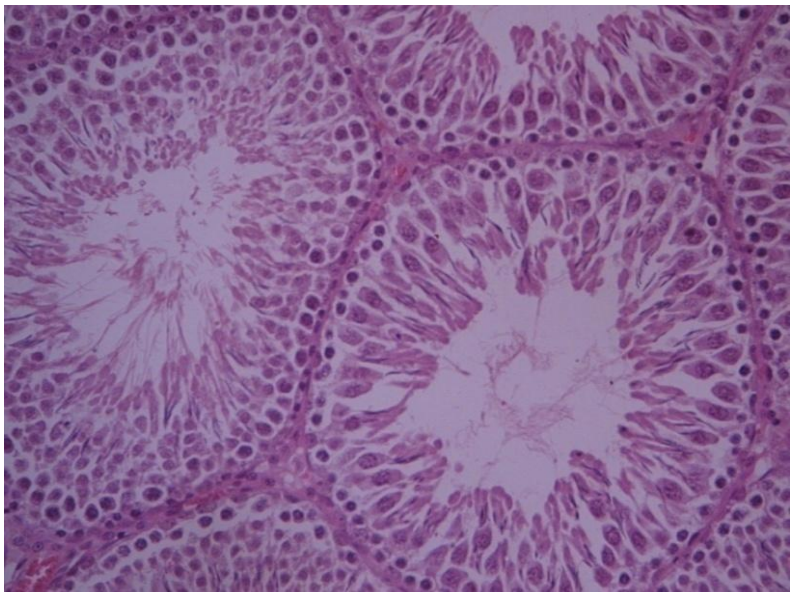

Fig 4: Histoculture of testis in normal control group, x200, scale bar 100µm. No significant changes

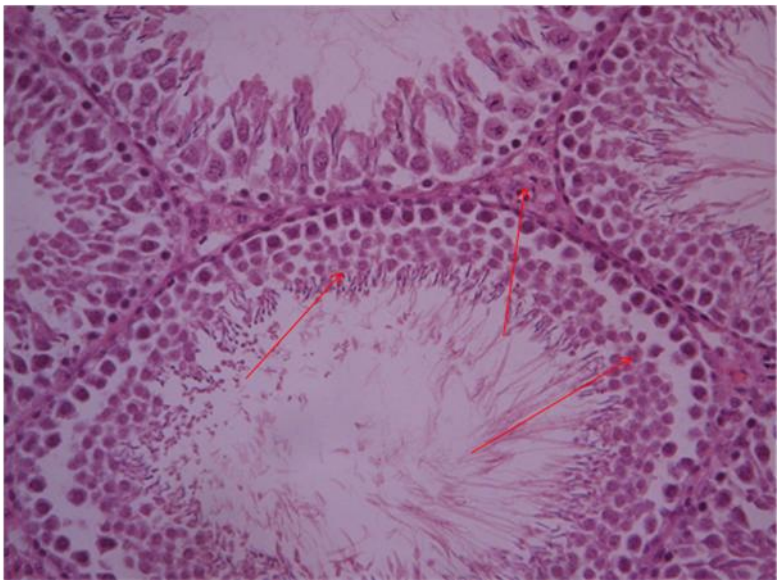

Fig 5: Histoculture of testis treated with 450mg/kg of aqueous extracts of C.articulata, scale bar 100µm. Hyperplasia of leydig cells and increased epithelium and spermatogonia

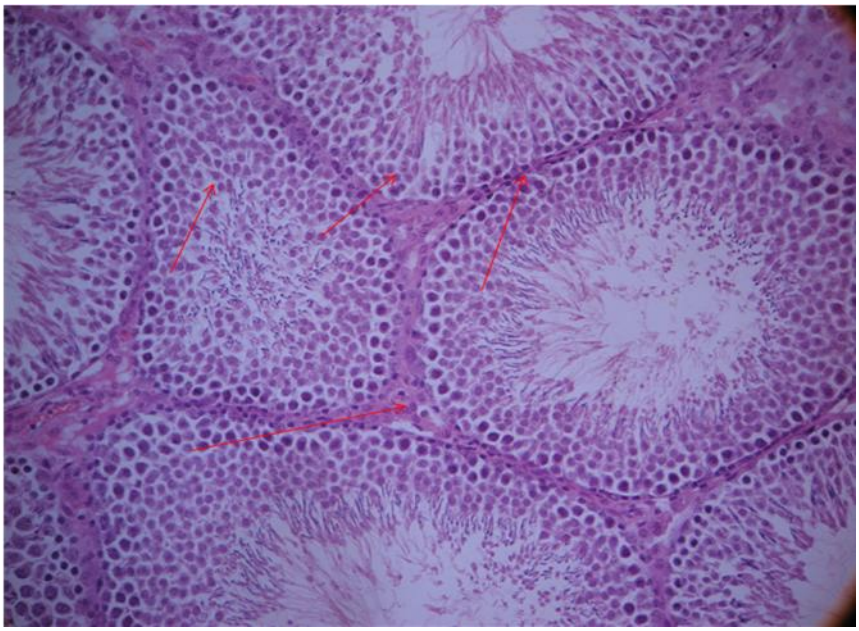

Fig 6: Histoculture of testis treated treated with 450mg/kg of aqueous extract of *M.aethiopicum*, x200, scale bar 100µm. Hyperplasia of leydig cells and increased epithelium and spermatogonia

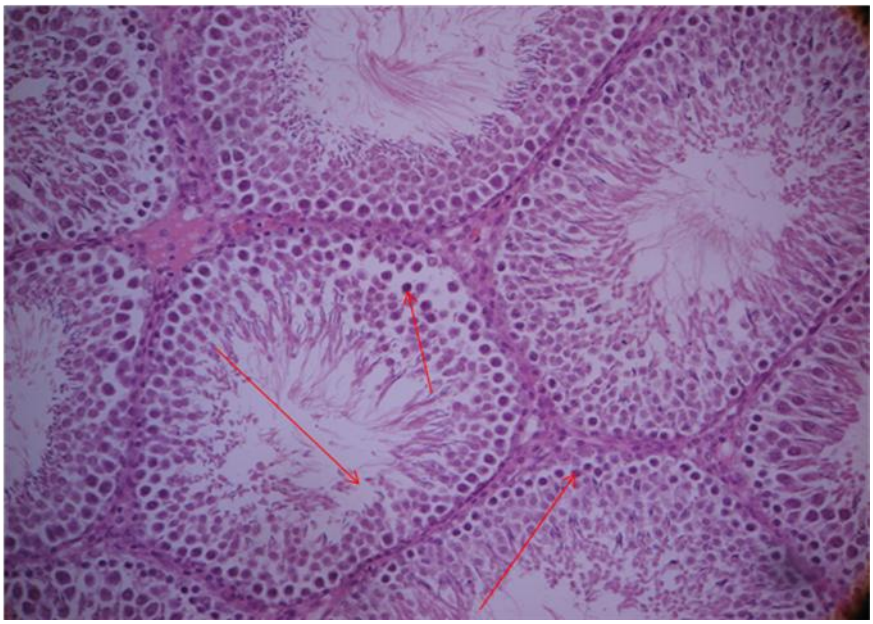

Fig 7: Histoculture of testis treated with 100µg/kg of Positive control (Testosterone), x200, scale bar 100µm. Less number of leydig cells, spermatid and spermatogonia**.**

IV. DISCUSSION

Since time immemorial man has used various parts of plants in the treatment and prevention of many ailments, including sexual impotence (Ayyanar & Ignacimuthu, 2009). Ancient people knew about herbal and animal aphrodisiacs, used in combinations like potions to mystical rites to infertility, to increase sexual performance, desire and pleasure (Malviya *et al*., 2011). The present study revealed that the use of aqueous leaf extracts of *C. articulata* and *M. aethiopicum* has a significant effect on the serum testosterone level and sexual performance in flutamide treated male rats which was a dose-dependent.

Generally, elevated testosterone level enhances the sexual behavior in humans. Sexual desire may be enhanced directly by increasing serum testosterone level or by having testosterone like effect. Luteinizing hormone (LH) and Follicle Stimulating Hormone (FSH) produced by anterior pituitary lobe are necessary for maintaining testosterone levels such that as LH and FSH increases so do the testosterone. Medicinal plants claimed to have aphrodisiac potential apart from being able to increase the concentration of free testosterone should also cause increase in the concentrations of serum LH and FSH. This study showed that *M. aethopicum* had a significant increase ($p<0.01$) in LH serum level and this agrees with the higher testosterone level observed for this plant extract when compared with that of *C. articulata* at the dose of 450mg/Kg. The dose–dependent increase in mounting and intromission frequencies in both aqueous extracts of *C. articulata* and *M. aethiopicum* can be attributed to the elevated serum testosterone levels in higher doses of the extract. Testosterone causes increase in libido which is manifested as increase in mounting and intromission frequencies which were significantly higher for *M. aethiopicum* than for *C. articulata* which correspond with its significant ability to increase serum testosterone level.

The dose-dependent increase in body weight, testicle and heart weight at high extract doses of both extracts shows their anabolic effects. This is attributable to the anabolic effects of raised testosterone levels which causes increase in metabolism, tissue generation and muscle building which results to general increase in body mass index. The results of anabolic effects correspond with those of the raised serum testosterone levels in the high doses of extract-treated groups.

The absence of increase in prolactin levels for treated groups is also in reflected in normal levels of FSH and increase in serum LH and testosterone levels. Normally, prolactin increases the production of breast milk and this suppresses secretion of LH and FSH. However, high levels of prolactin in men may cause hypogonadism, low blood testosterone levels and decrease in sex drive (libido) and sexual function. This agrees with elevated LH and testosterone levels in extract treated groups. Therefore, any plant associated with aphrodisiac tendency should produce reduction in the concentrations of prolactin in males which would enhance the levels of LH and FSH and by extension the testosterone concentration. Interestingly there was no statistically significant change in levels of FSH in both aqueous extracts irrespective of the use of anti-androgen drug (flutamide). Lack of significant effect of aqueous extracts on prolactin (dopamine inhibiting hormone) resulted in non-impairment of sexual activity. Stimulation and inhibition of dopamine receptors is reported to enhance and impair sexual behavior respectively [Dominguez and Hull, 2005]. According to Hull *et al*., (2004), dopamine activity increases in several sex-relevant brain regions before and/or during copulation and plays a key role in the genital reflex, motor pattern of copulation and possibly sexual motivation (Dominguez and Hull, 2005). In addition the extracts had aphrodisiac effects which were reflected as genital sniffing, grooming and chasing of females by males which accounts for the improved sexual desire (Morales, 1995).

In the histoculture of testis of rats treated with both extracts, all stages of spermatogenesis (primary and secondary spermatocytes, spermatogonia, spermatids, spermatozoa, basement membrane and leydig's cells) were more pronounced at the 450 mg/Kg dose when compared with positive and negative controls. In the extract-treated groups the spermatogonia are in their first stage of repetitive and multiplicative cell division, the lumen size is decreased and there is an observable vascularisation while the effect is slightly restrained in the testosterone treated group and is very minor in normal control group. There is hypertrophy of leydig cells and spermatid differentiation in extract-treated groups as compared to testosterone treated group which further support their androgenic activity. The *M. aethiopicum* extract-treated rats showed an increased number of spermatozoa in seminiferous tubules as compared to *C. articulata* and both control groups. This further confirms the increased spermatogenesis, which is highly elaborated by an increase in spermatogenic elements. Spermatogenesis involves a complex interplay between the structural element of testis and the endocrine system (Chauhan and Dixit, 2008). FSH stimulates spermatogenesis and LH stimulates synthesis and release of testosterone. Testosterone cause direct stimulation of spermatogenesis. Our results also show that there is increase in spermatogenesis and increase in testes weight in extracted treated groups as compared to control groups. According to Thakur and Dixit (2006) the androgenic effect of plant extracts could be attributable to the raised serum testosterone levels, which in turn could be due to increasing testosterone secretion, allowing better availability of the hormone to gonads which induces the occurrence of spermatogenesis**.**

The phytochemical screening revealed a number of phytochemicals in both extracts. The saponins present in the plant extracts might have assisted in stimulating an increase in the body natural endogenous testosterone levels by raising the levels of LH. Studies have implicated the saponins components of plants in enhancing aphrodisiac properties due to its androgen increasing property. LH released by pituitary gland helps to maintain testosterone levels; as LH increases, so does the testosterone (Gauthaman et al., 2002; Alevtina and Zerihun, 2009). Saponins also stimulate the leydig cells of the testes to directly increase testosterone production system (Ang *et al*., 2004). Alkaloids were also present in both extracts and according to Dimitris *et al.,* (1997) they increase blood flow in the sexual organs due to vasodilatation thus sustaining male erection leading to enhanced sexual performance. This is a similar mechanism of action to that of sildenafil citrate (Viagra®). This corresponds with erection hemodynamics which involve balance between inflow and outflow of blood within the corpus cavernosum. There is a relaxation of the smooth muscles and arterioles which allows blood supply to flow in the sinusoidal space. The increased flow of blood, compress venules between sinusoids and the tunica albuginea of the corpus cavernosum. The lack of the distension of tunica albuginea results in venous occlusion, which increases the intracavernosal pressure, generating and sustaining a full erection (Zanolari, 2003).

Studies conducted by Ko *et al*., (2004) indicate that flavonoids are phosphodiesterase (PDE) inhibitors. Phosphodiesterase enzyme breaks down cyclic AMP (cAMP) which is activates synthesis of nitric oxide leading to vasodilatation thus increasing blood flow which sustains an erection. In addition to their antioxidant, anti-inflammatory, hepatoprotective, cardio protective, antiulcer, anticancer, antimutagenic, antispasmodic and other effects, flavonoids inhibit xanthine oxidase, protein kinase C and PDE (Rahimi *et al*., 2009; Ko *et al*., 2004).

We have demonstrated that aqueous leaf extracts of *C. articulata* and *M. aethiopicum* have an androgenic activity in flutamide treated male rats. This agrees with reports from Kamatenesi and oryem, (2005), that leaves were the most preferred part to treat ED, constituting 57.6% of herbal remedies in western Uganda. This exceptionally high usage relate to their availability throughout the year. The use of leaf parts as an option of stem and root bark saves these plants from extinction and promotes environmental biodiversity. The 20 minutes methods of boiling used in this study does not seem to affect the active phyto-component of the plant extracts and this agrees with Van Wyk et al., (1997); De Wet et al., (2011); Yirga, 2010 and Tabuti *et al*., (2010).

V. CONCLUSION

The results of this study suggest that the *M. aethiopicum* aqueous extracts was more efficient in increasing serum testosterone, LH, mounting and intromission frequencies level when compared to *C. articulata*. Therefore this study validates the continuous use of this plant preparation in traditional herbal remedies in communities of Uganda in the management of erectile dysfunction. However, isolation and identification of active constituents from these plants may bring a dynamic change in the modern world.

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VII. CONFLICT OF INTEREST

The author's hereby declare no conflict of interest in this work

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