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Investigation of some pharmacological actions of the aqueous extract of *Paulinia pinnata* leaves

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Abstract

The plant *Paulinia pinnata* is traditionally used extensively in the treatment of leishmaniasis in some endemic communities in Nigeria with acclaimed success. The plant therefore needs to be evaluated for other beneficial and toxic effects while its antileishmanial activity is being studied. The aqueous leaf extract of the plant *Paulinia pinnata Linn.* (Sapindaceae) was tested for its diuretic and morphological effects in rats and its hypotensive effect in cat. These investigations revealed that the extract has considerable diuretic activity ($P<0.05$) 24h after treatment and also caused significant changes in liver and kidney morphologies. These changes suggest potential organ toxicity. The extract also demonstrated hypotensive effect in cat. Phytochemical studies revealed the presence of alkaloids, tannins, saponins and cardiac glycosides. The plant therefore possesses some important biological activities that could be harnessed and employed beneficially.

Keywords: *Paulinia Pinnata;* Diuretic; Hypotensive effect; Liver morphology; Kidney morphology.

Introduction

The plant *Paulinia pinnata* Linn. (Sapindaceae) is a climbing herb commonly found in West Africa. In Ghana, the dried root powder is mixed with oil and pepper (*Piper gineense*) and used for local application to open wounds to prevent infections. The leaves, roots and seeds are also powdered together with ginger or Guinea grains and applied to fractures to aid bone healing and to promote healing of open wounds (Dalziel, 1937).

The leaves of this plant were extensively used in the treatment of cutaneous Leishmaniasis in Idah community of Kogi State, Nigeria and Keana community of Plateau State, Nigeria during an outbreak of leishmaniasis in these communities between 1988-1995 (personal communication). Previous epidemiological studies in one of these communities proved that the infection was that of leishmaniasis (Agwale *et al.*, 1993).

The extensive use of this plant in these communities and its acclaimed therapeutic success has generated interest among researchers to investigate its pharmacological properties. Knowledge of these properties will help in advising the herbal practitioners and patients alike on the dangers or efficacy of the plant. This is in addition to contributing to the pharmacological profile of the extract.

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Previous preliminary antibacterial studies of the extract at 2.5 mg/mL, 5 mg/mL and 7.5 mg/mL against *Pseudomonas aeruginosa*, *Staph. aureus*, *Proteus mirabilis*, *Salmonella typhi* and *Klebsiella* spp demonstrated higher activity than gentamicin at equal doses (unpublished work). In this study, the aqueous extract of the dried leaves was used.

Experimental

Animals. Male albino rats (Wister strain) weighing between 46-150g were obtained from the animal house, Department of Pharmacology, University of Jos and maintained on standard pellet diet and water *ad libitum* and cats (1.65-2.05 kg) were used in this experiment. Animals were used according to ethical standards.

Preparation of plant material. The plant *Paulinia pinnata* was obtained fresh in Keana, Nassarawa State, Nigeria in August and identified by Mr. I. A. Kareem of the Federal College of Forestry, Jos. The leaves were harvested from the stalk, washed with water and dried. The dried leaves were pulverized into powder using mortar and pestle.

Extraction. 50g portion of the powdered material was subjected to exhaustive soxhlet extraction in 200mL of water at 70°C for 72 h. The resultant solution was evaporated to dryness in a water bath maintained at 50-60°C. The extract obtained was stored in a refrigerator until required for use.

Phytochemical screening. The aqueous extract of *Paulinia pinnata* was tested for the presence of various phytochemical constituents using the methods described by Sofowora (1982).

Diuretic activity in rats. Fifteen albino rats weighing about 150g each were divided into 3 groups of 5 rats each. Group 1 (negative control) rats received 0.2ml normal saline. Rats in-group 2 (positive control) received 960mg/kg body weight of urea dissolved in saline. Rats in group 3 (test) received 500 mg/kg of the extract dissolved in saline (chosen as an average dose after a pilot diuretic study in which 5 rats were given graded doses of the extract). All substances were administered orally using an orogastric tube. After dosing, the animals were housed individually in Nalgene metabolic cages and allowed free access to water. Urine outputs were measured after 4h, 18h and 24h (to see if diuresis was time-dependent). Sodium and potassium ion contents were estimated using a flame photometer.

Effects of acute administration of Paulinia pinnata on rat liver and kidney. Fifteen albino rats weighing 46-56g were divided into 3 groups of 5 rats each. Group 1 received distilled water as control. Groups 2 and 3 were given 500 mg/kg and 1000 mg/kg single oral doses of the extract respectively using an orogastric tube. Rats in each group were kept in separate cages and allowed water and food *ad libitum* for 48h. The rats were sacrificed and their liver and kidneys isolated and examined for any morphological changes. Sizes were measured with a graduated straight edge. Kidney lengths were measured from the capsular edge at the level of the 12^{th} thoracic vertebra longitudinally to the end at the 3^{rd} lumbar vertebra while the widths were measured from the hilum horizontally to the other end. Weights were measured on a Mettler analytical balance.

Effects on cat blood pressure. The cats (1.65-2.05 kg) were anaesthetized with 25% urethane at a dose of 6mL/kg body weight intraperitonially (IP). The femoral vein was canulated and connected to a 2mL syringe containing heparinised saline. This site was used for the injection of doses. The trachea was intubated and the carotid artery canulated and connected to a pressure transducer, which is connected to a Washington Recorder whose sensitivity was set at 0.5 and speed of 0.25 mm/s. A Condom Mercury Manometer was used to calibrate the responses before the administration of drugs. Extract doses were injected through the femoral vein and

responses were compared with baseline values.

Analysis of data. All results were presented as mean ± SEM. Student t-test was used to assess statistically significant differences. P values equal or less than 0.05 were considered to be significant.

Results

Phytochemical screening. The phytochemical tests revealed the presence of alkaloids, tannins, saponins and cardiac glycosides and the absence of flavonoids, phlobatannins and cyanogenetic glycosides in the aqueous extract.

Diuretic activity in rats. After 24 h the extract showed statistically significant ($P<0.05$) increase in urine output indicating a diuretic activity. The diuretic activity of urea was brief while that of the extract was of longer duration. The diuretic activity is independent of sodium and potassium metabolism as there was no significant difference in electrolyte concentrations between the treated and control animals (Table 1).

Morphological effects in rats. The extract caused very significant ($P<0.001$) decrease in liver weights at all the doses tested but insignificant decrease in kidney weights was noticed (Table 2a). Similarly, the extract caused very significant ($P<0.001$) decreases in liver sizes and kidney lengths at the doses tested (Table 2b)

Effect on cat blood pressure. The extract decreased blood pressure at lower dose (5.48 mg/kg) far more than at higher doses of 10.96 mg/kg and 16.44 mg/kg (Table 3). Both systolic and diastolic blood pressures were affected.

Table 1. Effect of aqueous leaf extract of *P. pinnata* on urine output and electrolyte excretion in rats.

Treatment (Dose)	Mean urine output(mL) ± SEM (n =5)			Mean electrolyte excretion (meq/L/kg) ± SEM (n =5)	
	4 h	18 h	24 h	Na^+	K^+
N/saline (2mL)	3.00 ± 1.82	13.17 ± 0.88	15.08 ± 0.00	1286.50 ± 206.54	1033.50 ± 140.65
Urea (960 mg/kg)	6.25 ± 0.50	13.17 ± 1.37	13.59 ± 1.33	1390.25 ± 133.41	891.75 ± 179.65
Extract (500 mg/kg)	3.92 ± 1.32	16.66 ± 2.34	21.50 ± 2.47*	1213.50 ± 205.61	1005.00 ± 219.65

*P<0.05

Table 2a. Effect of the leaf extract of *P. pinnata* on liver and kidney weights of rats.

Treatment (Dose)	Mean organ weights (g/kg) ± SEM (n = 5)		
	Liver	Left kidney	Right kidney
Water (2mL)	42.73 ± 2.48	5.43 ± 1.01	5.43 ± 0.99
Extract (500 mg/kg)	34.29 ± 2.71*	4.35 ± 0.16	4.38 ± 0.18
Extract (1000 mg/kg)	34.74 ± 1.37*	4.68 ± 0.22	4.69 ± 0.21

*P<0.001

Table 2b. Effect of the leaf extract of *P. pinnata* on liver and kidney sizes

Treatment (Dose)	Mean liver size (cm/kg) ± SEM (n = 5)		Mean Left kidney size (cm/kg) ± SEM (n = 5)		Mean Right kidney size (cm/kg) ± SEM (n = 5)	
	Length	Width	Length	Width	Length	Width
Water (2mL)	59.96 ± 3.89	38.25 ± 1.98	20.78 ± 0.35	10.12 ± 0.75	20.80 ± 0.52	10.25 ± 0.38
Extract (500 mg/kg)	41.96 ± 1.95*	27.42 ± 2.23*	19.36 ± 0.54	10.23 ± 0.51	19.36 ± 0.51	10.61 ± 0.72
Extract (1000 mg/kg)	34.53 ± 0.99*	25.59 ± 1.36*	17.02 ± 0.75*	8.32 ± 0.74	17.28 ± 0.54*	9.30 ± 0.81

*P<0.001

Table 3. Hypotensive effect of *P. pinnata* extract in cat.

Treatment (Dose)	Mean systolic BP (mmHg) ± SEM (n = 4)	Mean Diastolic BP (mmHg) ± SEM (n = 4)	Mean Pressure (mmHg) ± SEM (n = 4)
Basal	182.02 ± 1.16	133.32 ± 4.18	149.48 ± 2.99
Extract (5.48 mg/kg)	114.77 ± 2.01	73.04 ± 5.31	86.95 ± 4.07
Basal	184.35 ± 2.01	134.49 ± 2.32	151.11 ± 1.69
Extract (10.96 mg/kg)	157.68 ± 2.32	104.35 ± 4.02	122.12 ± 3.37
Basal	180.86 ± 0.00	147.76 ± 2.32	156.13 ± 1.54
Extract (16.44 mg/kg)	159.99 ± 4.02	122.89 ± 2.32	135.21 ± 2.79

Discussion

The idea that the responses of a cell, organ or whole organism to a drug is proportional to the dose of the drug administered is of fundamental importance in drug action (Williamson *et al.*, 1996). Pharmacological investigations therefore nearly always entail the measurement of responses in relation to the dose of the drug.

Crude extracts of medicinal plants are usually a complex mixture of different chemical constituents as evident by the presence of cardiac glycosides, alkaloids, saponins and tannins in this extract. These constituents are usually responsible for the diverse pharmacological actions of the crude extract. The results show that the aqueous extract of the leaves of *Paulinia pinnata* contained chemicals that have diuretic, hypotensive and organotoxic activities.

The diuretic activity lasted over 24h with a statistically significant ($P<0.05$) increase in urine volume, suggesting the presence of a long acting diuretic constituent whose effect and duration is greater than that of large dose (960mg/kg) of the standard diuretic - urea (Table 1). The induced diuretic activity was not accompanied by a corresponding increase in sodium or potassium excretions. This diuretic activity may be by an osmotic diuretic mechanism as put forward by Tanira *et al.*, (1996) in a similar work.

The observed morphological changes strongly suggest that the *Paulinia pinnata* extract caused very significant decrease ($P<0.001$) in liver weights and size and kidney lengths. The kidney weights and widths were insignificantly decreased as summarized in Tables 2a and 2b. These effects are indicative of the presence of organotoxic substances in the extract. However, these effects were obtained at doses far in excess of that used in humans.

Blood pressure measurements revealed that the aqueous extract of *P. pinnata* leaves decreased both systolic and diastolic pressures which were greater at lower (5.48mg/kg) than at higher (10.96mg/kg and 16.44mg/kg) doses. The direct actions of cardiac stimulants especially those mediated by muscarinic stimulation include an important increase in membrane potassium permeability in arterial muscle cells and probably in the cells of the sinoatral nodes as well (Katzung, 1989). These stimulants also decrease the slow inward calcium current in atral and nodal cells of some species.

The observed reduction in systolic and diastolic blood pressures exhibited by the extract may be attributed to either or both of these actions. Since whole animals were used, blood pressure changes may be due to effect of the extract on any of the blood pressure regulating sites from CNS (vasomotor center) to extra-CNS sites such as baroreceptors and actions on the vascular smooth muscles. Several plant extracts have been shown to decrease blood pressure in rodents by different routes (Uguru and Okwuasaba, 1998; Ibarrola *et al.*, 1996).

Plant constituents like tannins and cardiac glycosides have demonstrated diuretic properties which may account for the diuresis observed in this experiment. Alkaloids of

Ephedra roots have demonstrated hypotensive effects (Hikino *et al.*, 1983) which may be due to their vasodilatory effects. This in addition to effects of cardiac glycosides on the heart may contribute to the hypotensive effect on the heart in this experiment.

In conclusion, the results have provided information on the presence of substances in the leaves of *Paulinia pinnata* whose pharmacological actions can be exploited into beneficial effects with clinical applications. However, toxicity studies should be done in detail to exclude toxic substances, which may be present in the plant leaves.

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