

Original Research Article

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

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 norvegicus*).

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.

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 plants metabolites have been reported to be the probable source of their characteristic "healing virtue"; some secondary plant metabolites that make them highly beneficial to the 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*,

33 *Tecoma stans*, *Aegle marmelos* which have been reported to hinder testicular functions
34 [4][5][6] whereas *Fadogia agrestis*, *Rauvolfia vomitoria*, *Xylopi aethiopica* enhance
35 testicular functions [7][1][8].

36 Infertility is defined as the inability of a couple to have a child or carry a pregnancy till full
37 term after one year of regular sexual intercourse (three to four times a week) without
38 contraceptives [9][10][11]. 20 – 30% of infertility cases have been attributed to male-related
39 issues [12][13]. This includes problems with the testes since the testis is mainly involved in
40 the production of androgens and spermatogenesis. Although several approaches for male
41 infertility treatment have been investigated for a long period of time such as hormonal,
42 surgical and chemical approaches; however, the high cost of orthodox medicine and
43 unavailability of modern infrastructures exempt a large percentage of the population of
44 developing countries from modern healthcare. Consequently, alternative medicine remains a
45 cheap and reliable source of healthcare for most developing countries.

46 *Cissampelos mucronata* (A. Rich) belongs to the family *Menispermaceae*, it is a perennial
47 climbing plant with annual stem that scramble over the ground or twine into the surrounding
48 vegetation for support. *Cissampelos mucronata* is widely distributed all through tropical and
49 sub-tropical Africa, America and Asia [14][15]. The common English names are velvet leaf,
50 ivy vine and heart-leaved vine. In Nigeria, the aqueous leaf extract of *Cissampelos*
51 *mucronata* is commonly used in the treatment of female infertility issues especially in
52 preventing miscarriages. Different parts of *Cissampelos mucronata* A. Rich are commonly
53 used in the tropics and subtropics to treat infirmities such as malaria, venereal diseases,
54 gastro-intestinal complaints and menstrual problems, [15]. Fractions of methanolic leaf
55 extract of *Cissampelos mucronata* have been documented to demonstrate significant
56 protection against ulcer induced indomethacin in rats [16]. The effect of the methanolic
57 extract of the root of *Cissampelos mucronata* on embryofetal development of rats was
58 examined and the results showed that administration of the extract from gestation day 6 to
59 20 had no significant effect on the number of implantation sites while resorption sites were
60 significantly high in a dose dependent manner [17]. The effect of orally administered
61 aqueous extract of *Cissampelos mucronata* on the kidneys of adult female rats has also
62 been evaluated with results revealing ruptured blood vessels with distorted cytoplasm
63 compared with the control, exposing a deleterious effect on the kidneys of adult female
64 wistar rats [18]. However, there is paucity of data on the effect of *Cissampelos mucronata*
65 on the reproductive parameters of male wistar rats. This research was therefore aimed at
66 evaluating the effect of aqueous leaf extract of *Cissampelos mucronata* on some testicular
67 function indices of male wistar rats.

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69

70 2. MATERIAL AND METHODS

71

72 2.1 Plant extract

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

77 Prior to use, the sampled leaves of *Cissampelos mucronata* were first rinsed in distilled
78 water to remove dirt and air dried at room temperature. The dried leaves were then
79 pulverized, using an electric blender, out of which 188g was extracted in 1000 mL distilled
80 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 were give 1ml of distilled water
- roup B: administered 300 mg/kg body weight of the extract
- Group C: administered 600 mg/kg body weight of the extract
- Group D: adminisitered 1200 mg/kg body weight of the extract

The extracts (300, 600 and 1200 mg/kg body weight) were reconstituted in 1mL each of distilled water and orally administered to the animals once daily for 14 days between 09:00 – 09:45 hours using plastic syringes attached to a metal oropharyngeal cannula. The rats were sacrificed 24 hours after their last doses. The weight of the animals was taken and recorded once in two days.

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 $224 \times g$ 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 (15th 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 was thereafter blotted with tissue paper and weighed to allow for the calculation of the testes-body weight ratio. The testes was homogenized in ice-cold 0.25 M sucrose solution (1:5 w/v) using a Teflon homogenizer. The homogenates were centrifuged at $1340 \times g$ 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], [23] and [24] respectively. The activities of GGT, ACP and ALP were also determined using the assay techniques described by [25], [26] and [27] respectively.

2.4 Statistical Analysis

Results were expressed as mean $\pm$ 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

As shown in Table 1, 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.

Table 1: Effect of aqueous leaf extract of *Cissampelos 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 $\pm$ 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

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 in 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 2).

Table 2: Effect of aqueous leaf extract of *Cissampelos 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)

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

163 With respect to testicular protein and glycogen concentration, significant decreases in the
 164 concentration of testicular protein were only observed in Groups B and D. Generally, the
 165 lowest value testicular protein concentration of 6.35 mg/mL was observed in Group D while
 166 the highest value of testicular protein concentration 10.76 mg/mL was observed in Group C.
 167 In the case of testicular glycogen concentration, when compared with the control, a
 168 significant increase in Groups B and C and a decrease in Group D was observed (Table 3).

169 **Table 3: Effect of aqueous leaf extract of *Cissampelos mucronata* on total protein**
 170 **(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)

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

174 The result of the effect of aqueous leaf extract of *Cissampelos mucronata* on the activity of
 175 enzymes is displayed in Table 4. As shown in the table, Gamma glutamyl transferase (GGT)
 176 was found to show increase in concentration within the treatment groups in a dose
 177 dependent manner with Group B having the lowest concentration of 170.46 U/L while Group
 178 D had the highest concentration of 193.85 U/L. These increases were however not found to
 179 be significant. The concentration of Acid Phosphatase (ACP) displayed significant increases
 180 across all the treatment groups. The lowest concentration 3281.52 nM/min/ml was observed
 181 in Group D while the highest concentration was observed in Group C. Although there was a
 182 decrease in Alkaline Phosphatase (ALP) concentration across all the treatment groups, this
 183 decrease only observed to be significant in Group D (Table 4).

184 **Table 4: Effect of aqueous leaf extract of *Cissampelos mucronata* on the activity of**
 185 **enzymes – Gamma glutamyl transferase GGT (U/L), Acid Phosphatase ACP**
 186 **(nM/min/ml), Alkaline Phosphatase ALP (nM/min/ml)**
 187

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

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

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

209 Following the administration of aqueous extract of *Cissampelos mucronata* leaves at 300
210 and 600 mg/kg body weight, the significant increase in testicular glycogen may be ascribed
211 to the stimulation of phosphorylase and an inhibitory action of glycolysis [34] due to the
212 presence of cholesterol which is an alternative source of energy. However, the reduction in
213 glycogen concentration following the administration of the extract at 1200 mg/kg body weight
214 probably suggests that the extract has reached a level to start exerting toxic effect on the
215 testes and this may be attributed to its saponins content as reported by Gupta and
216 colleagues [35] where saponin content of the bark of *Albizia lebbek* (L.) Benth
217 (Mimosoideae) decreased glycogen and inhibited glycolysis during spermatogenesis.
218 The significant increase in testicular cholesterol concentration in the extract-treated rats
219 reflects the reduced conversion of cholesterol to androgens or the arrest of steroidogenesis
220 of testosterone [36], leading to the accumulation of cholesterol in the testis. This may be the
221 explanation for the low testosterone level observed in *Cissampelos mucronata* treated rats.
222 A similar result was reported by Sonalika and co-workers [37] with the aqueous extract of
223 *Calendula officinalis* on the reproductive function of adult male rats. This may be an
224 indication of the extract ability in impairing of steroidogenesis process.

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

233 The observed decrease in acid phosphatase in this study may be a result of impaired
234 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 *Tecoma stans* 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 lebbbeck* (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 *Cissampelos mucronata* aqueous leaf extract on male rats with the 1200mg/kg body weight of the extract exhibiting the highest anti-androgenic and anti-steroidogenic activities. This implies that the use of *Cissampelos 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 aqueous leaf extract of *Cissampelos mucronata* 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.

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