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Full Length Research Paper

Microanatomical effects of aqueous extract of the leaves *Cissampelos mucronata* on the kidneys of adult female Wistar rats (*Rattus norvegicus*)

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The crude extract of the leaves of *Cissampelos mucronata* A. Rich is generally used as an anti-abortifacient and known to posses uterine relaxant properties. The present study investigates the microanatomy of the kidneys of adult female wistar rats administered aqueous extract of *C. mucronata* orally. Twenty-four adult female Wistar rats were randomly divided into four groups A, B ,C and D (n =6) in the animal holding of the department of Human anatomy University of Ilorin, Nigeria and fed with pellets from Ladoke farms, Ibadan. They were administered varying doses of aqueous extract of the leaves of *C. mucronata* for two weeks at a concentration of 0.01g/ml/kg body weight. Group A was administered water *ad libitum*, group B received 0.6 ml, while group C received 1.0 ml, and group D received 0.8 ml crude extract of *C. mucronata* for two weeks. Result of study indicated by the photomicrographs of transverse sections of the kidneys of the rats that received 1 ml aqueous extract of the leaves of *C. mucronata* for two weeks showed ruptured blood vessels with distorted cytoplasm compared with control sections. The basement membrane of the sections treated with 0.6 ml of extract was seen to be collapsed. In conclusion, the aqueous extract of the leaves of *C. mucronata* had deleterious effects on the blood vessels of the kidneys of adult female wistar rats.

Key words: *Cissampelos mucronata*, blood vessels, kidneys, Wistar rats.

INTRODUCTION

The kidney is a structurally complex organ that has evolved to sub serve a number of important functions; excretion of waste products of metabolism, regulation of body water and salt, maintenance of appropriate acid balance, and secretion of a variety of hormones and autocoids in Man (Kumar et al., 2000). Diseases of the kidney are as complex as its structure, but their study is facilitated by dividing them into those that affect the four basic morphological components; glomeruli, tubules, interstitium, and blood vessels.

Cissampelos mucronata A. Rich (menispermaceae) is being used among indigenous populations all around the world as a medication. It was used in Uganda for treating stomach ache in children, the twigs and leaves have also been used in curing burns. This herb of study is locally called jokoje leaves in western Nigeria. In context 'jokoje' means 'time to stop miscarriages in Yoruba, and the extract is often used, and abused by mothers to be with concomitant cases of miscarriage locally, however an interplay between the extract of the leaves and the kid-neys of adult female Wistar rats is the focus of this study. *C. mucronata* extract possesses a powerful anti – arbortifacient effect suggesting probably a beneficial effect on other organ systems especially the kidneys which regulates body water and salt. It grows in damp places including waterlogged areas, Island, and Lakes. It is almost a water plant which places its leaflets showing a pentagonal structure (mucronate), always photosyn-thesizing even in dry seasons. The aqueous extract of *C. mucronata* A. Rich is popular among traditional healers in Nigeria as an antidiaorrhoeal and palliative in stomach ache. The description of the plant morphology has been documented (Hutchinson and Dalziel, 1963). The root Bark extract of *C. mucronata* and related species of *Cissampelo periera* and

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Cissampelo ovariensis are used in traditional medicine as an antihelmintic (Audu, 1995) to relieve dysmenorrheal, to prevent abortion and as a se-dative (Ogwal et al., 1996; Oliver, 1967). Previous investi-gation had reported antispasmodic (Offiah et al., 1996) and anti-Ulcer (Akah and Nwafor, 1999; Nwafor and Akah, 1999) activities of the crude leaf extract while the ethanolic root extract had been reported to exhibit potent uterine relaxant property (Nwafor, 2002). The fractions isolated from the methanolic leaf extract have also been shown to exhibit significant ulcer protection against ulcer induced indom-ethacin in rats (Nwafor and Akah, 1999). Oral LD50 of 8.5 /kg (Akah and Nwafor, 1999) and intra-peritoneal LD50 of 0.283 g/kg were demonstrated for crude ethanolic and methanolic extract respectively. Nwafor et al. (2002) further reported that the root extract of *C. mucronata* possess uterine relaxant property suggesting that it may probably be beneficial on the reproductive and consequently on the urinary system Investigated.

The aims and objectives of this study are to:

(a) Investigate the effects of *C. mucronata* leaf extract on the cytoarchitecture of the kidneys of adult female Wistar rats, and
(b) Establish these effects in relation to human subjects.

MATERIALS AND METHODS

ANIMALS

Experiments were carried out on twenty-four adult female Wistar rats (160 - 80 g) procured and maintained in the animal holdings of the Department of Human Anatomy, University of Ilorin, Nigeria. The animals were housed in metabolic cages under a controlled room temperature of about 25 - 28°C, relative humidity of about 60 - 80% and photo-periodicity of 12 h day /12 h night and fed with pellets from Ladokun Feeds, Ibadan Nigeria. Six rats in each group were examined and being put under close supervision. Three test group B, C, D and one control group A. The three test groups were administered repeatedly 0.6, 1.0 and 0.8 ml respectively of 0.01 g/ml stock concentration of the leaf extract of *C. mucronata* and the placebo was administered water *ad libitum*.

Preparation of extract of leaves of *C. mucronata*

100 g leaves of *C. mucronata* were weighed using a sensitive weighing balance and properly dried in the sun for three days. The leaves were homogenized in an electric blender and the resulting homogenate was filtered using a sterilized cheese cloth and concentrated in rotatory evaporator at room temperature, the filtrate was dried into a powdery substance. A stock concentration of 0.01 g/ml was prepared repeatedly and stored for use.

Experimental design

The crude extract was administered orally, repeatedly for 14 consecutive days at varying doses of 0.6, 1.0, and 0.8 ml to the treatment groups B, C, and D respectively. The animals were sacrificed 24 h after the last day of administration.

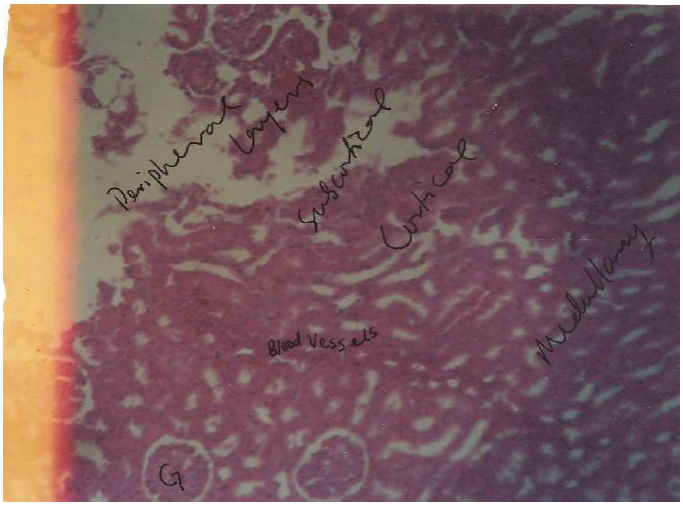

Figure A. Photomicrograph of a section through the kidney of group A rats (control group) given water *ad libitum* for 2 weeks. The glomeruli (G) is well distributed, and the peripheral to medullary layers are intact. H and E method of staining.

Histological procedure

Samples of the kidneys of the sacrificed animals were fixed in 10% formol saline and processed for light microscopy. These processing methods included dehydration through graded ethanol (50, 70, and 100%), Clearing in xylene and infiltration in the paraffin wax for 2 h at 56°C and embedding of the tissues in paraffin wax for 48 h. Sections were then obtained using a rotary microtome at 5 um thickness. The sections were finally subjected to a haematoxylin and eosin staining procedure by Drury and Wallington (1960).

Statistical analysis

Weights of Animals were recorded before and after administration of crude extract of the leaves of C. *mucronata* and tabulated to determine the mean weights and differences in respective mean weight before and after administration of the extract.

RESULTS

Histology

There was an alteration in the cytoarchitecture of the treated sections as compared to the control (Figures 2, 3 and 4 compared to Figure 1). Result of study indicated by the photomicrographs of the transverse section through the kidneys of the rats that received 1 ml extract for 14 days showed ruptured blood vessels with distorted inters-titial cellular layer compared with the control sections (Figure 3 compared to Figure 4). The Basement mem-brane thickness of section treated with 0.6 ml of extract was seen to be collapsed (Figure 4).

Table 1. Weights and mean weights of animals before and after oral administration.

Groups		Weights before treatment (g)	Weights after treatment (g)	Weight Difference (g)
A	1	162.00	163:00	1.00
	2	160.00	160:00	0.00
	3	168.00	170:00	2.00
	4	164.00	165:00	1.00
	Average	161.50	161.25	0.25
B	1	166.00	153.00	13.00
	2	158.00	146.00	12.00
	3	160.00	148.00	12.00
	4	162.00	150.00	12.00
	Average	163.00	154.00	11.00
C	1	165.00	154.00	11.00
	2	165.00	145.00	20.00
	3	162.00	157.00	5.00
	4	160.00	160.00	_
	Average	163.00	154.00	9.00
D	1	160.00	158.00	2.00
	2	162.00	150.00	12.00
	3	160.00	150.00	10.00
	4	158.00	146.00	12.00
	Average	158.00	151.00	7.00

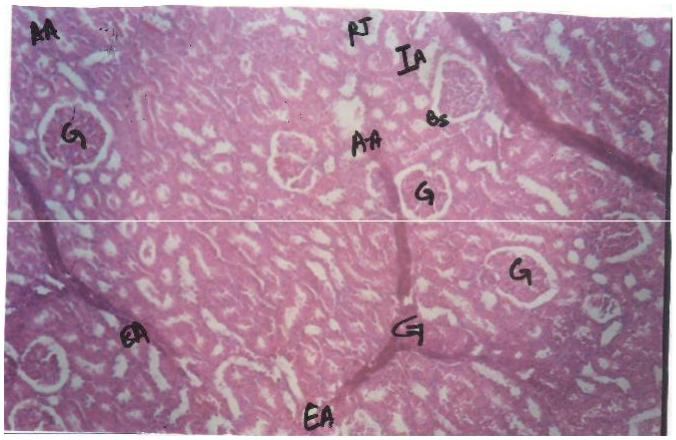

Figure B. Photomicrograph of a section through the kidney of group B rats (administered 0.6 ml of 0.01 g/ml crude extract of *Cissampelos mucronata*) for two weeks. H and E staining. Magnification × 100.

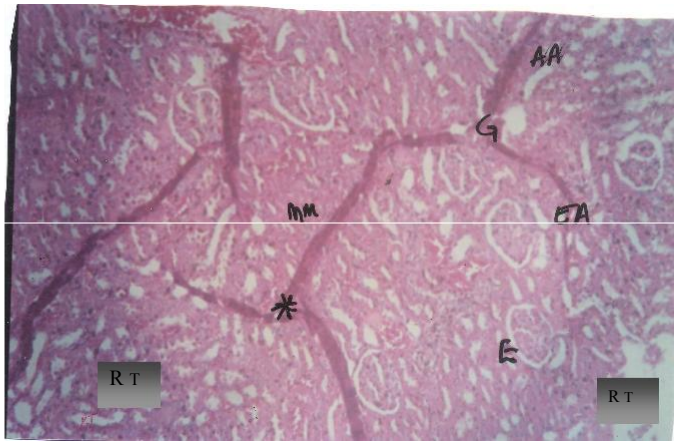

Figure C. Photomicrograph of a section through the kidney of Group C rats (administered 1 ml of 0.01 g/ml crude extract of *Cissampelos mucronata*) for two weeks showing ruptured blood vessels EA and AA and distorted glomeruli in the cytoplasm. Rt = renal tubules H and E staining. Magnification x 100.

From Table 1, it was observed that there is a significant average weight reduction in the animals in groups B and C after treatment as compared to the average weight difference in the placebo group. The observed weight loss may probably be due to a dehydrating effect of the extract on the animals, inability to feed properly / increased fat metabolism as a consequence of the treatment and / or suggesting presence of free radicals in the constituents/ active principles in the leaf extract of *C. micronata.*

Histological findings

(A) Photomicrograph of section of control group administered water *ad libitum* for 14 days (Figure 1) presents a well organized interstitium and glomerulus (G). The blood vessels are indicated by (Blood vessels) and the Capillaries are lined by fenestrated endothelial cells. From Figure 1 the peripheral regions are cortical arches and

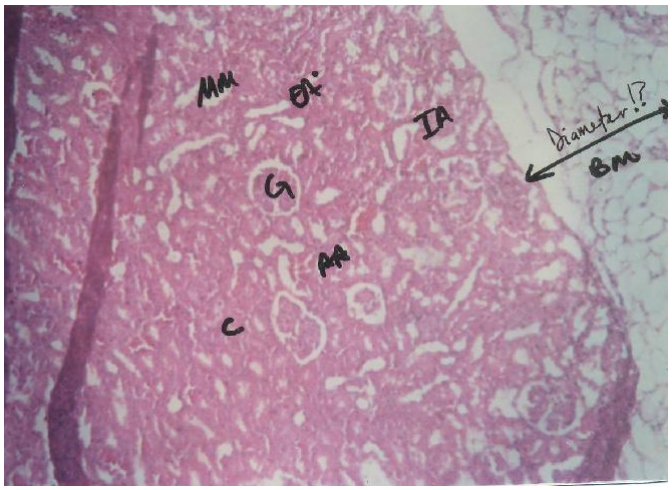

Figure D. Photomicrograph of a section through the kidney of Group D rats (administered 0.8 ml of 0.01g/ml crude extract of *Cissampelos mucronata*) for two weeks showing the diameter/ structure of the basement membrane and irregular histoarchitecture of the cytoplasm. H and E staining. Magnification × 100.

are traversed by medullary rays (Brightly stained sections). Ruptured blood vessels were not observed and the renal interstitium showed signs of high cellular activity. The Glomeruli is indicated by (G).

(B) Photomicrograph of sections from rats treated with 0. 6 ml extract (Figure 2) for 14 days showed distorted interstitium and glomerulus. The renal tubules are also iregularly arranged, and blood vessels were seen to be collapsed (indicated by EA and AA).

(C) Photomicrograph of section from rats treated with 1ml extract for 14 days showed ruptured blood vessels running from the subcortical regions to the medullary segment indicated by (EA and AA).

(D) The photomicrograph from sections of rats treated with 0.8 ml crude extract of *C*. *mucronat*a for 14 days showed a marked disarrangement of the cellular components (cytoplasm) that is, renal tubules and corpuscles. Thus the basement membrane thickness was observed to have been altered (Figure 4).

All these distortions in the microanatomy of the sectioned kidneys are more than enough reasons to awaken our concern on the effects of the administered extract which is still used and misused by female populations in Nigeria in particular and Africa at large for the treatment of miscarriages/infertility thus posing a possible threat of terror to their respective urinary systems and predisposing them to renal diseases which are a ultimate cause of deaths among women of child bearing age.

DISCUSSION

We investigated the effect of oral administration of crude extract of *C. mucronata* at varying doses. *C. mucronata* is used as an antiabortfacient, antispasmolytic and antiulcer ulcer among indigenous populations in Africa. This was with a view to providing useful information on the use and application of this herb of study among women of child bearing age. Our investigation revealed that the treated sections of the kidneys showed some histological changes that were at variance with what was obtained in the control sections. The result of this experiment presents a dose dependent derangement of the cellular components of the kidneys which could be associated with functional changes that may be detrimental to the health of these female Wistar rats. Cellular degeneration had been reported to result in cell death, which is of two types which differ biochemically and morphologically (Farber et al., 1981). Cell death in response to toxins occurs as a controlled event involving a genetic programme in which cascade enzymes are activated (Johnson, 1995). Johnson (1995) reported in his work that drug poisoning, water intoxication, hypoxia from asphyxia and acute hyponatremia could result in cellular distortion. Oral administration of crude extract of *C. mucronata* in this present investigation may have acted as poison to the renal cytoarchitecture, thus affecting the tubules, corpuscles and cellular integrity in general, thus causing a defect in the ionic channels of the kidneys and compromising the renal index. Acworth et al. (1997) revealed that lipid per oxidation can negatively affect membrane function by decreasing membrane fluidity and changing the activity of the membrane bound enzymes and receptors. The reported morphological alterations may be caused by oxidative stress. Oxidative stress can be caused by strenuous exercise, oxidation of food and other chemical reactions that occur in the cell (Coyle and Pultfarcken, 1993). Oxidative stress leads to the production of free radicals which damage and destroy cells as evidenced in Figures 2, 3 and 4. Our investigation concluded that oral administration of crude extract of *C. mucronata* can lead to per oxidation injury. Though the Crude extract has been reported beneficial to the reproductive systems of animals and Man. It may also result in alteration/impairment of renal function. It is therefore suggested that the herb be prescribed with caution or used in patients with renal diseases/ hypertension, thus self-medication and abuse involving this in expendable herb should be discouraged as these may lead to prolonged exposure or overdose and subsequent renal failure/ damage as observed in this investigation.

Conclusion

It was concluded that the oral administration of the aqueous extract of the leaves of *C. mucronata* had profound negative effects on the microanatomy of the kidneys of adult female Wistar rats, thus should not be abused and misused in the local treatment of miscarriages/infertility in women despite its potent beneficial pharmacological effects in the female reproductive system.

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