Journal of Biological Sciences and Bioconservation
Volume 6, Number 2, 2014
ISSN: 2277-0143

TESTICULAR AND ANDROLOGICAL EFFECTS OF THE METHANOL EXTRACT OF THE ROOT OF *CISSAMPELOS MUCRONATA* (A. RICH) IN RATS

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ABSTRACT

This study was designed to determine the potential toxicological effects of the methanol root extract of *Cissampelos mucronata* (A. Rich) on testicular tissue and some andrological parameters in the male rats. A total of 20 male rats weighing 200-253 g were used for the study which was randomly divided into four groups of 5 rats per dosage group (I-IV). Group I served as the control group and were administered normal saline equivalent to the volume administered to the highest dosed experimental rats. Rats in Groups II, III and IV were administered with 100 $mgkg^{-1}$, 200 $mgkg^{-1}$ and 300$mgkg^{-1}$ doses of the extract respectively for 28 days. At the end of the experimental period the rats were anaesthetized, sacrificed and orchidectomy performed to obtain testicular tissues for semen and histopathological analysis while blood obtained was analysed to determine the serum levels of Testosterone, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH). Administration of 100, 200 and 300 $mgkg^{-1}$ of the extract for 28 days caused a significant ($p<0.01$) decrease in both body and testicular weights in a dose dependent manner. There was a decrease in sperm count, motility and the percentage of total viable sperm and an increase in the percentages of non motile and abnormal sperm cells that was not biologically significant and dose dependent. Hormonal analysis also revealed that the extract elicited a decrease in testosterone levels at 200 and 300 $mgkg^{-1}$ all the changes were not biologically significant while LH and FSH levels were not affected. Histopathological assessment of the testicular tissue revealed paucity in the number of spermatogenic cells, atrophy of Leydig cells and moderate basement membrane thickening. The study suggests that prolonged and constant use of the extract has testicular toxicity potentials but further studies to assess if the damages caused are reversible or not is suggested.

Keywords: Paucity, Atrophy, Leydig Cells and Basement Membrane, LH and FSH.

INTRODUCTION

The biggest challenge faced in many African or developing countries is that of health care as such many have resorted to the healing properties of medicinal plants and herbs, this had led to an increasing interest on traditional medicine

by many researchers in an attempt to discover more potential drugs to combat emerging new disease or diseases that developed resistances to conventional drugs. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Farnsworth, 1989; Eisner, 1990). The plant *Cissampelos mucronata* (A. Rich) belongs to the family *Menispermaceae* and is used worldwide in traditional medicine to treat varieties of ailments and conditions. Its use as an emmenagogue and diuretic have been reported (Burkill, 1997). The plant is also used for the treatment of abdominal pains, swollen stomach and gastro-intestinal upset (Gelfand *et al.*, 1985; Van Wyk and Gericke, 2000). It's hepatotoxic, Nephrotoxic effect and its ability to decrease foetal weight, placental weight, crown rump length and cause resorptions in pregnancy in a dose dependent manner has also been reported (Garba *et al.*, 2014a,b). Its indigenous names in Nigeria include *Jibdar Kasa* or *Damarji* (Hausa) and *abakenwo* in Igbo, *Jokoje* (Yoruba), *Barwada* (Kanuri), *Magirahi* (Fulfulde), *Zagaduwa* (Marghi) and *Kwahara* or *Kwahirka* (Babur/Bura). Despite the widespread use of *Cissampelos mucronata* in traditional medicine for the treatment of various ailments, its testicular and andrological effects has not been elaborated thus, it is imperative to evaluate the possible effects of *Cissampelos mucronata* on testicular and andrological parameters using adult Wistar rats as the model.

MATERIALS AND METHODS

Collection and Identification of Plant Materials: This study was conducted in the Department of Human Anatomy, University of Maiduguri. The plant was collected around Giwa Military Barracks in Maiduguri metropolis latitude 11° 50' 42" North and longitude 13° 9' 36" East and identified/authenticated by a botanist with a specimen voucher (CM.01) prepared and deposited at the herbarium of the Department of Veterinary Physiology and Pharmacology, University of Maiduguri, Borno state. The collection, identification and storage of the plant material were carried out according to World Health Organization's standards (WHO, 1993; 1998). The root was then sun-dried, pulverised into powdered form using a pestle and mortar and then stored in cellophane bags at room temperature.

Extraction Procedures: A total of one hundred grams (100g) of the pulverised root was subjected to exhaustive soxhlet extraction in methanol (500ml) for 72 hrs at 60°C. The extract obtained was then concentrated in a water bath until a constant dark sticky residue was obtained (11.34g w/w). The extract was

further oven dried and maintained in a desiccator until a constant weight was obtained and then stored in a stoppered container in refrigerator at - 4°C. Stock solution was prepared by dissolving 2g of the extract in 50ml distilled water in the presence of 1 drop (0.05ml) of dimethylsulfoxide (DMSO).

Animals and Husbandry: This study was carried out in the Departments of Human Anatomy, University of Maiduguri, Nigeria. Male Wister strain rats were purchased from the animal house of the Department of Pharmacology and Pharmaceutical Sciences, University of Jos, Plateau State, Nigeria. Following an acclimatization period of 2 weeks, the rats were individually identified by colour tattoo and weighed. The rats were kept in plastic cages at room temperature with a 12 h light/dark cycle. They had access to standard laboratory diet (Pelletised growers Feed by Grand Cereals and oil Mills Limited, Jos) and drinking water *ad libitum*. The rats were cared for according to the Guiding Principles for the Care and Use of Animals based on the Helsinki Declaration as amended by World Medical Assembly; Venice, Italy (Helsinki Declaration, 1996). Prior ethical approval was obtained from the ethical committee on the use of animals of the College of Medical Sciences University of Maiduguri, Nigeria.

Experimental Protocol

A total of 20 male rats weighing 200-253 g were used for this study according to standard protocols (U.S.EPA., 2000). The rats were randomly divided into four groups of 5 rats per dosage group (I-IV). Group I served as the control group and were administered normal saline equivalent to the volume administered to the highest dosed experimental rats. Rats in Groups II, III and IV were administered orally with 100 $mgkg^{-1}$, 200 $mgkg^{-1}$ and 300 $mgkg^{-1}$ doses of the extract respectively for 28 days. Dosages were calculated based on an initial acute toxicity study that gave an LD_{50} of above 2000mg/kg/oral (Garba *et al.,* 2014a).

Sample collection: The rats were anaesthetized using ether and afterward sacrificed by cervical dislocation. Orchidectomy was performed by open castration method. A midline or pre-scrotal incision was made and the testicles were milked out of the incision site. The testicles were exposed by incising the tunica vaginalis. The spermatic cord was exposed, ligated and incised. Semen samples were thereafter collected from the cauda epididymis and the samples were analyzed immediately after collection.

Sperm count: Sperm count was performed as reported earlier by Narayana *et al.,* 2005 with minor modifications. Briefly, cauda, caput and corpus epididymis were carefully separated from the testis and minced in 2 ml of normal saline followed by filtration through a nylon mesh. The suspension was then stained with 2% eosin in normal saline and sperm heads were counted using a Neubauer haemocytometer chamber (WHO, 1987). Counts for the sperm head in eight haemocytometer chambers (except the central erythrocyte chamber) were averaged and expressed as the number of sperm per cauda, caput and corpus epididymis.

Sperm Morphology: A drop of stained sperm suspension (which was prepared for sperm count) was smeared on a glass slide, air-dried and visualized microscopically at a magnification of x40. From each rat, 200 sperms were screened and the percentages of total abnormalities of heads (such as microcephalus, detached head, flattened head, doubled head and bent neck) and/or tails (such as coiled tail, bent tail and doubled tail) were determined (Narayana *et al.,* 2005; WHO, 1999).

Sperm motility: Assessment of sperm motility was done according to WHO protocol i.e. 10 µl of the sperm suspension was placed on a microscopic slide and coversliped. A minimum of five microscopic fields were assessed to evaluate sperm motility on at least 200 sperm for each animal WHO, 1999). The percentage of sperm motility was analyzed for the following motion parameters: percentage of progressively motile sperm (PMS), nonprogressively motile sperm (NPMS) and nonmotile sperm (NMS).

Hormonal Assay: levels of Testosterone, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) were determined in serum of the male rats using Randox Laboratory kits at the Department of Chemical Pathology, University of Maiduguri Teaching Hospital, Maiduguri.

Histopathological analysis: Testicular tissue obtained were carefully dissected out, weighed, fixed in Bouins fluid, embedded in paraffin and sectioned at 5 µm. Sections were stained with Haematoxylin and Eosin and mounted in Canada balsam. Light microscopic examination of the sections was then carried out.

Statistical Analysis: Data obtained from this study were expressed as the mean value ± standard error of mean. The data were analysed using one way analysis of

variance (ANOVA) and differences between means of control and treated groups were determined using Statistical Package for Social Scientist (SPSS 11.0). p values less than 0.05 or 0.01 were considered statistically significant.

RESULTS

Effect of the Extract on Mean Body and Testicular Weights of Male Rats

The effects of the administration of the methanolic extract of the root of *Cissampelos mucronata* on mean body and testicular weights are presented in Table 1. The male rats in the control group administered only equivalent volume of vehicle (normal saline) had a significant ($p<0.001$) increase in their body weights while male rats that were administered 100, 200 and 300 $mgkg^{-1}$ of the extract significantly ($p<0.001$) lost weight. Testicular weights were also significantly ($p<0.01$) decreased in a dose dependent manner.

Effect of the Extract on Mean Sperm Parameters and Hormone Levels in Male Rats

The effects of the administration of the methanolic extract of the root of *Cissampelos mucronata* for 28 days in male rats following the administration of 100, 200 and 300 $mgkg^{-1}$ of the extract were observed to have caused a decrease in sperm count, motility and the percentage of total viable sperm and an increase in the percentages of non motile and abnormal sperm cells that was not biologically significant and dose dependent (Table 2). Hormonal analysis also revealed that the extract elicited a decrease in testosterone levels at 200 and 300 $mgkg^{-1}$ all the changes were not biologically significant while LH and FSH levels were not affected (Table 2).

Table 1: Effects of 28 Days Administration of Methanolic Extract of Root of *Cissampelos Mucronata* on Mean Body and Testicular Weights in Male Rats

	Doses Administered mgkg^{-1})			
Parameter (g)	**0**	**100**	**200**	**300**
Initial body weight.	212.10±0.96	214.74±0.74	224.00±1.05	201.10±1.79
Final body weight.	253.36±1.68***	201.60±0.86***	208.50±0.80***	180.99±0.90***
Body weight difference	41.26	13.14	15.51	20.11
Left Testis	1.99±0.03	1.84±0.05	1.77±0.04*	1.72±0.03**
Right Testis	2.00±0.12	1.83±0.04	1.71±0.09*	1.55±0.03**

Significance relative to control (0 mgkg^{-1})
***p<0.001, **p<0.01,*p<0.05, ^{a}p<0.05 groups. N=5,
Results are presented as Means ± SEM.

Table 2: Effect of the Administration of the Methanolic Extract of the Root of *Cissampelos mucronata* on Mean Sperm Parameters and Hormonal Levels in Male rats.

	Doses Administered (mgkg^{-1})			
Parameters	**0**	**100**	**200**	**300**
Sperm count (x10^{6}/mL)	57.50±1.05	56.66±1.19	55.26±1.62	52.50±2.28
Sperm motility (%)	91.77±1.63	88.43±0.83	87.95±1.00	87.85±1.29
Non motile sperm (%)	8.23±1.63	11.57±0.84	12.05±1.00	12.15±1.29
Total viable sperm (%)	92.78±1.07	89.82±0.84	84.70±1.42	87.37±2.10*
Total sperm abnormality (%)	2.97±0.26	3.90±0.31	3.57±0.36	3.75±0.61
LH(mIU/ml)	8.36±0.50	8.20±0.73	8.65±0.48	7.58±0.52
FSH(mIU/ml)	0.82±0.15	0.67±0.10	0.55±0.11	0.64±0.04
Testosterone(ng/mL)	5.69±0.65	4.84±0.32	4.63±0.12	5.03±0.37

Significance relative to control (0 mgkg^{-1})
***p<0.001, **p<0.01,*p<0.05, N=5,
LH = Luteinizing Hormone and **FSH** = Follicle Stimulating Hormone

Histopathologic Findings: Light micrographs of the paraffin section of the testis from control rats showed a normal testicular tissue composed of seminiferous tubules with spermatogenic cells at various stages of maturation with the Leydig cells appearing normal and the arrangements in both the basement membrane and interstitial connective tissue appearing normal (figure 1). Administration of 100, 200 and 300mgkg^{-1}of the extract caused some degree of paucity in the number of spermatogenic cells undergoing various stages of maturation, there was atrophy of Leydig cells which was mild in the 100mgkg^{-1} dosed group with the severity increasing in the 300mgkg^{-1}dosed group and basement membrane thickening that was moderate to severe (figures 2, 3 and 4).

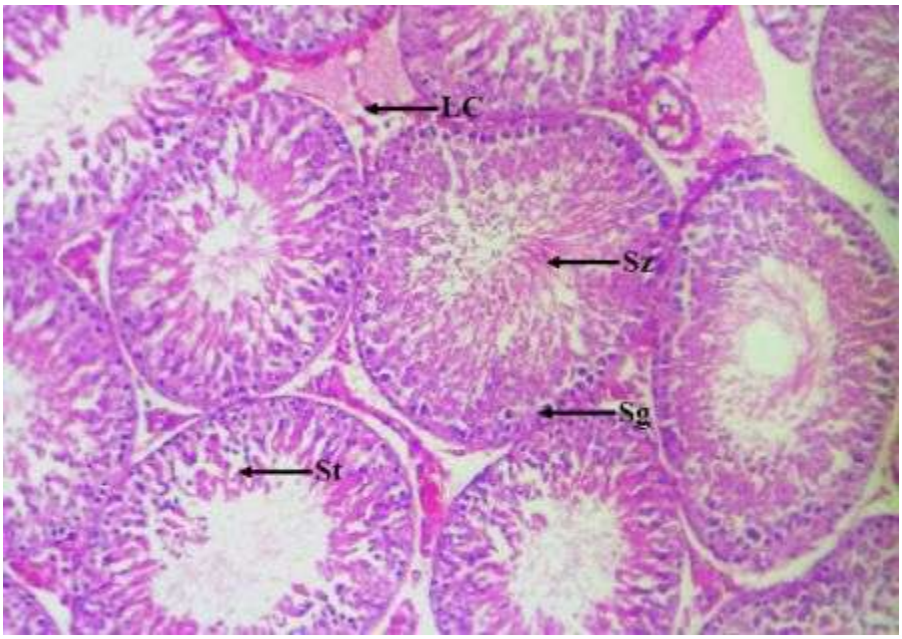

Figure 1: Photomicrograph of the paraffin section of the testis of a control rat showing testicular tissue composed of seminiferous tubules with spermatogenic cells at various stages of maturation showing spermatogonia (Sg), spermatocytes (St), spermatozoan (Sz) and Leydig cells (LC) appearing normal . H and E stain. (magnification x 100).

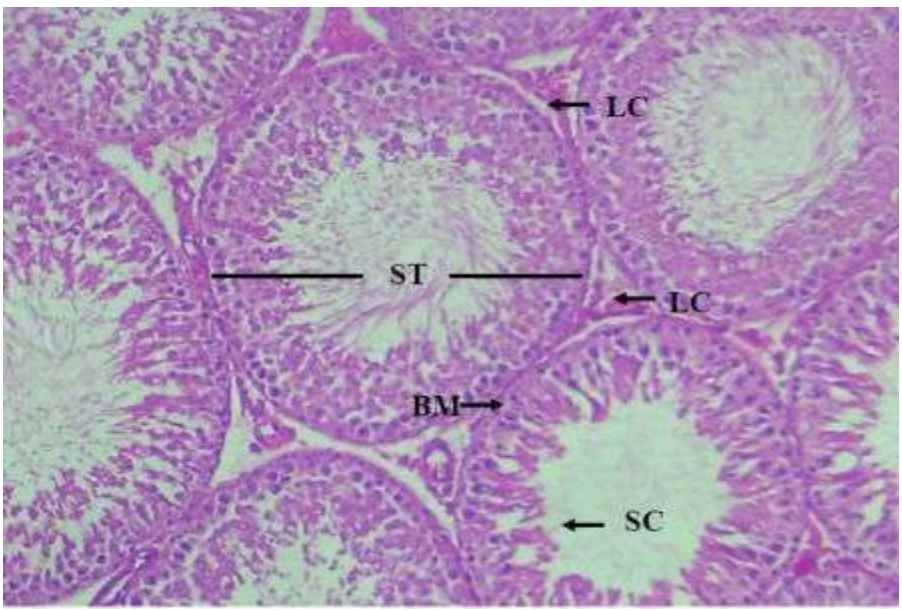

Figure 2: Photomicrograph of the paraffin section of the testis of a rat administered 100 mgkg^{-1} of the extract showing seminiferous tubules (ST) with spermatogenic cells at various stages of maturation, mild atrophy of Leydig cells (LC) and a moderate basement membrane thickening (BM) and spermatogenic cells (SC).H and E stain. (magnification x 100).

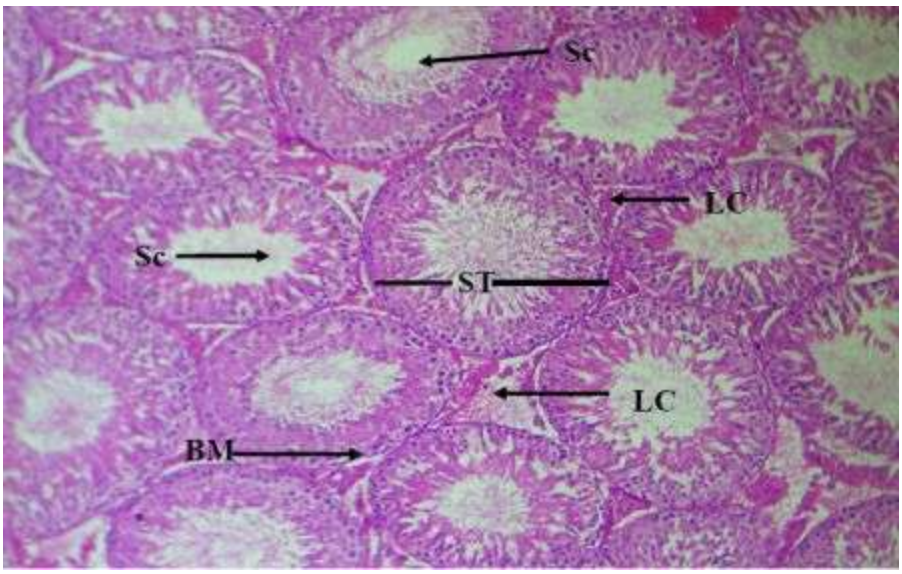

Figure 3: Photomicrograph of the paraffin section of the testis of a rat administered 200 mgkg^{-1} of the extract showing seminiferous tubules (ST) with paucity of spermatogenic cells (Sc) at various stages of maturation, mild atrophy of Leydig cells (LC) and a moderate basement membrane thickening (BM).H and E stain. (magnification x 100).

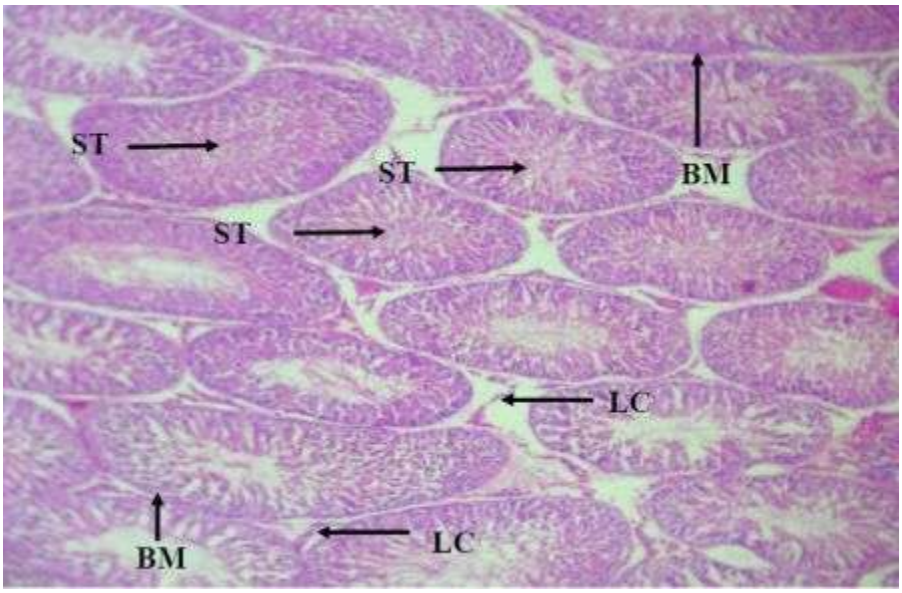

Figure 4: Photomicrograph of the paraffin section of the testis of a rat administered 300 mgkg^{-1} of the extract showing seminiferous tubules (ST) with paucity of spermatogenic cells at various stages of maturation, atrophy of Leydig cells (LC) and basement membrane thickening (BM).H and E stain. (magnification x 100).

DISCUSSION

The body weight loss observed in the treated groups might be attributed to the loss of appetite or effects phytochemical constituent polyphenols present in the plant extract that have been linked with reduction in body weight in other studies (Aherne and O'Brien, 2002; Han *et al.,* 2003; Dang and Lowik, 2004; Tsuda *et al.,* 2005; Yu *et al.,* 2006). This observation also agrees with our previous findings of loss of appetite observed during initial and sub acute toxicity study that led to body weight loss and a subsequent body weight gain following withdrawal of the extract (Garba *et al.,* 2014a).

The male reproductive system is extremely sensitive to various toxicants and Several natural and synthetic products are reported to target the testis at the hormonal level or spermatogenesis or both (Saradha and Mathur, 2006). These factors may have led to the decrease in sperm count, motility and the percentage of total viable sperm and an increase in the percentages of non motile and abnormal sperm cells that was observed in this study . The Sertoli cell plays a critical role in supporting spermatogenesis and as such is one of the most common target cells for toxicity, as a result any functional deficit in its function leads to secondary effects on the survival of the dependent germ cells.

The study showed that administration of 100, 200 and 300mgkg^{-1}of the extract caused some degree of paucity in the number of spermatogenic cells undergoing various stages of maturation with atrophy of Leydig cells which was mild in the 100mgkg^{-1} dosed group with the severity increasing in the 300mgkg^{-1}dosed group and basement membrane thickening that was moderate to severe . The paucity in the number of spermatogenic cells undergoing various stages of maturation is attributable to the testicular damages arises from the death of specific populations of germ cells from agents that affect only the mitotic divisions of spermatogonia (Meistrch, 1984 ; Creasy *et al.,*1985; Meistrch, 1986). Leydig cell function is susceptible to any agent that interferes with the steroidogenic pathway or with circulating levels or receptor binding of its regulatory hormones, luteinizing hormone and prolactin (Clegg *et al.,* 1997). It can also be a direct target for toxicity, as illustrated by the Leydig cell– specific toxicant ethane dimethane sulfonate (Bartlett *et al.,* 1986; Kerr *et al.,* 1993). Blood flow and fluid balance are other targets for disturbance because the seminiferous tubules are avascular, all oxygen and nutrients have to pass through the interstitial space, then through the peritubular myoid cells, and finally through the Sertoli cells to reach the germ cells. This places them on the boundary of

hypoxia and makes them very susceptible to vasoactive chemicals, such as 5-hydroxytryptamine or histamine (Bocabella *et al.,* 1962; O'Steen, 1963).
The decrease in testosterone levels observed following administration of 200 and 300 $mgkg^{-1}$ corroborates with the mild atrophy of Leydig cells observed since the major function of the Leydig cell is steroidogenesis, any substance that interferes with this pathway produces functional disturbances in hormone balance (Creasy, 1999; Creasy, 2001).

Conclusion

In conclusion the results of this study shows that histopathological and androlological analysis reveals that the effect of the methanolic extract of the root of *Cissampelos mucronata* was observed to cause paucity in spermatogenic cells, atrophy of Leydig cells, basement membrane thickening in testicular tissue and a decrease in testasterone levels.

ACKNOWLEDGEMENTS

We wish to acknowledge the technical assistance of Ibrahim Wiam and Ephraim Ayuba of the Departments of Veterinary Anatomy and Human Anatomy, University of Maiduguri, Nigeria and Professor S.S. Sanusi (Botanist) of the Department of Biological Sciences, University of Maiduguri, Borno state, Nigeria for plant identification and authentication.

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Reference to this paper should be made as follows: S.H. Garba *et al.,* (2014), Testicular and Andrological Effects of the Methanol Extract of the Root of *Cissampelos mucronata* (A. Rich) in Rats. *J. of Biological Science and Bioconservation,* Vol. 6, No. 2, Pp. 18 – 30.