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HYPOTENSIVE EFFECT OF AQUEOUS EXTRACT OF THE LEAVES OF *PHYLLANTHUS AMARUS* SCHUM AND THONN (*EUPHORBIACEAE*).

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Abstract: The plant of *Phyllanthus ama*rus is used as diuretic and to lower blood pressure in traditional medicine practice. The effect of the aqueous extract of the leaves of *Phyllanthus amarus* on blood pressure was evaluated in normotensive male rabbits. Intravenously administered aqueous doses (5 mg to 80 mg/kg) of the extract to anaesthesized normotensive male rabbits produced a significant fall in mean diastolic, systolic and mean arterial pressures in a graded dose response manner. The dose of 5 mg/kg produced the least hypotensive effect, causing a fall in mean diastolic, systolic, and mean arterial pressure of 13.3 ± 3.1, 19.7 ± 5.4, and14.3 ± 3.4 mmHg, respectively, while the dose of 80 mg/kg produced the greatest fall in mean diastolic, systolic, and mean arterial pressure of 49.7 ± 7.9, 45.5 ± 9.5, and 48.00 ± 6.5 mmHg, respectively. The extract had a greater blood pressure depressant effect on the diastolic blood pressure than on the systolic blood pressure. The highest dose of 80 mg/kg caused 62.5% fall in diastolic blood pressure, compared to the 33.2% fall in systolic blood pressure caused by the same dose. Atropine at the dose of 1 mg/kg blocked the hypotensive effect of the aqueous extract in a competitive manner. Promethazine at the dose of 1 mg/kg did not block the hypotensive effect of the aqueous extract, but potentiated the effect of the extract. After the administration of promethazine, the maximum tolerable dose of the extract was 40 mg/kg as compared to the initial dose of 80 mg/kg. The extract was found to decrease both the force and rate of myocardial contraction in a concentration dependent manner. The extract also dose dependently inhibited the intrinsic myogenic contraction of isolated rat portal vein.
The results obtained show that the extract has blood pressure lowering effect which may be by the combined effects of myocardial depression, muscarinic receptor mediated vascular smooth muscle relaxation and by the calcium channel ion blockade in vascular smooth muscle.

Keywords: *Phyllanthus amarus*, extract, mean arterial pressure, hypotensive effect, myogenic contraction, normotensive

Medicinal plants have over the years constituted indispensable tools for research and development of new drugs (1, 2), and coupled with the fact that there are still many plants whose medicinal values have not been exploited, it is reasonable to describe the plant kingdom as a sleeping giant for potential drug development (3).

The *Phyllanthus* genus (family *euphorbiaceae)* has a central role in the development of new drugs. It contains over 600 species of shrubs, trees, and annual and bi-annual herbs distributed throughout the tropical and subtropical regions of the world (4). The most popular species are *Phyllanthus niruri, Phyllanthus sellowianus, Phyllanthus fraternus and Phyllanthus amarus*.

Phyllanthus amarus is a small erect annual herb that grows up to 15 – 50 cm high. It is indigenous to the rainforest of the Amazon and other tropical areas of the world including the Bahamas, southern India, and Africa (4).

Phyllanthus amarus has a long history of use in herbal medicine in every country where it grows. Most times it is employed for the same purpose in different places. The natural remedy is usually an infusion or a decoction of the whole plant or its aerial parts. Traditionally in herbal medicine, it is employed in the treatment of biliary and urinary conditions, such as gall bladder stones, viral hepatitis, flu, cold, jaundice, liver cancer, tuberculosis, prostatitis, venereal diseases, urinary tract infections, diabetes, hypertension, and pains, it is also used as an antispasmodic (4).

Many works have been published on the pharmacological properties of the *Phyllanthus* genus, especially on *Phyllanthus amarus*. The infusion of the aerial parts has been reported to be used to manage non-insulin dependent diabetes mellitus, for the relief of stomach ache and to treat dysentery (5). The plant extract has also been reported to have antitumor and anticarcinogenic activity (6). As a con-

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stituent of a multiherbal preparation, *Phyllanthus amarus* inhibited gastrointestinal tract motility in mice (7). The plant was reported to lack hypotensive and cardiac depressant effects (8). The powdered leaf materials of *Phyllanthus amarus* formulated into capsules and given to human subjects has been reported to lower blood pressure and increase urine volume in human subjects (9).

This work reports the hypotensive effect of the aqueous extract of the leaves of *Phyllanthus amarus* on normotensive adult male rabbits, and the possible mechanism by which the extract affects blood pressure.

EXPERIMENTAL

Preparation of plant extract

Fresh leaves of *Phyllanthus amarus* were collected around the premises of University of Benin between the months of July and September (2001). A sample of the leaf was identified in the of Department of Pharmacognosy, and it was confirmed at the Forestry Research Institute of Nigeria (FRIN), Ibadan, where a herbarium specimen (number SH107456) has been deposited. The leaves were air-dried for 7 days. The dried leaves were reduced to fine powder using a Viking Joncod electric milling machine (model: 9809005). The powdered plant material (500 g) was extracted using a Soxhlet apparatus and this gave a yield of about 17%. The extract was concentrated to a solid mass. A specific weight of the extract was dissolved in an appropriate volume of normal saline to obtain a stock solution of the desired concentration from which calculated doses were administered to the animals during the various experimental procedures.

Phytochemical tests

Phytochemical tests were carried out on the plant to identify the presence of plant secondary metabolite such as glycosides, alkaloids, saponins, tannins and flavonoids.

Animals

Adult male rabbits weighing between 1.2-2.0 kg, and adult albino Wistar rats weighing between 150-200 g were used for the experiments. Approval was obtained from the Faculty of Pharmacy Ethical Committee, University of Benin, Benin City, Nigeria. The animals were obtained from the animal house of the Department of Pharmacology and Toxicology, University of Benin, where they were fed with standard rabbit pellets and mouse cubes (Livestock Feed) and allowed free access to water.

Effect of extract on blood pressure and heart rate

Each rabbit was anaesthetized with pentobarbital at the dose of 40 mg/kg, administered intravenously through the marginal ear vein, previously canulated with 21-G butterfly canula. The trachea was canulated with a plastic canula to ease respiration. The vagus nerve was located and separated from the carotid artery, which was cleared of connective tissues and canulated with a plastic canula connected to a pressure transducer via a three-way tap. The pressure transducer transmitted the blood pressure to Ugo Basile twin channel recorder (model 7090), which recorded the blood pressure and heart rate. The channel recorder was always calibrated before and after each experiment, using a mercury sphygmomanometer. When the animal had stabilized and the blood pressure and heart rate had remained constant, the extract was administered in graded doses of 5 mg/kg, 10 mg/kg, 20 mg/kg, 40 mg/kg and 80 mg/kg. The effects of the graded doses of the extract on blood pressure and heart rate were recorded.

Atropine sulfate and promethazine maleate were each administered at the dose of 1 mg/kg to the rabbits. Twenty minutes after each drug administration, the extract was again administered in graded doses as above and the effects on blood pressure and heart rate were recorded.

Effect of extract on isolated perfused heart of rabbit

This experiment was carried out in accordance with the Langendorff, procedure (10). A rabbit was injected with 1000 IU of heparin intravenously through the marginal ear vein. Five minutes later, the rabbit was sacrificed, dissected and the heart with about 1 cm of the aorta attached, was removed as quickly as possible and transferred into a Petri-dish containing Ringer-Locke solution (NaCl; 45.0 g, $NaHCO_3$; 1.0 g, D-glucose; 5.0 g, KCl; 2.1 g, $CaCl_2 \cdot 2H_2O$; 1.6 g, in 5 L of distilled water). The heart preparation was gently squeezed several times to remove as much of residual blood as possible. The aorta was cut just below the carotid bifurcation. The heart was transferred to the perfusion apparatus and tied to the glass canula. A small spring clip was attached to the apex of the ventricle and connected to a force displacement transducer (model 82125) via two pulleys. The perfusion fluid was Ringer-Locke solution, which was continuously bubbled with oxygen. The fluid was applied at constant flow rate from a reservoir maintained at 37^{o}C by water circulated through thermostated water bath. The extract was administered in the perfusion in graded concentrations of 0.1 mg/mL, 0.2 mg/mL and 0.4 mg/mL.

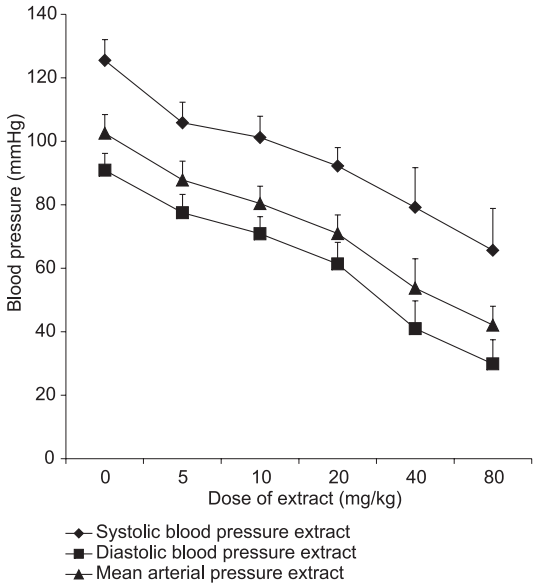

Figure 1. The effect of extract on systolic, diastolic, and mean arterial pressure.
The extract has a graded dose lowering effect on both the systolic, diastolic blood pressures, and also on mean arterial pressures, with a greater blood pressure lowering effect on the diastolic than on the systolic blood pressure. The effect was significant at 40 mg /kg for the systolic blood pressure, and at 20 mg/kg for the diastolic blood pressure and the mean arterial pressure, respectively. ($p < 0.05$), n = 5. Each point represents the mean ± S.E.M.

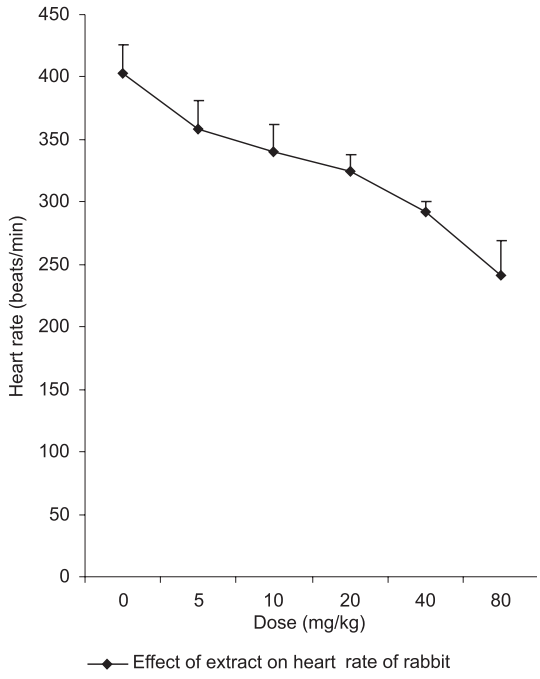

Figure 2. The effect of extract on heart rate.
The corresponding depressant effect of the extract on the heart rate is dose dependent and significant as from the dose of 20 mg/kg, ($p < 0.5$). Each point represents the mean ± S.E.M. (n = 5).

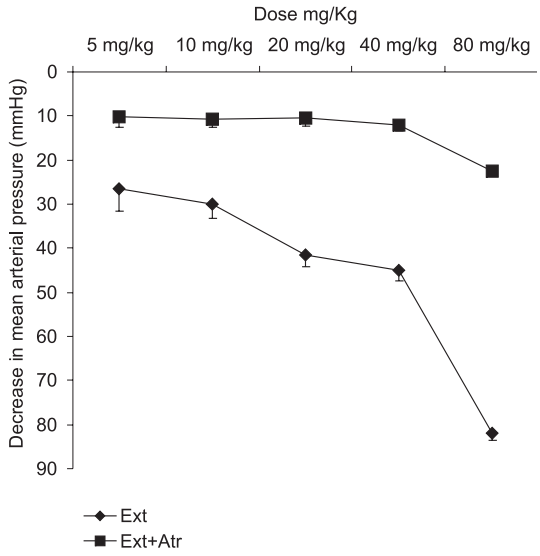

Figure 3. The effect of extract on mean arterial pressure of rabbit, before and after administration of atropine.
This shows a complete obliteration of the hypotensive effects of the extract by 1 mg/kg atropine up to 40 mg/kg dose of extract, beyond which the hypotensive effect of the extract started manifesting, suggestive of competitive blockade. Each point represents the mean ± S.E.M. (n = 5)

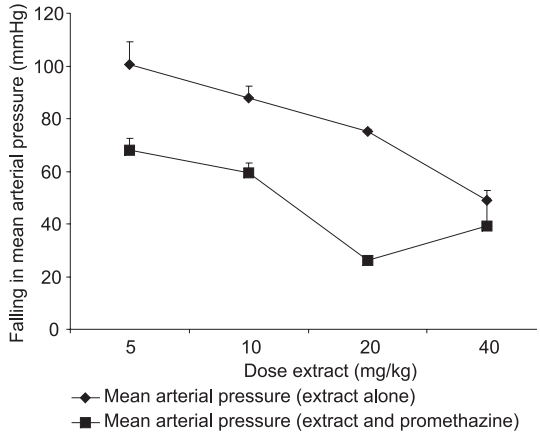

Figure 4. The effect of extract on mean arterial blood pressure in before and after the administration of promethazine maleate (1 mg/kg).
Promethazine did not block the hypotensive, but tend to potentiate the toxicity of the extract because the maximal tolerable dose as shown above after the administration of promethazine was 40 mg/kg. Each point represents the mean ± S.E.M. (n = 5).

Effect of extract on myogenic contraction of isolated rat portal vein

Portal vein was isolated from rat (10) and set up in organ bath containing Krebs solution (NaCl; 34.5 g, $NaHCO_3$; 10.5 g, D-glucose; 10.0 g, NaH_2PO_4; 0.72 g, $MgSO_4$; 1.45 g $CaCl_2 \cdot 2H_2O$; 3.7 g in 5 L of distilled water). The tissue was continu-

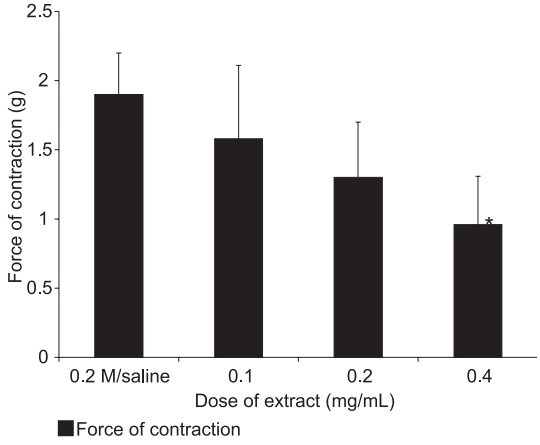

Figure 5. The effect of extract on force of myocardial contraction of an isolated rabbit heart.
The extract only had a minimal depressant effect on force of contraction of isolated heart at the concentrations used, being significant at 0.4 mg/mL ($p < 0.05$), n = 4. Each point represents the mean ± S.E.M.

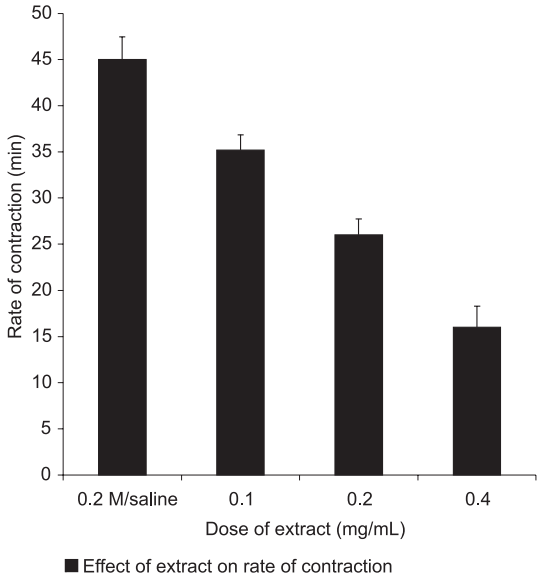

Figure 6. The effect of the extract on the rate of myocardial contraction of isolated rabbit heart.
The extract has a greater effect on rate of contraction of isolated rabbit heart, compared to the effect on force of contraction in Fig. 6, which was significant as from the concentration of 0.2 mg/mL, ($p < 0.05$), n = 4. Each point represents the mean ± S.E.M.

ously bubbled with a mixture of 95% oxygen and 5% carbon dioxide. The tissue was allowed to stabilize and the myogenic rhythmic contraction became steady. The extract was then added into the organ bath to give graded bath concentrations of 0.71 mg/mL, 1.4 mg/mL, 2.9 mg/mL, 5.7 mg/ml, 11.4 mg/mL and 22.9 mg/L.

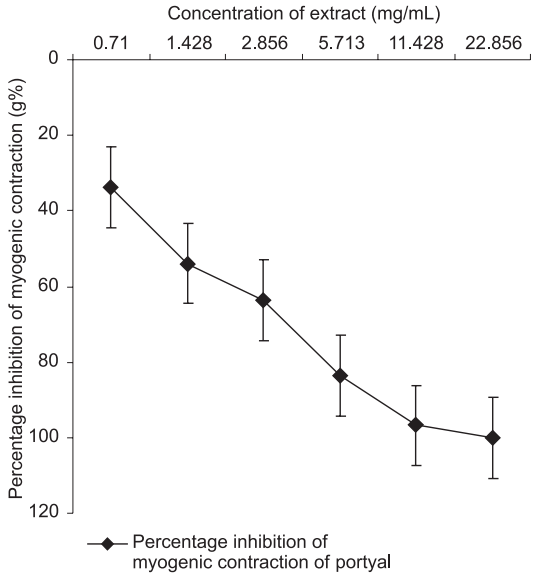

Figure 7. The percentage inhibition effect of the extract myogenic contraction of isolated rat portal vein.
This shows the concentration dependent relaxation and percentage inhibition of force of myogenic contraction of rat portal vein by extract, with complete relaxation and 100% inhibition occurring at 22.9 mg/mL. Each point represents the mean ± S.E.M. The inhibition was significant as from the dose of 2.856 mg/mL, ($p < 0.05$), n = 4.

RESULTS

The phytochemical tests gave positive reactions for the presence of glycosides, saponins, tanins and flavonoids, while the test for alkaloids was negative.

The extract lowered the systolic, diastolic and the mean arterial pressure of normotensive rabbits in a dose dependent manner (Figure 1). The dose of 5 mg/kg caused the least fall in blood pressure, reducing the systolic blood pressure from initial 125.5 ± 9.4 mmHg, to 105.8 ± 6.5 mmHg, and the diastolic from an initial level of 90.83 ± 7.4 mmHg to 77.5 ± 5.9 mmHg. While the dose of 80 mg/kg caused the greatest fall from an initial 111.2 ± 14.2 mmHg to 45.5 ± 9.5 mmHg for the systolic, and from 79.5 ± 12.4 mmHg to 29.8 ±.4 mmHg for the diastolic blood pressure (Figure 1). The mean arterial pressure dropped from an initial level of 102.5 ± 4.7 mmHg to 87.8 ± 5.2 for 5 mg/kg, and from 90.1 ± 6.4 mmHg to 42.1 ± 6.9 mmHg for the dose of 80 mg/kg (Figure 2). There was also a significant decrease in the heart rate of the rabbits following the administration of the graded doses of the extract. The heart rate dropped from an initial level of 402.4 ± 23.5 beats/min to 358.9 ± 22.8 beats/min for 5 mg/kg, and to 240.9 ± 28.7 beats/min, for 80 mg/kg (Figure 2). Atropine sulfate (1 mg/kg) blocked the

hypotensive effect of the extract, while promethazine at the dose of 1 mg/kg did not block the effect of the extract (Figures 3 and 4). The extract reduced the force and rate of contraction of isolated rabbit heart, with a greater effect on the rate than on the force of contraction (Figures 5 and 6). The extract also inhibited the intrinsic myogenic contraction of the isolated rat's portal vein in a concentration dependent manner (Figure 7).

DISCUSSION

The results of various experiments carried out showed that the extract has blood pressure lowering effect. The extract lowered the systolic, diastolic and mean arterial pressure of normotensive rabbits in a dose dependent manner. The extract exerted a greater blood pressure lowering effect on the diastolic blood pressure than on the systolic blood pressure (Figure 1). The highest dose of 80 mg/kg caused 62.5% fall in diastolic pressure, compared to 33.2% fall in systolic pressure by the same dose. It is interesting to note that this kind of blood pressure lowering effect has been observed with isoprenaline, a beta-receptor agonist with a potent vasodilating property, marked increase in cardiac output and a greater fall in diastolic blood pressure (12, 13). The extract depresses the heart rate of rabbits in a graded dose response manner, from an initial level of 402.4 ± 23.5 beats/min, to 240.9 ± 28.7 beats/min at the maximal intravenous dose of 80 mg/kg (Figure 3). Both the rate and force of myocardial contractility were depressed in a concentration dependent manner, with a greater effect on the rate than on the force of contraction (Figures 5 and 6). The myogenic contraction of rat portal vein has been described to result from the mobilization and utilization of extra-cellular calcium ions through the calcium channels (11). The extract also inhibited this myogenic contraction of rat portal vein, in a concentration dependent manner (Figure 7). Pharmacologically, orthodox anti-hypertensive and crude drugs exert hypotensive effects by various mechanisms. Anti-hypertensive drugs could lower blood pressure by a direct effect on the heart, in which case the force and rate of myocardial contraction are decreased, e.g. propranolol, a beta-receptor antagonist, some may relax the vascular smooth muscle, in which case the total peripheral resistance is decreased; e.g. alpha adrenoceptor antagonists like prazosin, and nifedipine a calcium ion channel blocker. This study has shown that the hypotensive effect of the aqueous extract of the leaves of *Phyllanthus amarus* may be by a combination of direct decrease in myocardial contractility, and by the relaxation of vascular smooth muscle, through various mechanisms. The blood pressure lowering effect of the extract was blocked by atropine sulfate (1 mg/kg), but not blocked by promethazine maleate (1 mg/kg). This is an indication that the extract may be relaxing vascular smooth muscle via muscarinic receptor stimulation, with the possible release of endothelium derived relaxing factor (EDRF), and possibly by the inhibition of the influx and utilization of extra-cellular calcium ions through the calcium channels.

With the results obtained from the various experiment carried out it follows that the extract has blood pressure lowering effect, which may be by the combined effects of vascular smooth muscle relaxation, occasioned by both the muscarinic receptor mediated release of endothelium derived relaxing factor, inhibition of influx and utilization of extracellular calcium ion through the vascular smooth muscle, and by the depression of both the force and rate of contraction of myocardium.

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