Hypotensive Activity of the Ethanol Extract of *Pavetta crassipes* Leaves

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**Pavetta crassipes** leaf is routinely used locally in Nigeria for the management of respiratory disorders and hypertension. The hypotensive and other cardiovascular effects of *Pavetta crassipes* were investigated in cats and rats. The effects of the extract on rat and cat blood pressures, isolated rat atria, rat portal vein, isolated rat aorta and rat vas deferens were studied. Specific receptor antagonists (atropine, mepyramine, phentolamine, propranolol) were used to elucidate the underlying mechanism(s) involved in the cardiovascular changes induced by *P. crassipes*. The results revealed that the ethanolic extract of *Pavetta crassipes* lowered the blood pressures of cats and rats in a dose dependent manner. The extract also caused a concentration-dependent decrease in the force of contraction of the isolated rat atria and rat portal vein. The decreases in blood pressure values were attenuated in the presence of a β-adrenoceptor antagonist, propranolol. The extract also attenuated isoprenaline-induced contraction of the rat atria. However, the extract did not affect contractions evoked by KCl, norepinephrine and 5-HT on the rat aorta. *Pavetta crassipes* contains biologically active substances that may be useful in the management of hypertension.

**Key words** *Pavetta crassipes*; hypotension; rat atria; rat portal vein; cardiovascular activity

Medicinal herbs constitute the cornerstone of traditional medicinal practice worldwide. These herbs are relatively cheap, available and their use depends on ancestral experience.1–3) Medicinal plants represent a great deal of untapped reservoir of drugs and the structural diversity of their component molecules makes a valuable source of novel lead compounds.2,3) There is a growing interest in the utilization of phytoceuticals because many compounds of plant origin are known to possess important phytoceuticals or nutraceutical traits. Natural product scientists are now intensifying efforts towards scientific evaluation of medicinal plants used in traditional remedies. *Pavetta crassipes* K. Schum (Rubiaceae) is widely distributed in the West African sub-region. The leaf is routinely used in Nigeria for the management of respiratory disorders and hypertension in ethnomedical practice (Azija, personal communication). In Nigeria, the plant commonly known as Gadu (Hausa) and Lolubo (Yoruba) is used for food. The inhibitory effects of *Pavetta crassipes* on the gastrointestinal and uterine smooth muscles have been studied.4,5) Amos et al.,5) also reported the anti-inflammatory and muscle relaxant effects of the aqueous extract of *Pavetta crassipes*. There are no reports in the literature on the hypotensive activity of *Pavetta crassipes*. The present study was therefore undertaken to investigate the hypotensive effect of the ethanolic extract of *Pavetta crassipes* leaves in cats and rats as a scientific rationale for their folkloric use as antihypertensive.

**MATERIALS AND METHODS**

**Animals** Swiss albino mice (20—25 g each), Wistar rats (180—220 g each) of either sex maintained at the Animal Facility Centre (AFC) of National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria were used. Adult cats (2.0—3.0 kg each) obtained from Samaru local market were quarantined and used after 28 d. All animals were housed under standard condition of temperature, light (12 h light/12 h dark cycle) and fed on standard diet (Ladokun Feeds Plc, Ibadan) and water *ad libitum*.

**Drug** Propranolol, isoprenaline, pentobarbital, sodium, verapamil, yohimbine, norepinephrine, potassium chloride, chlorpromazine, atropine, acetylcholine, histamine, serotonin were all obtained from Sigma Chemical Co., U.S.A. Mepyramine (M & B, Lagos Nigeria) and methylene blue (Aldrich Chemical Co. Milwaukee, WI, U.S.A.).

**Plant Material** The fresh leaves of *Pavetta crassipes* were collected from Minna, Niger State, Nigeria. The plant material was collected between the months of April and June 2001. Preliminary studies with different collection times were done and activity was highest within the months of April and June. The plant was identified and authenticated by Mr. Abraham Ohaeri (late) and Mallam Ibrahim Muazzam of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. Herbarium specimen (number 4745) was made and deposited at National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria.

**Preparation of Extract** The leaves were cleaned, air-dried for 7 d and crushed into coarse powder using a pestle and mortar. The powdered leaf (500 g) was cold macerated with 2.5 l of ethanol for 24 h with constant shaking using the GFL Shaker (No. 3017 MbH, Germany). The resulting mixture was filtered using Whatman filter paper. The filtrate was concentrated to dryness in vacuo at 40 °C using a rotary evaporator to give a yield of 20% w/w of the extract.

**Pharmacological Evaluation. Direct Blood Pressure Measurement in Cats and Rats** Normal rats and cats were used in this study. These animals were anaesthetized using sodium pentobarbital (40 mg/kg i.p.). At the stage of...
surgical anaesthesia, the trachea was intubated to facilitate spontaneous respiration. The femoral vein was cannulated with heparinized polyethylene tubing (PE-50) for intravenous injection of the test materials and drugs, while the carotid artery was cannulated and connected to a Bentley Trantec Blood Pressure Transducer for blood pressure recording on Ugo Basile Microdynamometer 7050. Temperature was maintained at 37 ± 1°C by means of thermostatically controlled dissecting table. Animals whose blood pressure fluctuates by more than 10% within the first 30 min of recording were discarded. After a 30 min equilibration, intravenous administration of the extract at doses of 1, 2, 4, 8, 16 and 32 mg/kg and other drugs were carried out slowly for about 30 s. The maximum volume of extract injected was not greater than 0.4 ml. This is to help check medullary influences on the blood pressure.6—9)

**Pharmacological Studies** Rats and cats were anaesthetized and prepared for experimentation as outlined above. α-adrenoceptor, β-adrenoceptor, cholinergic receptor, histamine and serotonin receptor blockades were established by using their specific blockers—phenotolamine (2 mg/kg), propranolol (2 mg/kg), atropine (2 mg/kg), mepyramine (1 mg/kg) and cimetidine (10 mg/kg) and methysergide (1 mg/kg) respectively. The antagonist was given slowly, as soon as the mean arterial pressure stabilized, the agonist was then administered. If the agonist action was blocked, a test dose of the extract (4 mg/kg) was then administered immediately. Methoxamine (50 mg/kg), isoprenaline (0.2 mg/kg), acetylcholine (2 µg/kg), histamine (2 µg/kg) and serotonin (2 µg/kg) served as the α, β, cholinergic, histaminergic and serotoninergic receptor agonists respectively.7,10) The possible interaction between the hypotensive action of the extract and nitrous oxide (NO) release was studied by infusing methylene blue (0.7 mg/kg) and after 10 min, the extract was administered. If the hypertensive effect of the extract was blocked, a test dose of the extract at doses of 1, 2, 4, 8, 16 and 32 mg/kg and other drugs were carried out slowly for about 30 s. The maximum volume of extract injected was not greater than 0.4 ml. This is to help check medullary influences on the blood pressure.6—9)

**Studies on Isolated Rat Atria** Adult Wistar rats of either sex were killed and exsanguinated. The thoracic region was opened, the heart was rapidly removed and placed in Locke’s solution of the following composition (mM): NaCl 153.8, KCl 5.6, CaCl₂ 2.1, NaHCO₃ 5.9 and glucose 5.5. This was kept at a temperature of 30 ± 1°C and aerated with 100% oxygen. The atria both right and left were carefully dissected out and mounted in an organ bath containing 20 ml Locke’s solution. A resting load of 0.5 g was applied and the tissue was allowed to equilibrate for a period of 60 min during which the physiological solution was changed every 15 min. The effect of the extract was recorded on the Ugo Basile Unirecorder 7050 via an isometric transducer 7004. Furthermore, the inhibitory effect of the extract on isoprenaline and calcium chloride-induced contractions were investigated.

**Studies on the Rat Isolated Portal Vein** Adult Wistar rats were used. The rats were killed by a blow on the head and exsanguinated. The abdomen was opened and the portal vein was isolated. Each isolated portal vein was mounted in a 20 ml organ bath containing Kreb’s solution at 37 ± 1°C and aerated continuously with 95% O₂ and 5% CO₂. The tissue was allowed to equilibrate for a period of 60 min during which the physiological solution was changed every 15 min. A resting load of 0.5 g was applied. The spontaneous rhythmic myogenic contractions of the tissue and the effect of the extract on the intrinsic myogenic activity and on KCl, norepinephrine-induced contractions of the preparations were recorded isometrically by means of an Ugo Basile Unirecorder.12,13)

**Studies Using Isolated Rat Aortic Rings** Aortic rings (2—4 min) were obtained free from connective tissue and fat. The tissues were suspended by platinum hooks for isometric recording in Kreb’s-Henseleit solution of the following composition (mM): NaCl 118.8, KCl 4.7, MgSO₄ 1.18, KH₂PO₄ 1.18, NaHCO₃ 25, glucose 11, CaCl₂ 1.25. This was maintained at 37 ± 1°C and gassed with a mixture of 95% O₂ and 5% CO₂. The rings were allowed to equilibrate for 1 h under a resting tension of 1 g. During this time, the bathing medium was changed every 15 min to prevent against interfering metabolites.14) The effect of the extract on the aortic rings were recorded on Ugo Basile Unirecorder 7050 via an isometric transducer 7004. The effect of the extract on KCl-induced and norepinephrine-induced contractions of the aorta was investigated.15) In another set of experiments, the effect of the extract on phenylephrine and 5-HT were investigated.16)

**Studies on the Isolated Rat Vas Deferens** Adult male rats were killed and bled. The abdomen was opened along the midline and the intestine moved to one side. The vas deferens on each side was cut. The mesentery was trimmed off. Each vasa was suspended in a 20 ml organ bath containing Kreb’s solution aerated with 95% O₂ and 5% CO₂. The tissue was maintained at 37 ± 1°C. Responses due to norepinephrine alone and in combination with increasing concentrations of the extract were recorded on Ugo Basile Unirecorder 7050 via an isometric transducer 7004.

**Statistical Analysis** Data were represented as mean±S.E.M. and analyzed using one-way analysis of variance (ANOVA) and further comparisons done using the Student’s t-test. The level of significance was taken at p<0.05.

**RESULTS**

**Effect on Cat and Rat Blood Pressure** The ethanol extract of *Pavetta crassipes* at doses of 1—32 mg/kg i.v. caused an immediate hypotensive response. The decrease in blood pressure was dose-dependent and significantly (p<0.05) dif-

**Fig. 1.** Physiological Tracing of the Dose Dependent Effect of the Ethanol Extract of *Pavetta crassipes* (1—32 mg/kg i.v.) on Normotensive Cats

Filled circles (●) represent the point of drug administration.
different from control. A dose of 4 mg/kg i.v. caused a reduction in mean arterial pressure of $36.67 \pm 1.5$ mmHg and $32.5 \pm 2.3$ mmHg in cats and rats, respectively (Figs. 1, 2). To determine the underlying mechanism(s) mediating the hypotensive effect of *P. crassipes* we used specific receptor antagonists. In the studies on cholinergic transmission, $\alpha$-adrenoceptor, histaminergic, serotonergic and nitrous oxide activities, it was observed that the decrease in mean arterial blood pressure in the presence of these antagonists was not significantly different from control. In the presence of $\beta$-adrenoceptor blocker propranolol, the decrease in mean arterial pressure caused by the extract was blocked (Fig. 3).

**Effect on the Rat Atria** Studies on the atria were carried out to further investigate direct effect on heart contractility and beta-adrenergic influence on the heart. It was observed that the extract of *Pavetta crassipes* (0.05—3.2 mg/ml) caused a concentration-dependent decrease in the force of contraction of the spontaneous beating atria. The extract did not affect the frequency of contraction (Fig. 4). The extract (1.6—3.2 mg/ml) did not attenuate calcium-induced increase in contraction of the atria. However, the extract attenuated isoprenaline-induced contraction of the atria (Figs. 5, 6).

**Effect on the Rat Portal Vein** The extract at concentration of 0.05—3.2 mg/ml caused a concentration-dependent fall in the force of myogenic rhythmic contraction of the rat
portal vein. The extract also prolonged the frequency of contraction at higher concentrations (0.8—3.2 mg/ml) (Fig. 7). The extract did not attenuate KCl-induced contractions of the portal vein (Fig. 8).

**Effect on Rat Aorta and Rat Vas Deferens** These tissues are rich in α-adrenergic and serotoninergic receptors. They were studied to investigate any possible α-adrenergic and serotoninergic inhibitions that might be mediated by the extract. Added alone, the extract did not show any observable response on the rat aorta. The extract did not attenuate 5-HT,
phenylephrine-induced contractions of the rat aorta (Figs. 9, 10). It was also observed that the extract of *Pavetta crassipes* at concentrations of 1.6 mg/ml and 3.2 mg/ml did not affect norepinephrine-induced contractions of the rat vas deferens (Fig. 11).

**DISCUSSION**

The present study was designed to evaluate the cardiovascular effects of the ethanol extract of *Pavetta crassipes* and to try to ascertain the possible mechanism of its hypotensive action. The ethanol extract of *Pavetta crassipes* extract exhibited a significant dose-dependent decrease in the mean arterial pressure in cats and rats. The decrease in blood pressure was immediate upon intravenous administration of the extract and was sustained at high doses. The immediate effect on blood pressure seems to suggest action on the blood vessels or directly on the cardiac muscles. The results obtained in this study were similar to other reports on hypotensive effects of some naturally occurring plant extracts.  

As evidenced from the pharmacological antagonists studies, the hypotensive effects of the ethanol extract of *Pavetta crassipes* was not attenuated by cholinergic, α-adrenoceptor, histaminergic, serotonergic receptor activity. This suggests that the hypotensive effect is not mediated via any of these receptor mechanisms. Furthermore, it was observed that the hypotensive effect of the extract was not significantly affected by methylene blue suggesting that the extract did not act by enhancing release of NO. The hypotensive effect of the extract was blocked by pre-treatment with propranolol suggesting a beta adrenoceptor activity.

![Fig. 7. Effect of *Pavetta crassipes* (0.05—3.2 mg/ml) on the Intrinsic Myogenic Contraction of the Rat Portal Vein](image)

![Fig. 8. Effect of *Pavetta crassipes* on KCl (40 mM) Induced Contraction of the Rat Portal Vein](image)

![Fig. 9. Effect of *Pavetta crassipes* on 5-HT Induced Contraction of the Rat Aorta](image)

5-HT (■), 5-HT + *Pavetta crassipes* 1.6 mg/ml (▲), 5-HT + *Pavetta crassipes* 3.2 mg/ml (●), 5-HT + methysergide 10 ng/ml (●). Each point represents a mean of 7 readings.
Calcium chloride-induced contraction of the rat atria was not attenuated by pre-incubation with the extract, but it was observed that the extract attenuated isoprenaline-induced contraction, thus further strengthening the involvement of beta adrenoceptor mechanism or a system that synergizes with beta adrenoceptor. Furthermore, the extract did not affect KCl and norepinephrine-induced contraction of the portal vein and the rat aortic strips indicating that extract did not block calcium influx.\textsuperscript{21,22} Similarly, the extract did not attenuate phenylephrine and 5-HT-induced contractions of the rat aortic strips, suggesting that the extract lack $\alpha_1$-adrenoceptor and serotonergic receptor activity. The activation of the rat aortas by phenylephrine is $\alpha_1$-adrenoceptor mediated,\textsuperscript{23,24} thus ruling out the involvement of $\alpha_1$-adrenoceptor mecha-
Nakaki et al.\textsuperscript{25} reported that serotonin-induced contraction of the rat aorta was due to activation of the 5-HT\textsubscript{2}-receptor subtype. Since the extract could not attenuate 5-HT-induced contraction of the rat aorta, could suggest that 5-HT\textsubscript{2} subtype might not be involved in the observed pharmacological effects of the extract.

On the rat vas deferens, the extract did not attenuate noradrenaline-induced contractions. Activation of \(\alpha\textsubscript{1}\)-adrenoceptors by agonist is known to open receptor-operated calcium channels leading to influx of extracellular calcium and release of intracellular calcium,\textsuperscript{26} thus suggesting the non-involvement of \(\alpha\textsubscript{1}\)-adrenoceptors or interference with the mobilization of calcium or the catecholamine-induced contraction.

In conclusion the extract of \textit{Pavetta crassipes} possesses hypotensive activity and this might be mediated via \(\beta\)-adrenoceptors mechanisms or a system that synergizes with beta adrenoceptors. The hypotensive action of \textit{Pavetta crassipes} described in this study may explain the traditional use of this herb in the management of hypertension.

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