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Original Research Article

Blood pressure lowering effect of the aqueous extract of the aerial parts of *Euphorbia hirta* Linn (Euphorbiaceae) in normotensive Wistar rats

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Abstract

Purpose: The aqueous extract of *Euphorbia hirta* (*E. hirta*) was evaluated for hypotensive effect in normotensive Wistar rats at the dose of 0.625-40 mg/kg.
Methods: The aqueous extract of the aerial parts of *E. hirta* was evaluated for hypotensive effects in thiopentone/urethane (20/1250 mg/kg) anaesthetized normotensive Wistar rats in the presence and absence of atropine (1.0 mg/kg), at the graded doses of 2.5-40 mg/kg.
Results: The aqueous extract of *E. hirta* caused a dose dependent decrease in the systolic, diastolic and mean arterial pressure in normotensive Wistar rats. The extract significantly reduced the systolic blood pressure from a resting blood pressure levels of 125.63 ± 4.27 to 1363.6 ± 2.92 ($p < 0.01$), 107.41 ± 5.45 ($p < 0.05$), 102.03 ± 4.61 ($p < 0.01$), 98.93 ± 4.57 ($p < 0.01$) at the doses of 0.625, 5.0, 10.0, 20.0 and 40.0 mg/kg respectively, while the effect of the extract at the doses of 1.25 and 2.5 mg/kg respectively of 114.43 ± 6.36 and 113.8 ± 5.76 mmHg were not significant ($p > 0.05$).
Atropine (1 mg/kg) abolished the dose dependent hypotensive effect of the extract.
Conclusion: We conclude that the aqueous extract of *E. hirta* has hypotensive effect in normotensive Wistar rats.

Keywords: Blood pressure, normotensive, *Euphorbia hirta*, mean arterial pressure, thoracic aorta

Indexing: Index Copernicus, African Index Medicus

Introduction

Euphorbia hirta (*E. hirta*) Euphorbiaceae is an annual hairy plant with a slender stem that has many branches at the base [1], and indigenous to the tropical Americas, with a worldwide distribution to many temperate and tropical countries of the world, like India, Asia, Australia and Africa. Its common names include: asthma and milk weed [2], Nonan kurchiya in Hausa, Udani in Igbo and Akun esan in Yoruba [3]. *E. hirta* is listed as an official drug in African Pharmacopeia for the treatment of itching, to facilitate child birth, and treat dysentery [5].

E. hirta is an important valuable medicinal plant used by various communities in China, Malaysia, South and West Africa [6], prepared in various forms to treat respiratory disorders like asthma, bronchitis, laryngeal spasm, coughs and colds, and gastrointestinal disorders like worm infestation among others [5]. The plant has also been reported to have anxiolytic, analgesic, antipyretic and anti-inflammatory activities [1]. Some communities in Western, Mid-Western, and Eastern parts of Nigeria; Ghana in West Africa, and India [3; 6], have reported the use of *E. hirta* to treat jaundice,

hypertension, anemia and malaria, cough, asthma, and anti-fertility. The plant has also been reported to be used as an aphrodisiac, to facilitate childbirth by traditional birth attendants, and also induce lactation as a galactagogue [7; 8].

The effect of *Euphorbia hirta* on blood pressure was first evaluated between 1968 and 1971, with conflicting reports. While a group of Indian researchers reported the hypotensive effect of 250 mg/kg ethanol-water (1:1) extract of *Euphorbia hirta* in dogs [8], a separate group of researchers in Thailand reported that the ethanol-water (1:1) extract of *Euphorbia hirta* had no blood pressure lowering effect in dogs, but demonstrated cardiotonic effect in human subjects [9]. This paper, therefore, reports the results of the comprehensive evaluation of the effect of the aqueous extract of *Euphorbia hirta* on the blood pressure of normotensive Wistar rats.

Methods

Collection and extraction of plant material

The whole plant of *E. hirta* was collected within the premises of the University of Benin, Benin City, Edo State, between the months of June through to October 2013, and between November 2014 and January 2015. Sample of the plant was identified by Mr. Sonny Nweke of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, and authenticated by Professor Akinibosun of the Department of Plant Biology and Biotechnology with a herbarium specimen voucher UBH-E023. The plant material was washed to remove earthy materials and air dried under shade for two weeks. The aerial parts (the stalk, leaves and the flowers) was pulverized with an electric milling machine at the Department of Pharmacognosy, University of Benin.

About 350 g of the pulverized material was extracted exhaustively with 1.5 L of distilled water for about 5 hours, using Soxhlet apparatus. The extract obtained was concentrated under pressure with a rotary evaporator to a slurry semi-solid paste, which was further concentrated to dryness in an oven at about 35°C for 72 hours. A weighed quantity of the extract was dissolved in normal saline and 0.1% Tween 80 to obtain a stock solution of 50 mg/mL, from which dilutions of desired concentration were made for the administration of calculated doses to the experimental animals.

Animal experiment

Evaluation of extract for hypotensive effect

Adult Wistar rats (220-250 g) were used for the evaluation of extract for hypotensive activity. The rats were obtained from the animal house of the Department of Pharmacology and Toxicology, University of Benin. Ethical approval was obtained from the Ethical Committee of the Faculty of Pharmacy, University of Benin on the use of animals for laboratory experiments, and the animals were handled according to standard protocols for the use of animals (National Institute of Health, USA; Public Health Service Policy on Human Care and Use of Laboratory Animals, 2002).

Each rat was anaesthetized with Urethane/Thiopentone (1250/20 mg/kg) administered intraperitoneally and prepared for blood pressure evaluation following the procedure previously described [10]. Under full anaesthesia, the rat was fastened to the dissecting table with the aid of masking tape. The cervical region was dissected and the trachea was isolated, cleared of connective tissues and intubated with a polythene cannula to complement the respiration of the animals.

One of the carotid arteries was isolated and carefully cleared of connective tissues. The end of the carotid artery towards the head of the rat was ligated with a soft cotton thread to prevent flow back of blood from the head, while a bulldog artery clip was applied to occlude the carotid artery at a small distance away from the heart. A portion of the carotid artery between the ligated and the occluded ends was nipped open and cannulated with heparinized saline filled Teflon tubing (22 Ga. Lightweight- 0.28”, Wall-0.006”).

The Teflon tubing was connected via a three-way tap to a Ugo Basile Bentley Trantec pressure transducer (Model: 800; no: 62327), which was connected to Ugo Basile Uni-channel recorder (Model 7040) for blood pressure recording. The Uni-channel recorder was always calibrated before and after each experiment using a mercury sphygmomanometer. When the

animal had stabilized and all the measurable variables were constant, the extract was administered to the animal at the graded doses of 0.625, 1.25, 2.5, 5, 10, 20 and 40 mg/kg.

The extract was evaluated for hypotensive effect in the presence of atropine, to ascertain the possible involvement of the parasympathetic pathway. A group of seven (7) Wistar rats were used for this screening. The animals were prepared for blood pressure as described above, and when the animals had stabilized under anesthesia and all measureable variables were observed to be constant, atropine (1.0 mg/kg) was administered intravenously to the animals [10]. After 10 minutes following the administration of atropine, the extract was administered in graded doses to the animals as earlier described, and the effect on blood pressure was monitored and recorded.

Preliminary evaluation of the effect of the extract on rat thoracic aorta

A preliminary evaluation of the effect of the extract on the contractility of rat thoracic aorta with intact endothelium, and denuded endothelium was carried out for the purpose of hypothesis on the possible mechanism of action following the protocol as previously described [11]. A rat was sacrificed by cervical decapitation. The abdominal region was dissected and the thoracic aorta isolated. The aorta was placed in a petri dish previously filled with Krebs solution (NaCl: 6.9g; $NaHCO_3$: 2.1g; D-glucose: 2.0g; KH_2PO_4: 0.16g; KCl: 0.36g; $MgSO_4.7H_2O$: 0.29g and $CaCl_2.2H_2O$: 0.74g; in 1 L) and cleared of connective and fatty tissues as previously described [11].

About 2 mm of the aorta was mounted in a 10 mL organ bath containing Krebs solution aerated continuously with Carbogen (95% Oxygen and 5% Carbon dioxide) under 1 G tension and maintained at 37ºC. The aorta was attached to an Ugo Basile isometric force transducer (Model: 7004 and 7003), which was connected to an Ugo Basile 2 Channel Data Capsule (Model: 17400) for the measurement and acquisition of variation in tension. The aorta was allowed to equilibrate for 60 minutes, during which the Krebs solution was replaced at 15 minutes' interval [9].

After equilibration, concentrations of 0.3125, 0.625, 1.25, 12.5 and 25 mg/mL administered cumulatively in volume of 25, 50, 100, 250 and 500 µL to evaluate the effect of the extract on the contractility of endothelium intact, and endothelium denuded rat thoracic aorta at resting tension (1.0 g).

Statistical analysis

Data was analyzed using GraphPad Prism version 6.0 Results were presented as mean ± SEM, One-Way Analysis of Variance (ANOVA) was used to determine differences in means of variables. *P-values* < 0.05 were considered significant.

Results

The extract dose dependently reduced the mean systolic and diastolic blood pressure in all the doses used for the experiment. The blood pressure lowering effect was significant at the doses of 0.625 ($p < 0.01$), 5 ($p < 0.05$), 10 ($p < 0.01$), 20 ($p < 0.01$) and 40 ($p < 0.01$) mg/kg respectively for the systolic blood pressure. The decrease in the diastolic blood pressure was significant only at the doses of 20 and 40 mg/kg ($p < 0.01$).

The dose of 40 mg/kg reduced the blood pressure from a resting value of 125.63 ± 4.27 to 96.69 ± 4.57 mm Hg for the systolic blood pressure, and from a resting value of 85 ± 3.13 to 52.48 ± 5.43 mm Hg for the diastolic blood pressure respectively (n = 8), (*$p < 0.05$; **$p < 0.01$) (Fig. 1). The extract reduced the mean arterial blood pressure in a dose dependent manner, with the reduction being significant at the doses of 20 mg/kg ($p < 0.05$) and 40 mg/kg ($p < 0.01$) respectively. The dose of 40 mg/kg reduced the mean arterial blood pressure from a baseline level of 100.21 ± 4.85, to a level of 69.9 ± 6.4. The extract increased the pulse pressure only at the dose of 0.625 mg/kg, from a resting value of 45 to 52.7 mm Hg respectively, while the other doses produced a steady reduction in the mean pulse blood pressure which was however not significant ($p > 0.05$), (n = 8) (Fig. 2).

Atropine (1.0 mg/kg) blocked the hypotensive effect of the extract up to the dose of 5 mg/kg, after which the blood pressure increased significantly from the dose of 10 mg/kg ($p < 0.05$), through to 20 and 40 mg/kg ($p < 0.01$) respectively, as shown by the mean arterial

pressure (n = 7) (Fig. 3).The extract relaxed the rat thoracic aorta with intact endothelium in a concentration dependent manner in the preliminary evaluation on the effect of the extract on vascular reactivity (Fig. 4), but was not able to relax the rat thoracic aorta with denuded endothelium (Fig. 5).

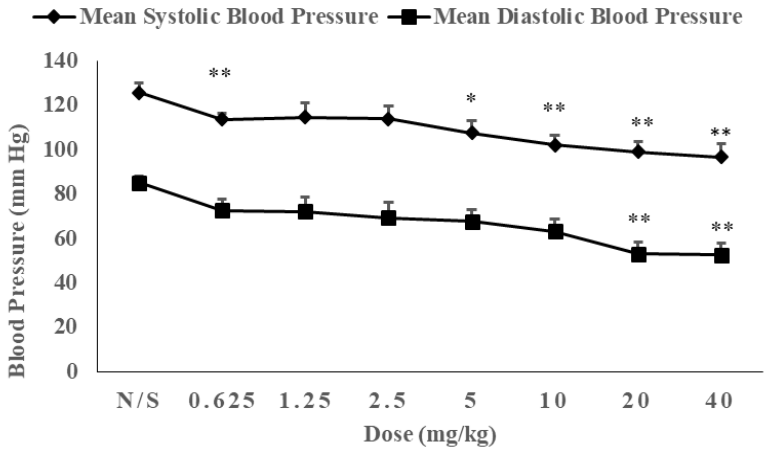

Fig. 1: The effect of the aqueous extract of *E. hirta* on systolic and diastolic blood pressure.

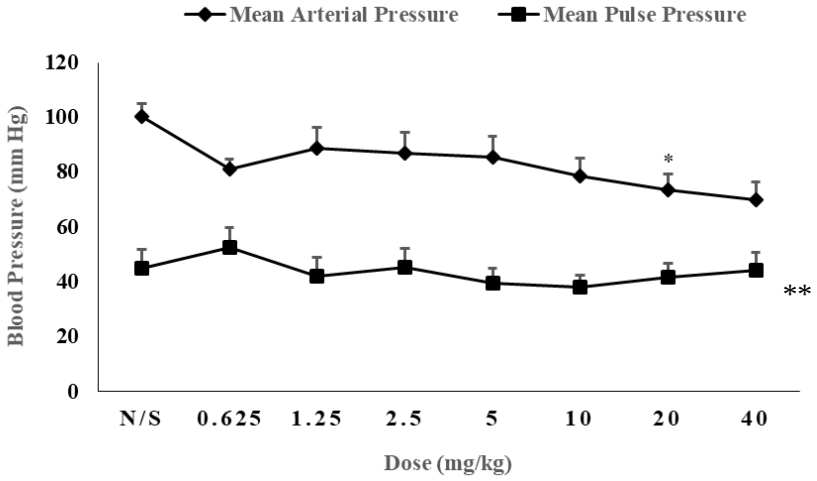

Fig. 2: The effect of the aqueous extract of *E. hirta* on the mean arterial blood and pulse pressures

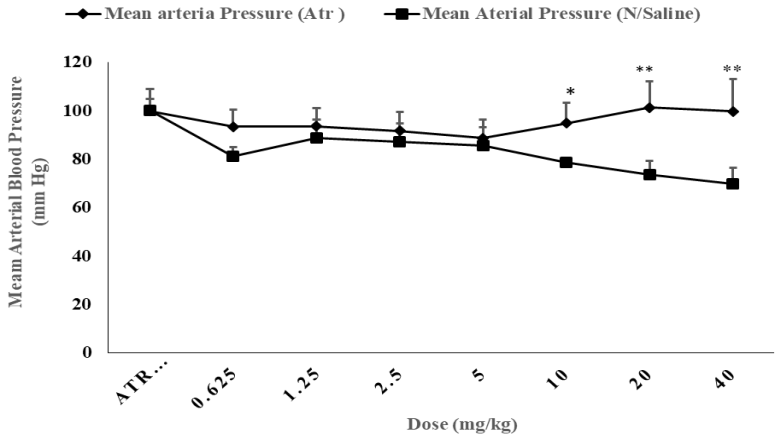

Fig. 3: The effect of the extract of *E.hirta* on the Mean Arterial Blood pressure of normotensive Wistar rats in the presence and absence of atropine

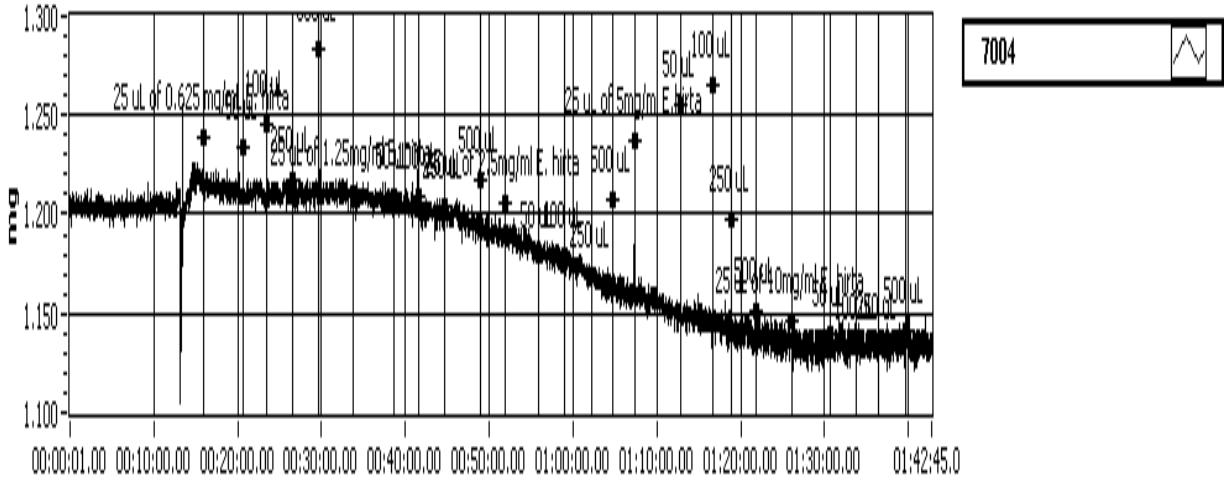

Fig. 4: The chart of the preliminary evaluation of the relaxation effect of the extract of *E. hirta* on the thoracic aorta of Wistar rat with intact endothelium. The extract relaxed the thoracic aorta in a concentration dependent manner. The relaxation effect was highest at the concentration of 25 mg/mL, (n = 1 aortic ring).

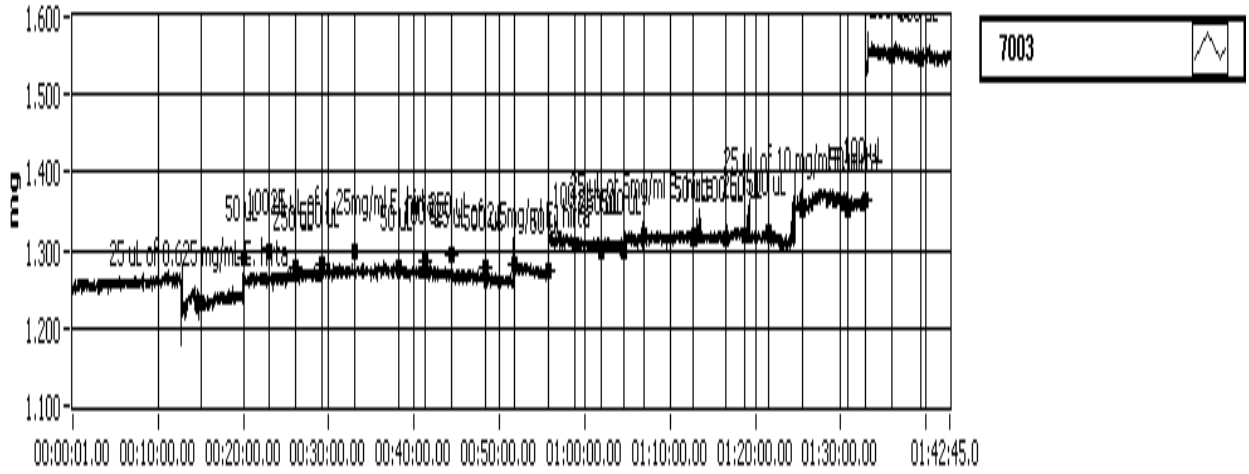

Fig. 5: The effect of extract on rat thoracic aorta with denuded endothelium. The smooth muscle relaxant effect of the extract was completely abolished in endothelium denuded rat thoracic aorta (n = 1 aortic ring).

Discussion

The aqueous extract of *E. hirta* has blood pressure lowering effect, and dose dependently reduced the mean systolic, diastolic and mean arterial pressures in normotensive Wistar rats.

Systolic and diastolic blood pressure

The results of the evaluation of the extract of *E. hirta* on blood pressure showed that the extract caused a dose dependent decrease in the systolic, diastolic and the mean arterial blood pressure. While all the doses of the extract effectively reduced blood pressure, it was importantly observed that the dose of 10 mg/kg comparatively to 20 and 40 mg/kg, reduced the systolic and the diastolic pressure to levels that are within the optimal clinical normotensive range of 102.02 and 63.19 mm Hg respectively which do not tend towards hypotension, which has been one of the major side effects and cause for concern with most of the conventional antihypertensive drugs like the angiontensin receptor antagonists [12].

Mean arterial and mean pulse pressure

The mean arterial pressure (MAP) is a critical haemodynamic factor in cardiovascular medicine

[13]. It accounts for the flow, resistance and pressure within the arteries, and also facilitates the evaluation of the efficiency of blood flow from the heart through the body to the vital organs, which should be adequately perfused [13]. The optimal clinical range of MAP is between 70-110 mm Hg, and should at all times be maintained at not less than 60 mm Hg [14]. MAP should be properly regulated to ensure that it does not fall below the least clinically acceptable level (60 mmHg) because lower MAP values have been shown to adversely compromise blood flow to organs leading to ischaemia, and infarction, and by extension, the perfusion of vital organs especially the cerebral tissues, leading to syncope and hastened neuronal death [14].

The results of our research with the extract of *E. hirta* showed a dose dependent reduction of the mean arterial pressure of normotensive Wistar rats within the normal range. The dose of 40 mg/kg reduced the MAP from a resting value of 100.02 mm Hg to 69.9 mm Hg, which is still within the normal MAP range suggesting that the extract of *E. hirta* at the most effective hypotensive dose does not depress the MAP of normotensive Wistar rats below the minimal clinically accepted value of 60 mm Hg, and therefore may not predispose individuals to further cardiovascular risk and complication of hypotension.

Pulse pressure is a determinant of susceptibility to cardiovascular risks. The normal pulse pressure range is between 40-60 mmHg, and pulse pressure that is less than 25% (<35 mmHg) of systolic blood pressure is considered low or narrow and would predispose individual to aortic stenosis or cardiogenic shock [15]. A low pulse pressure is independently predictive of cardiovascular collapse, especially in people with heart failure [16].

While lowering blood pressure, the extract caused a dose dependent increase in the mean pulse pressure in all the administered doses but within the normal range [16]. The dose of 1.25 mg/kg that caused a transient decrease in pulse pressure maintained the pulse pressure within the normal range. This showed that the blood pressure lowering effect of the extract did not compromise the functionality of the cardiovascular system by depressing myocardial contraction, with the possibility of causing cardiovascular collapse.

The dose dependent decrease in MAP caused by the extract was completely abolished after the administration of atropine (1.0 mg/kg). This result suggests that the blood pressure lowering effect of the extract may not be through the possible stimulation of muscarinic (M_3-receptor) on the vascular smooth.

Preliminary evaluation of the effect of the extract of *E. hirta* on rat thoracic aorta

The preliminary evaluation of the relaxant effect on the extract of *E. hirta* showed that the extract relaxed the rat's thoracic aorta with intact endothelium in a graded concentration dependent manner, but was not able to relax the rat's thoracic aorta with denuded endothelium. Based on these preliminary results, it can be inferred that the extract of *E. hirta* might be lowering blood pressure partly by relaxing the vascular smooth muscle as a vasodilator, possibly in an endothelium intact vascular smooth muscle, among other possible mechanism(s) like reduction in cardiac output or blockade of adrenergic receptors (α-$_1$ and β1) that may directly affect some other anatomical sites that modify and regulate blood pressure. However, further works need to be carried out to extensively evaluate the effect of the extract on vascular reactivity in greater details.

Conclusion

Following the results reported and discussed in this paper, we have established that the aqueous extract of *E. hirta* has blood pressure lowering effect in normotensive Wistar rats. We have also established that the extract lowers blood pressure in normotensive rats, without the possibility of causing severe hypotension that might predispose individual to cardiogenic shock, syncope and hastened neuronal death.

Conflict of Interest

No conflict of interest is associated with this work.

Contribution of Authors

FCA conceived, designed and supervised the study. BAA and JOO supervised the collection

and the extraction of the plant material. PAU and BM carried out the biological evaluations. EEB supervised the analysis of the data generated during the study and EKO coordinated the research team.

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The authors wish to acknowledge Mrs. Josephine Oyibo Amaechina for suggesting *Euphorbia hirta* in the year 2001 as a plant that has potentials to treat varieties of ailment.

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