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Article *in* Journal of Ethnopharmacology · June 2006

DOI: 10.1016/j.jep.2005.11.027 · Source: PubMed

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Journal of ETHNO-PHARMACOLOGY
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Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of *Musanga cecropioides* in rats

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Received 19 May 2005; received in revised form 17 June 2005; accepted 17 November 2005
Available online 18 January 2006

Abstract

These studies were designed to determine the preliminary oral toxicity profile of the crude aqueous stem bark extract of *Musanga cecropioides* (MCW) in adult Sprague–Dawley rats and its active chemical constituents by way of phytochemistry. The acute oral toxicity study was conducted using limit dose test of Up and Down Procedure according to the OECD/OCDE Test Guidelines on Acute Oral Toxicity (AOT425statPgm, version: 1.0) at a limit dose of 3000 mg/kg body weight/oral route. Repeat dose oral toxicity studies were conducted by daily oral dosing of 750 mg/kg body weight of MCW dissolved in 1 ml of 0.9% saline and 1 ml of 0.9% saline to rats in the test and control groups, respectively, for 28 days. On day 29, blood samples for bioassays were collected by cardiac puncture under diethyl ether anesthesia. The phytochemical analysis was conducted using standard procedures. The LD_{50} estimate of the extract was calculated to be greater than 3000 mg/kg body weight/oral route. The extract caused a significant ($P < 0.05$) decrease in weight gain, differential eosinophil count and increase in serum creatinine but did not affect the organ weights, other serum electrolytes (Na^+, K^+, HCO_3^-), liver enzymes and other hematological indices in test rats. Its phytochemical analysis showed it contains saponins, flavonoids, alkaloids, tannins, phlobatannins, glycosides, reducing sugars and anthraquinones. These results show that the aqueous extract of *Musanga cecropioides* is relatively safe toxicologically when administered orally. Thus, its use in folkloric medicine as an oral antihypertensive is relatively safe when used over the tested period.

Keywords: *Musanga cecropioides*; Rats; Oral toxicity; Phytochemical analysis

1. Introduction

The plant, *Musanga cecropioides* (family: Cecropiaceae) is a rapidly growing tree, ubiquitous to the tropical rainforest, particularly of West Africa, where it has a reputation for medicinal value (Bunkill, 1985). In south-eastern Nigeria, the Igbo boil the leaves and use it as powerful oxytocic to induce or augment labour while in the south-western Nigeria, the leaves are boiled and the decoction used as remedy for hypertension (Bunkill, 1985). Among the Ijebus in the south-west of Nigeria, the boiled decoction of the stem bark sheaths of the plant is equally used as hypertension remedy (Adeneye, 2005).

Different parts of the plant are used by traditional healers in the treatment of array of human diseases which include lumbago, rheumatism, leprosy, chest infections, trypanosomosis (Bunkill, 1985) but information on its toxicity in man and animal models is lacking. This study, therefore, was designed to evaluate its toxicity in adult Sprague–Dawley rats and to identify the active principles in the extract by way of phytochemistry, as toxicity may result from drug overdose or the active principles contained in the plant.

2. Materials and methods

2.1. Collection and processing of plant material

About 3 kg of the stem bark sheaths of *Musanga cecropioides* was collected from the deciduous forest of Ijebu-Igbo in Ijebu

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doi:10.1016/j.jep.2005.11.027

North Local Government Area of Ogun State, Nigeria, in the months of March and June 2004.

Samples were authenticated by Mr. T.I. Adeleke of Pharmacognosy Department, University of Lagos and the voucher specimen deposited in the herbarium of the department, with the specimen no. PCLHCEC 01.

The stem bark sheaths were sorted, cleaned and air-dried at room temperature for 2 weeks. These were ground to powder using the laboratory Hammer mill. Powdered samples were collected and stored in air- and water-proof containers protected from direct sunlight and heat until required for extraction.

2.2. Animal and management

Investigations using experimental animals were conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the U.S. guidelines (NIH publication no. 85-23, revised in 1985).

A total of 38 adult rats of the Sprague–Dawley strain, 8–9-weeks old and weighing between 150 and 250 gm were obtained from the laboratory animal house of Nigerian Institute for Medical Research and National Agency for Food Administration and Control (NAFDAC), Yaba, Lagos. The animals were obtained after approval for the study was obtained from the College of Health Sciences ethical committee of Usmanu Danfodiyo University, Sokoto.

The rats were kept in metal cages in the metabolic laboratory with uniform temperature of 22–25 °C, 12 h light and12 h dark periodicity. The rats were fed standard rat chows (Neimeth Livestock Feeds, Ikeja, Lagos.) and water ad libitum and allowed to acclimatize for 14 days before the procedure.

2.3. Preparation of aqueous crude extract

1.6 kg of the powdered sample was extracted with 3.2 l of distilled water using Soxhlet extraction procedure. The extraction was done over a period of 6–8 h to ensure complete extraction. The liquid extract obtained was then filtered using Whatman filter paper no. 1. The filtrate was then concentrated to dryness (deep-brown solid residue) using digital aeration oven preset at 50 °C and the solid residue was later stored in the refrigerator at 4 °C. From this a fresh stock solution was prepared whenever required. The yield of extract was 14.87 ± 0.85%.

2.4. Acute oral toxicity study

Acute toxicity study was carried out in vivo. All solutions were prepared using 2 ml of 0.9% Saline solution and administered per os using gastric tube.

The Acute oral toxicity study was conducted using the limit dose test of up and down procedure according to OECD/OCDE Test Guidelines on Acute Oral Toxicity under a computer-guided Statistical Programme-AOT425statPgm, version 1.0 (AOT, 2001), at a limit dose of 3000 mg/kg body weight/oral route and default of Sigma at 0.5.

A total of five rats of either sex (three females, two males) were systematically selected out of a population of 38 rats by systematic randomization techniques. The population sample was selected such that the weight differences do not exceed ±10% of the mean initial weight of the sample population. The rats were fasted of rat chow overnight prior to dosing on each occasion.

A rat was picked at a time, weighed and dosed with equivalent 3000 mg/kg body weight of the crude extract dissolved in 1 ml of 0.9% Saline used as the vehicle. Feeding was done using gastric feeding tube.

Each animal was observed each time for the first 5 min after loading for signs of regurgitation and then kept in a metabolic cage. Each was watched for every 15 min in the first 4 h after dosing, then every 30 min for the successive 6 h and then daily for the successive 38 h for the short-term outcome and the remaining 12 days for the long-term possible lethal outcome which in this case was “death”.

Behavioural manifestations of acute oral toxicity were also noted. All observations were systematically recorded with individual records being maintained for each rat.

2.5. Repeat dose oral toxicity study

The experimental rats consisting of a total of 20 rats (16 males and 4 females) were systematically randomized so that the weight differences from one another do not exceed 10%. These were randomly divided into two groups of 10 rats (8 males and 2 females) namely the control and test groups.

At the start of the experiment, the rats were weighed and their weights recorded.

The crude extract of Musanga stem bark was administered on daily basis to the tested group by gastric feeding at the dose of 750 mg/kg of body weight dissolved in 1 ml of 0.9% normal saline. They were fed daily for a total of 28 days. Drug administration was terminated on the 28th day, after which the rats were fasted for 24 h. The control group which consisted of 10 rats was fed with 1 ml of the vehicle only, under the sample experimental conditions and sham-handling.

On the 29th day, the rat in each group was weighed, anesthetized with diethyl ether and blood samples for biochemical and hematological analyses were collected.

2.6. Collection of blood for bioassay

Blood samples were collected by cardiac puncture under diethyl ether anesthesia. Whole blood for FBC and Hb determination was collected into bottles containing the anticoagulant, ethylene diamine tetra-acetic acid (EDTA) while samples for LFT and electrolytes, urea and creatinine were collected into plain sample bottles.

The PCV was determined by the micro-hematocrit method while total and differential leucocytes count, platelets count were made from blood smears stained with Giemsa (Schlam et al., 1975). The hemoglobin concentration, Hb, was determined by cyanomethemoglobin method using the Beckman Model Spectrophotometer (Drabkin and Austin, 1932). The plasma activities of liver enzyme, alkaline phosphatase was estimated by method of Sigma Diagnostic (1987) while those of aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

Table 1
Sequence and results of limit dose test of MCW in rats

Test sequence	Animal ID	Dose (mg/kg)	Short-term result (48 h)	Long-term result (12 days)
1	01	3000	Survival	Survival
2	02	3000	Survival	Survival
3	03	3000	Survival	Survival
4	04	3000	Survival	Survival
5	05	3000	Survival	Survival

ID = identification number.

were estimated by colorimetric method (Sigma Diagnostic, 1985). Sodium and potassium were estimated by flame photometer while urea was estimated by modified diacetylmonoamine method (Marsh, 1965). Estimation of creatinine was done by the Jaffe's reaction method (Biod and Sirota, 1948). The total protein was estimated by Biuret method (Treitz, 1970) while that of albumin was determined by bromocresol green (Lowry et al., 1957). The total bilirubin and the conjugated bilirubin were determined by Jendrassik–Grof method (Spencer and Price, 1977). The serum cholesterol was determined by the Lierberman–Burchard quantitative test (Odesanmi et al., 2000).

2.7. Phytochemical analysis

The presence of saponins, tannins, anthraquinones, alkaloids, triterpenes, flavonoids, glycosides, reducing sugars and phlobatannins were detected by simple qualitative and quantitative methods of Trease and Evans (1989) and Sofowora (1993).

3. Results

3.1. Acute toxicity study

There were no deaths of rats administered 3000 mg/kg body weight of plant extract within short- and long-term outcome of the limit dose test of Up and Down Procedure (Table 1). However, the observed behavioural signs of toxicity include irritation, restlessness, tachypnoea, anorexia, bilateral narrowing of the eyelids and abnormal posture (which was characterized by tugging of the head in-between the hind-limbs). The LD_{50} was calculated to be greater than 3000 mg/kg body weight/oral route (Table 1).

3.2. Repeat dose toxicity study

3.2.1. Effect of MCW on weight gain of rats

Table 2 shows the initial mean body weight, final mean body weight, mean weight changes and the dosage of the extract per kilogram body weight.

MCW administered to rats for 4 weeks of this study decreased the weight gain of the animals significantly ($P < 0.05$) when compared to control.

3.2.2. Effects of extract on the weight of body organs

Table 3 shows the effects of the water extract on the weights of some vital body organs in rats. MCW administration did not cause a significant difference in the organ weights of rats in both control and test groups.

3.2.3. Hematological responses of rats to MCW

Table 4 shows the PCV, Hb, MCHC, Platelets count responses and dose in gm/kg body weight to the extract. The extract caused no statistically significant ($P > 0.05$) difference on the hematological parameters being investigated at the tested doses.

However, there was a remarkable decrease in the differential eosinophils count in the treated group when compared to control (Table 5).

3.2.4. Biochemical responses of rats to MCW

3.2.4.1. Effect of the extract on plasma electrolytes, urea and creatinine. Table 6 shows the effect of the aqueous extract of *Musanga cecropioides* stem bark on the plasma electrolytes, urea and creatinine. The extract elevated the plasma creatinine significantly ($P < 0.05$) while no significant increase occurred in other plasma electrolytes and urea ($P > 0.05$).

3.2.4.2. Effect of MCW on plasma proteins, cholesterol, bilirubin and liver enzymes. The extract caused no significant changes in the plasma proteins, cholesterol and bilirubin in rats in both groups (Table 7). So also, was for its effect on the circulating liver enzymes (Table 8).

3.3. Phytochemical analysis

Phytochemical analysis of the MCW showed it contains saponins, flavonoids, alkaloids, tannins, phlobatannins, cardiac

Table 2
Effect of MCW on weight gain in rats

Dose (mg/kg)	No. of rats	Mean initial weight on day 0 (gm)	Mean final weight on day 28 (gm)	Mean weight changes (gm)
0	10	175.4 ± 10.0	209.2 ± 33.4	35.0 ± 28.2
750	10	172.4 ± 7.8	197.4 ± 7.9*	25.0 ± 20.0*

Values are expressed as mean ± S.D. of 10 rats.
* Values significant at $P < 0.05$.

Table 3
Effect of MCW on the organ weights

Dose (mg/kg)	No. of animals	Mean weight of liver (gm)	Mean weight of kidneys (gm)	Mean weight of lungs (gm)	Mean weight of heart (gm)
0	10	7.9 ± 0.6	1.7 ± 0.1	1.6 ± 0.3	0.8 ± 0.1
750	10	7.3 ± 0.3	1.4 ± 0.1	1.5 ± 0.0	0.7 ± 0.0

Table 4
Effect of MCW on PCV, Hb, MCHC and platelet counts

Dose (mg/kg)	No. of rats	PCV (%)	Hb (gm/dl)	MCHC (%)	Platelets count ($\times 10^3$ mm^{-3})
0	10	37.8 ± 0.6	12.3 ± 0.2	32.5 ± 0.6	219.8 ± 15.2
750	10	37.8 ± 0.7	12.3 ± 0.3	32.6 ± 0.2	172.0 ± 10.2

Table 5
Effect of MCW on total and differential white blood cells

Dose (mg/kg)	No. of rats	Total WBC ($\times 10^9$ l^{-1})	Neutrophils (%)	Monocytes (%)	Lymphocytes (%)	Eosinophils (%)
0	10	2.1 ± 0.6	30.8 ± 6.0	6.8 ± 1.7	62.0 ± 7.2	0.4 ± 0.2
750	10	1.8 ± 0.2	31.0 ± 3.1	4.6 ± 1.6	64.2 ± 3.0	0.2 ± 0.1*

* Values significant at $P < 0.05$.

Table 6
Effect of MCW on serum electrolytes, urea and creatinine in rats

Dose (mg/kg)	No. of rats	Na^+ (mmol/l)	K^+ (mmol/l)	HCO_3^- (mmol/l)	Urea (mmol/l)	Creatinine (mmol/l)
0	10	148.8 ± 3.9	5.5 ± 0.3	27.6 ± 1.0	6.2 ± 1.3	0.5 ± 0.1
750	10	150.2 ± 4.0	5.4 ± 0.3	25.0 ± 1.1	6.0 ± 0.1	0.9 ± 0.1*

* Values significant at $P < 0.05$.

Table 7
Effect of MCW on serum proteins, cholesterol and bilirubin in rats

Dose (mg/kg)	No. of rats	Total proteins (gm/dl)	Albumin (gm/dl)	Cholesterol (mmol/l)	Total bilirubin (μmol/l)	Conjugated bilirubin (μmol/l)
0	10	90.8 ± 2.8	24.6 ± 1.0	1.5 ± 0.1	1.1 ± 0.1	0.2 ± 0.0
750	10	101.6 ± 4.8	25.4 ± 1.1	1.5 ± 0.1	1.0 ± 0.2	0.15 ± 0.0

glycosides, reducing sugars and anthraquinones (as shown in Table 9).

3.4. Statistical analysis

Results were analyzed using matched paired *t*-test on SYSTAT 10.2 software programmer and were expressed as mean ± S.E.M.

4. Discussion

Acute toxicity study of *Musanga cecropioides* aqueous stem bark extract showed that no mortality of rats occurred, at a limit dose of 3000 mg/kg body weight given per os. This is an indication the extract has low acute toxicity when administered per os. According to Clarke and Clarke (1977), substances with LD_{50} of 1000 mg/kg body weight/oral route are regarded as being safe or of low toxicity. The high LD_{50} obtained is an indication that the extract could be administered with a high degree of safety where the absorption might be incomplete due to inherent factors impeding absorption along the gastrointestinal tract (Dennis, 1984).

Blood is an important index of physiological and pathological status in man and animals and the parameters usually measured are hemoglobin, packed cell volume, white blood cell count, platelets count (Schlam et al., 1975). The normal range of these parameters can be altered by the ingestion of some toxic plants (Abatan and Arowolo, 1989; Ajagbonna et al., 1999). These

Table 8
Effect of MCW on the liver enzymes in rats

Dose (mg/kg)	No. of rats	Alkaline phosphatase (IU/l)	ALT (IU/l)	AST (IU/l)
0	10	52.0 ± 3.7	8.6 ± 2.7	18.0 ± 2.4
750	10	44.4 ± 2.5	8.6 ± 1.2	22.8 ± 2.3

Table 9
Chemical constituents of *Musanga cecropioides* stem bark aqueous extract

Tests	Result
Saponins	
(i) Benedict's test	+
(ii) Emulsion test	+
(iii) Frothing test	+
Tannins	
(i) Bromine water test	++
(ii) Ferric chloride test	++
Phlobatannins	+
Alkaloids	
(i) Draggendoff's test	++
(ii) Mayer's test	+
(iii) Wagner's test	++
Flavonoids	
(i) Lead acetate test	+++
(ii) Ferric chloride test	+++
(iii) Sodium chloride test	+++
Cardiac glycosides	
(i) Keller–Kelliani test	+++
(ii) Salkowski's test	++
(iii) Legal's test	+++
Reducing sugars	
Hexose sugar	+
Ketosugar	+
Pentosugar	+
Monosaccharide	+
Anthraquinones	
(i) Free anthraquinones	+
(i) Bound anthraquinones	++
Anthocyanides	−

−: not detected; +: present in low concentration; ++: present in moderate concentration; +++: present in high concentration.

blood indices were all measured in this present study after 28 days of oral administration without any significant alterations from the control values (except for eosinopenia), still corroborating the wide safety margin of the extract. Administration of the aqueous extract for 28 days in this study did not affect most of the biochemical parameters except for creatinine which was significantly elevated. However, the serum cholesterol was not lowered significantly as against earlier study by Odesanmi et al. (2000) where the aqueous extract of the bud sheath of same plant lowered the fasting serum cholesterol dose-dependently in female rats treated with the extract over a period of 27 days. This discrepancy may be explained by the fact that variation may sometimes occur in bioactive compounds of the different parts of the same plant and even in same plant parts found in different environment (Elujoba, 1989). Differences in the sex of experimental animals and differences in the experimental protocol despite the same species of experimental animal was used, could account for the discrepancy as well. The increase in the serum level of creatinine may be an reflection of an increased catabolic state in the rats resulting from prolonged reduced appetite as evidenced in the behavioural manifestation of acute oral toxicity or could be a manifestation of some degree of renal insult. However, these assertions need to be clarified. The lack of significant alterations of liver enzymes is also remarkable.

It is known that many toxic plants compounds accumulate in the liver where they are detoxified (Clarke and Clarke, 1977). A study of liver function tests may therefore prove useful in assessing especially the toxic effects of medicinal plants on the liver. These tests involve mainly determination of AST and ALT (Tilkian, 1979) and any marked necrosis of the liver cells lead to a significant rise of these enzymes in the blood serum. The lack of this effect on these liver enzymes shows that the extract is non-toxic on the hepatocytes.

The toxicity observed in these studies could have resulted from various active organic constituents like saponins, tannins, alkaloids, flavonoids and glycosides as shown by the result of phytochemistry. These findings are in concordance with those of other workers like Lontsi et al. (1987, 1989, 1990, 1991) and Dongmo et al. (2002) for other parts of the plant. Although it is known that variations may sometimes occur in bioactive compounds of the same plant found in different environments (Elujoba, 1989), but, this was not the case. Flavonoids possess a wide spectrum of biological actions such as hypoazotaemic, hypotensive, oestrogenous, spasmolytic, cholagogue, anti-inflammatory and antioxidant activities (Oladele et al., 1995). The aqueous extract of the stem bark of *Musanga cecropioides* have earlier been found to lower blood pressure and heart rate dose-dependently in rabbits (Ayinde et al., 2003) and rats (Adeneye, 2005). The presence of flavonoids in high concentration in the extract may account for its hypotensive effects.

5. Conclusions

The apparent lack of clinical signs of acute toxicities in human when administered the extract orally as an antihypertensive, may be a reflection of the oral route of administration, low dose administration as well as short duration of exposure. The extract, in folkloric medicine is administered two to three times per day for 1–2 weeks as an antihypertensive remedy. However, these studies only serve as a template for future research into plant extract's toxicity profile.

Acknowledgement

This work was partly funded by Federal Scholarship Board, Abuja, Nigeria, under the auspices of Federal Government Scholarship Award for Studies in Nigerian Tertiary Institutions: postgraduate (2001/2002), Award No. FSBA/FGSS:/PG/276 AI, which was awarded to the corresponding author.

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