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Evaluation of Aqueous Leaf Extract of *Cissampelos mucronata* on Testicular Function Indices in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author TDO designed the study, wrote the protocol and supervised the work. Author OGA carried out all laboratories work and performed the statistical analysis. Author APO managed the analyses of the study. Author TDO wrote the first draft of the manuscript. Author OGA managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study aimed at investigating the effect of aqueous leaf extract of *Cissampelos mucronata* on testicular function indices in male wistar rats (*Rattus novergicus*).
Methodology: A total of 20 rats, grouped into four, with a group consisting of five rats were used for the study. The groups were a control administered 1.0 mL of distilled water and three other groups that were respectively administered - 1.0 mL of 300, 600 and 1200 mg/kg body weight of the plant extract. The different groups were administered plant extract orally for 14 days, using metal oropharyngeal cannula.

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Results: The results revealed a significant increase (P <0.05) in body weight, testes-body weight ratio, cholesterol and glycogen concentrations at all the doses except at 1200 mg/kg body weight of the animals where decreased glycogen level was observed. There was also significant decrease (P <0.05) in the concentrations of acid phosphatase, alkaline phosphatase and total testicular protein especially in rats administered with 1200 mg/kg body weight of extract while there was no significant change in the activities of gamma glutamyl transferase. Serum testosterone concentration was observed to decrease significantly (P <0.05) only in the rats administered with 300 mg/kg body weight of extract.
Conclusion: The results indicate anti-androgenic and anti-steroidogenic activities of *Cissampelos mucronata* aqueous leaf extract on males.

Keywords: Cissampelos mucronata; testicular function indices; anti-androgenic; anti-steroidogenic.

1. INTRODUCTION

Medicinal plants are the main source of healthcare for most developing countries [1] as a result of their low cost, safety, cultural acceptability and closer to nature properties. The World Health Organization reported that 70 – 80% of the entire world population depends on traditional medicine as their primary healthcare and this is also inclusive of developed countries [2]. There has been an increase in the use of herbs and medicinal plants in developed countries and metropolitan areas of recent contrary to the fact that it was previously associated with rural areas [3].

Plants and plant products have been documented to possess curative properties including anti-sickling, anticancer, antimalarial, anti-diabetic, anti-hypertensive amongst others. Some secondary plant metabolites have been reported to be the probable source of their characteristic “healing virtue”; some secondary plant metabolites that make them highly beneficial to human health include alkaloids, flavonoids, tannins, glycosides, phenols, anthocyanins and several others. Several plants are known to possess the ability to modulate reproductive functions to various degrees in both males and females consequently leading to enhanced fertility or infertility. In males, some plants have been reported to enhance or hinder testicular functions, some of these include; *Chromolaena odorata*, *Tecoma stans*, *Aeglemarmelos* which have been reported to hinder testicular functions [4-6] whereas *Fadogia agrestis*, *Rauvolfia vomitoria*, *Xylopia aethiopica* enhance testicular functions [1,7,8].

Infertility is defined as the inability of a couple to have a child or carry a pregnancy till full term after one year of regular sexual intercourse (three to four times a week) without contraceptives [9-11]. 20 – 30% of infertility cases have been attributed to male-related issues [12,13]. This includes problems with the testes since the testis is mainly involved in the production of androgens and spermatogenesis. Although several approaches for male infertility treatment have been investigated for a long period of time such as hormonal, surgical and chemical approaches; however, the high cost of orthodox medicine and unavailability of modern infrastructures exempt a large percentage of the population of developing countries from modern healthcare. Consequently, alternative medicine still remains a cheap and reliable source of healthcare for most developing countries.

Cissampelos mucronata (A. Rich) belongs to the family *Menispermaceae,* it is a perennial climbing plant with annual stem that scramble over the ground or twine into the surrounding vegetation for support. *C. mucronata* is widely distributed all through tropical and sub-tropical Africa, America and Asia [14,15]. The common English names are velvet leaf, ivy vine and heart-leaved vine. In Nigeria, the aqueous leaf extract of *C. mucronata*is commonly used in the treatment of female infertility issues especially in preventing miscarriages. Different parts of *C. mucronata* A. Rich, are commonly used in the tropics and subtropics to treat infirmities such as malaria, venereal diseases, gastro-intestinal complaints and menstrual problems [15]. Fractions of methanolic leaf extract of *C. mucronata* have been documented to demonstrate significant protection against ulcer induced indomethacin in rats [16]. The effect of the methanolic extract of the root of *C.mucronata* on embryofoetal development of rats was examined and the results showed that administration of the extract from gestation day 6 to 20 had no significant effect on the number of implantation sites while resorption sites were significantly high in a dose dependent manner [17]. The effect of orally

administered aqueous extract of *C. mucronata* on the kidneys of adult female rats has also been evaluated with results revealing ruptured blood vessels with distorted cytoplasm compared with the control, exposing a deleterious effect on the kidneys of adult female wistar rats [18]. However, there is paucity of data on the effect of *C. mucronata* on the reproductive parameters of male wistar rats. This research was therefore aimed at evaluating the effect of aqueous leaf extract of *C. mucronata* on some testicular function indices of male wistar rats.

2. MATERIALS AND METHODS

2.1 Plant Extract

The plant samples were obtained from Landmark University, Omu-Aran, Kwara State, Nigeria, and authenticated at the Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, Ilorin, Kwara state. A voucher specimen (UIH001/442) was deposited at the herbarium.

Prior to use, the sampled leaves of *C. mucronata* were first rinsed in distilled water to remove dirt and air dried at room temperature. The dried leaves were then pulverized, using an electric blender, out of which 188 g was extracted in 1000 mL distilled water for 48 h at room temperature. The resulting extract from the distillation was filtered with whatman No. 1 filter paper and the filtrate concentrated with a rotary evaporator to give a yield of 35 g of residue, brownish-black slurry. This was later reconstituted in distilled water to give the required doses of 300, 600 and 1200 mg/kg body weight before oral administration to experimental animals.

2.2 Experimental Design

Male albino rats (*Rattus norvegicus*) of wistar strain weighing 140-200 g were obtained from the Animal Holding Unit of the Department of Biochemistry, Faculty of Science, University of Ilorin, Ilorin, Kwara state. Nigeria. Before the commencement of the experiment, the rats were acclimatized to the laboratory conditions (28–30°C; 12 h natural light and 12 h dark; Humidity: 50 – 55%) for two weeks with free access to rat chow and water *ad libitum*. The rats were used according to the Guide for the Care and Use of Laboratory Animals [19].

For the setup, the 20 rats were completely randomized into four groups of five rats each as follows:

- Group A: Control group administered 1ml of distilled water
- Group B: administered 300 mg/kg body weight of the extract
- Group C: administered 600 mg/kg body weight of the extract
- Group D: administered 1200 mg/kg body weight of the extract

The extracts (300, 600 and 1200 mg/kg body weight) were reconstituted in 1 mL each of distilled water and orally administered to the animals once daily for 14 days between 09:00 – 09:45 hours. The rats were sacrificed 24 hours after their last doses. The weight of the animals was taken and recorded once in two days. The oral administration of the extract on the rats was carried out by force-feeding, using cannula attached to graduated syringe.

2.3 Experimental Assay

The assay kits used for the determination of Cholesterol, Gamma-glutamyl transferase, and serum testosterone were products of Agappe Diagnostics Ltd, Switzerland. All other reagents used were of analytical grade and prepared in all glassware.

For serum preparation and testicular supernatant, the procedure described by [20] was employed. Under ether anesthesia, the neck areas were quickly cleared of fur and skin to expose the jugular veins. The jugular veins was slightly displaced from the neck region (to prevent contamination of the blood with interstitial fluid) and then cut with a sharp sterile blade. The rats were made to bleed into clean, dry corked centrifuge tubes which were left at room temperature for 10 min. After that, the tubes were centrifuged at 224 × *g* for 15 min using Laboratory Centrifuge (LW scientific centrifuge; Model: C5). The serum was thereafter collected using Pasteur pipettes into clean, dry, sample bottles and then used within 12 hours of preparation for the determination of the concentrations of the hormone.

Also, 24 h after the last day's administration (15^{th} day), each animal was weighed and put under diethyl ether anesthesia. The rats were thereafter sacrificed and the testes carefully removed, and placed in cold 0.25 M sucrose solution to maintain the integrity of the tissue of the testes. The testes were thereafter blotted with tissue paper and weighed to allow for the calculation of the testes-body weight ratio. The testes were homogenized in ice-cold 0.25 M sucrose solution

(1:5 w/v) using a Teflon homogenizer. The homogenates were centrifuged at 1340 x g for 15 minutes using a refrigerated centrifuge (Anke centrifuge; Model: TDL-5000B).The supernatant was kept frozen overnight before being subject to various biochemical assays.

In the determination of testicular parameters, the testes: body weight ratio was calculated as described by [21]. The concentration of testosterone was determined in accordance to the procedure outlined by the assay kit manufacturer's protocol; the analyzer was calibrated for use with animal sera instead of human sera. The concentrations of testicular total protein, total cholesterol and glycogen were determined using the procedures described by [22-24], respectively. The activities of GGT, ACP and ALP were also determined using the assay techniques described by [25-27] respectively.

2.4 Ethical Consideration and Statistical Analysis

For ethical consideration, before commencement of the study, the research proposal was submitted to the relevant research committee at the Landmark University, Omu Aran, Nigeria for approval to carry out the study. All ethical guidelines as required by the university were strictly adhered to.

Results were expressed as mean ± standard deviation. Differences between normal and treated groups were the criteria for the pharmacological activities. Statistical analysis of results was carried out using the SPSS statistical software. Comparison of means were done using the One-Way Analysis of Variance (ANOVA). Differences with values of $P < 0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

The qualitative phytochemical analysis of the aqueous leaf extract of *C. mucronata* is shown in Table 1. As shown in the table, the plant extract was observed to contain high concentration of saponins and tannins while the flavonoids and steroids were found in moderately high concentration and trace amount, respectively.

As shown in Table 2, when compared with the control groups, the body weights of all the experimental animals were observed to increase significantly across all the groups from day 1 to day 14. A similar trend was also observed with the testes-body weight ratio.

When investigating the effects of the extract on testicular cholesterol and serum testosterone concentration, the observation in the study revealed a significant increase in testicular cholesterol concentration than that of the control. The increase across all the groups was in a dose dependent manner from 3.93 to 4.34 mmol/L except in the case of Group C, which was 3.27 mmol/L. The serum testosterone was observed to display a reduction in concentration across all groups compared with the control, although this reduction was only observed to be significant in Group B (Table 3).

With respect to testicular protein and glycogen concentration, significant decreases in the concentration of testicular protein were only observed in Groups B and D. Generally, the lowest value testicular protein concentration of 6.35 mg/mL was observed in Group D while the highest value of testicular protein concentration 10.76 mg/mL was observed in Group C. In the case of testicular glycogen concentration, when compared with the control, a significant increase in Groups B and C and a decrease in Group D was observed (Table 4).

Three concentrations of the extract (300 mg, 600 mg and 1200 mg per kg-body weight) were used for the study. The choice of these concentrations was deliberate. An ethnobotanical survey of the plant carried out prior to the study revealed that the recommended safety oral dose of the extract was observed to be 600 mg/kg. The choice of the higher and lower concentrations was to see if such doses have similar or divergent effect on the indices investigated in the study.

Table 1. The phytochemical composition of the aqueous leaf extract of *C. mucronata*

Phytochemical	Ethanol leaf extract
Alkaloids	-
Flavonoids	++
Tannins	+++
Saponins	+++
Cardiac glycosides	-
Steroids	+
Anthraquinones	-
Terpenoids	-

+++ = Present in high amount, ++ = present in moderately high amount, + = present in trace amount, - = absent

The result of the effect of aqueous leaf extract of *C. mucronata* on the activity of enzymes is displayed in Table 4. As shown in the table, Gamma glutamyl transferase (GGT) was found to show increase in concentration within the

treatment groups in a dose dependent manner with Group B having the lowest concentration of 170.46 U/L while Group D had the highest concentration of 193.85 U/L. These increases were however not found to be significant. The concentration of Acid Phosphatase (ACP) displayed significant increases across all the treatment groups. The lowest concentration 3281.52 nM/min/ml was observed in Group D while the highest concentration was observed in Group C. Although there was a decrease in Alkaline Phosphatase (ALP) concentration across all the treatment groups, this decrease was only observed to be significant in Group D (Table 5).

The results of this study show that the oral administration of aqueous extract of *C. mucronata* brought about a significant increase in the body weight of the animals, the testes-body weight ratio was also observed to increase significantly. The increase in the testes-body weight ratio observed following the administration of the plant extract may be due to improved secretory activity of the testes which may be explained by the rise in the testicular cholesterol and glycogen concentrations. This observation corroborates with the work of [28,29] following the administration of aqueous extract of *Massularia acuminata* (G. Don) stem, *Zingiber officinale* and *Pentadiplandra brazzeana* in male rats respectively.

Table 2. Effect of aqueous leaf extract of *C. mucronata* on body weight (g) and testes-body weight ratio (%)

Groups	Body weight (g)(n=5)			Testes-body weight ratio (%)
	Day 1	Day 7	Day 14	
Group A	193.93 (±15.67)	196.32 (±15.20)	202.88 (±17.07)*	1.28 (±0.19)
Group B	159.52 (±8.81)	175.23 (±8.77)*	181.91 (±12.86)*	1.66 (±0.22)*
Group C	148.31 (±2.10)	165.46 (±2.54)*	177.62 (±3.32)*	1.67 (±0.07)*
Group D	139.94 (±6.40)	155.40 (±8.33)*	162.31 (±15.24)*	1.72 (±0.09)*

*n = number of rats; values are mean ± SD for each group of five rats. * represent means significantly different at P<0.05. Group, A, B, C and D represent administered with control (administered distilled water), 300 mg/kg, 600 mg/kg and 1200 mg/kg body weight of the extracts, respectively*

Table 3. Effect of aqueous leaf extract of *C. mucronata* on testicular cholesterol (mmol/L) and serum testosterone concentration (ng/ml)

Groups	Cholesterol concentration (mmol/L)	Serum testosterone concentration (ng/ml)
Group A	3.60 (±0.24)	1.70 (±0.71)
Group B	3.93 (±0.77)*	0.50 (±0.42)*
Group C	3.27 (±0.35)*	1.50 (±0.28)
Group D	4.34 (±0.31)*	1.50 (±0.57)

*Values are mean ± SD for each group of five rats. *Means significantly different at P<0.05. Group, A, B, C and D represent administered with control (administered distilled water), 300 mg/kg, 600 mg/kg and 1200 mg/kg body weight of the extracts, respectively.*

Table 4. Effect of aqueous leaf extract of *C. mucronata* on total protein (mg/ml) and glycogen concentration (mg/ml)

Groups (mg/kg b. wt)	Testicular Protein (mg/ml)	Testicular Glycogen concentration (mg/ml)
Group A	10.46 (±3.24)	3.46 (±2.02)
Group B	7.65 (±1.43)*	5.86 (±1.96)*
Group C	10.76 (±2.33)	5.56 (±2.77)*
Group D	6.35 (±0.80)*	2.31 (±0.43)

*Values are mean ± SD for each group of five rats. *Means significantly different at P<0.05. Group, A, B, C and D represent administered with control (administered distilled water), 300 mg/kg, 600 mg/kg and 1200 mg/kg body weight of the extracts, respectively*

Table 5. Effect of aqueous leaf extract of *C. mucronata* on the activity of enzymes – Gamma glutamyl transferase GGT (U/L), Acid Phosphatase ACP (nM/min/ml), Alkaline Phosphatase ALP (nM/min/ml)

Groups (mg/kg b. wt)	GGT (U/L)	ACP (nM/min/ml)	ALP (nM/min/ml)
Group A	167.14 (±27.64)	4090.76 (±910.16)	3627.53 (±1132.42)
Group B	170.46 (±38.06)	3332.00 (±286.03)*	2848.49 (±294.99)
Group C	187.50 (±29.99)	3894.02 (±223.82)*	2955.81 (±695.47)
Group D	193.85 (±52.15)	3281.52 (±595.34)*	2430.30 (±287.49)*

*Values are mean ± SD for each group of five rats. *Means significantly different at P<0.05. Group, A, B, C and D represent administered with control (administered distilled water), 300 mg/kg, 600 mg/kg and 1200 mg/kg body weight of the extracts, respectively*

The significant decline in testicular protein at 1200 mg/kg body weight of the plant may indicate the absence of spermatozoa in the lumen since the luminal fluid of the epididymis contains a number of proteins [6]. Testicular proteins are required for sperm maturation and spermatogenesis [30], therefore the significant decline in testicular protein observed in this study may imply animpairment in sperm maturation indicating the anti-androgenic nature of the plant extract, this may be further explained by the significant decrease in testosterone concentration observed in the present study which is a marker of androgenicity [31]. Similar result was observed with *Nyctanthes arbor-tritis* stem bark [32] and aqueous root extract of *Lecaniodiscus cupanioides* [33].

Following the administration of *Cissampelos mucronata* aqueous leaf extract (CMALE) at 300 and 600 mg/kg body weight, the significant increase in testicular glycogen may be ascribed to the stimulation of phosphorylase and an inhibitory action of glycolysis [34] due to the presence of cholesterol which is an alternative source of energy. However, the reduction in glycogen concentration following the administration of the extract at 1200 mg/kg body weight probably suggests that the extract has reached a level to start exerting toxic effect on the testes and this may be attributed to its saponins content as reported by Gupta and colleagues [35] where saponin content of the bark of *Albizia lebbeck* (L.) Benth (Mimosoideae) decreased glycogen and inhibited glycolysis during spermatogenesis.

The significant increase in testicular cholesterol concentration in the extract-treated rats reflects the reduced conversion of cholesterol to androgens or the arrest of steroidogenesis of testosterone [36], leading to the accumulation of cholesterol in the testis. This may be the explanation for the low testosterone level observed in *C. mucronata* treated rats. A similar result was reported by Sonalika and co-workers [37] with the aqueous extract of *Calendula officinalis*on the reproductive function of adult male rats. This may be an indication of the extract's ability in impairing the steroidogenesis process.

The decrease in the testicular alkaline phosphatase activity at 1200 mg/kg body weight may imply reduction in the recruitment of carbohydrates, lipids and other metabolites necessary for steroidogenesis and for use by other accessory sex structures. This may seem to explain the decrease in testosterone concentration observed in this study, showing the ability of the extract to inhibit intracellular and intercellular transport of metabolites required for steroidogenesis. This correlates with the report of Nurudeen and Ajiboye [33] where *Lucaniodiscus cupanioides* restores sexually impaired male rats by significantly attenuating paroxetine-mediated decrease in alkaline phosphatase.

The observed decrease in acid phosphatase in this study may be a result of impaired steroidogenesis and anti-anabolic effect of the plant extract; this is because improvement in steroidogenesis has been associated with increase in acid phosphatase [5]; increased level of acid phosphatase has also been linked to anabolic effect of the plant [38].

The non-significant increase in the activity of testicular GGT may indicate that the plant extract had no significant influence on the sertoli cells function, this may not be favorable for proper functioning of the sertoli cells and consequently spermatogenesis by hindering its supportive role and the carriage of spermatozoa into the rete testes [28].

The decrease in testosterone concentration in this study may be due to decreased number of Leydig cells, this is line with the report of Marthur and colleagues [34], where the number and nuclei diameter of Leydig cells were reduced in *Tecomastans* treated rats leading to a depletion of the testosterone levels. This may also be attributed to the high saponin content of the extract as supported by the report of Gupta *et al.* [35], where saponin content of the bark of *Albizia lebbeck* (L.) Benth (Mimosoideae) decreased Leydig cell nuclear area and number of mature Leydig cells. The decreased testosterone concentration may also be explained by the inhibition of steroidogenesis as a result of testicular cholesterol accumulation observed in this study. Testosterone is known as a very important marker of androgenicity, therefore this result may imply the anti-androgenic nature of the plant extract on male wistar rats.

4. CONCLUSION

The results of this study indicate anti-androgenic and anti-steroidogenic effect of CMALE on male rats with the 1200 mg/kg body weight of the extract exhibiting the highest anti-androgenic and anti-steroidogenic activities. This implies that the use of *C. mucronata*in the management of male sexual dysfunction and infertility just as it had previously been used for female infertility issues is not supported. Therefore, the oral administration of CMALE may help to regulate fertility in males due to its ability to arrest steroidogenesis and interfere with levels of androgens and other testicular function indices; this could result in impaired spermatogenesis in the testes hindering reproductive activity in male rats.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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