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Effect Of Aqueous Leaf Extract From Gynura Procumbens (Lour.) Merr. (Asteraceae) On L-Name-Induced Hypertension In Wistar Rats

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Abstract

This study is part of ongoing research into natural alternatives for thetreatment of hypertension, a major public health problem.The objective is to evaluate the effects of the aqueous extract of Gynura procumbens (Lour.) Merr. leaves on L-NAME-induced HTA in Wistar rats. The methodology involved inducing hypertension in rats with L-NAME (20mg/kg, oral) for 7 days. The rats were then randomly divided into 5 groups. The first group was sacrificed, and the other 4 were treated with distilled water (1 ml/100 g), aqueous extract of G. procumbens (250 and 500 mg/kg), captopril (50mg/kg) orally once daily for 7 days. In addition, one group was used as a control. At the end of the 14th day, body weight was measured, and DBP, SBP, and HR were measured using the invasive method. The results showed that administration of L-NAME led to a significant increase of 37.19% in SBP and 63.88% in DBP compared to the control group. In contrast, treatment with G. procumbens extract at doses of 250 and 500 mg/kg, resulted in significant reductions in SBP (28.62% and 30.68%, respectively) and DBP (35% and 45.98%). Furthermore, administration of this extract also reduced heart rate, demonstrating a cardiosuppressive effect. The aqueous extract of G. procumbens appears to have an antihypertensive effect comparable to that of captopril. This study supports its traditional use and calls for further research to explore its mechanisms of action and clinical application.

Keywords: Gynura procumbens, hypertension, L-NAME, Wistar rat.

Introduction

Cardiovascular diseases, particularly hypertension, are among the leading causes of morbidity and mortality. Exploring traditional medicines can offer new therapeutic options, enrich the medical arsenal through the discovery of new drugs, and provide less expensive alternatives in developing countries. In Africa, for example, traditional healers are widespread,and up to 80% of the population uses traditional medicine to meet theirhealthcare needs (1). In the Republic of the Congo, many researchers are turning to ethnopharmacology to identify new active natural compounds, particularly in the cardiovascular field, by studying experimental models of hypertension. This is the case for Etou Ossibi and al.(2), who studied the effects of the aqueous extract of Lippia multiflora Moldenke on DOCA-salt-induced hypertension in rats. Ngolo and al.(3) studied the effects of an extract from a medicinal formula on alcohol- and tobacco-induced hypertension. In this context, it is important to note that hypertension is often linked to a deficiency in nitric oxide (NO), a key mediator of vasodilation. Thus, repeated administration of N(ω)- Nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthesis, constitutes a relevant experimental model for reproducing hypertension in rats [4]. This allows for both a better understanding

of the human pathology and an effective evaluation of antihypertensive treatments. We therefore set out to evaluate the effects of G. procumbens aqueous leaf extract in an experimental model of hypertension induced by L-NAME, comparing them to the effects of a standard antihypertensive drug, captopril.

Materials and Methods

Plant Material

The leaves of *G. procambens* were collected in Brazzaville, Republic of the Congo, in April 2024. Taxonomic identification was performed by Dr. Victor KIMPOUNI, a systematic botanist affiliated with the National Forest Research Institute (IRF).

Animal material

Healthy 4- to 5-month-old male and female Wistar rats, weighing between 180 and 259 g, were used in this study. The test subjects were housed separately in standard cages equipped with top grilles. These animals were supplied by the animal facility of the Life and Earth Sciences (S.V.T.) laboratory at the École Normale Supérieure (E.N.S.), where they were kept under controlled lighting conditions, alternating 12 hours of light and 12 hours of darkness, at an ambient temperature maintained at 25 ± 1 °C. The animals had free access to water as well as a standard diet.

Preparation of the aqueous extract

The leaves were thoroughly washed twice under running tap water, then dried at room temperature (28 ± 1 °C), away from sunlight, for a period of 14 days, and then ground into a powder using a wooden mortar. Subsequently, an aqueous extraction of the leaves was performed using a maceration process at a concentration of 10%. To this end, 100 g of powder obtained from the leaves of G. procambens were macerated in 1000 ml of distilled water for a period of 48 hours. The resulting macerate was subjected to a three-step filtration process using cotton wool, yielding a filtrate that was then concentrated at a temperature of 80 °C using a rotary evaporator under vacuum.

The aqueous extract thus obtained was stored in the refrigerator in a sterile, light-resistant bottle for subsequent pharmacological testing, after determining the yield using the following formula: Extraction yield = (weight of extract / weight of initial powder used for extraction) ×100.

Effect of aqueous extract of Gynura procumbens leaves on L-NAME-induced increases inblood pressure (BP) and heart rate (HR) in Wisrar rats.

This study was conducted according to the method described by Quenum [5] to evaluate the effect of aqueous extract of *G. procumbens* leaves on hypertension induced by N(ω)-nitro-L-arginine methyl ester (L-NAME), an L-arginine analogue, whose chronic oral administration inhibits nitric oxide (NO) synthesis and consequently induces an increase in blood pressure in rats. On the first day (D0), eighteen (18) Wistar rats with normal blood pressure were weighed and randomly assigned to six groups (G1 to G6) of three (3) rats each, treated as follows:

Group 1 (G1): Control rats received distilled water (1 ml/100 g, orally).

- Groups (G2 to G6): Rats received L-NAME (20 mg/kg, orally) via gavage, as a

single daily dose for seven days. On the eighth day, after reweighing the animals, BP and HR were measured in the animals of Group 2 (G2) to verify the induction of hypertension by L-NAME, and those in Groups (G3 to G6) were subjected for seven days to the following treatments, respectively:

Group 3 (G3): rats treated with distilled water (1 mL/100 g, oral);

- Group 4 (G4) and Group 5 (G5): rats treated with the aqueous extract of G. procumbens at doses of 250 and 500 mg/kg p.o. per day, respectively;
- Group 6 (G6): rats treated with Captopril® (50 mg/kg p.o.) per day. The day after the end of the various treatments, i.e., on Day 14, the body weight of the rats was recorded one last time. The effect of each dose on cardiovascular parameters, particularly blood pressure (BP) and heart rate (HR), was measured for one hour during the experiment using the invasive method.

Measurement of Cardiovascular Parameters

The rats were anesthetized with 15% urethane (ethyl carbamate) at a dose of 1.5 g/kg, administered intraperitoneally at a rate of 1 ml per 100 g of body weight. The femoral vein and then the carotid artery were subsequently catheterized using fine polyethylene catheters according to the protocol for measuring blood pressure (BP) and heart rate (HR) using the invasive method (Nguelefack, 2002). A "Biopac Student Lab" MP 36 transducer and recorder were used to display the various recorded waveforms on the computer screen. After a stabilization period of approximately 30 minutes for cardiovascular parameters, all substances were administered through the catheter attached to a syringe at the level of the femoral vein of the rat. These substances, dissolved in a 0.9% NaCl solution, were administered at a dose of 0.1 ml per 100 g of rat body weight (Dimo, 2003).

Results

Effects of an aqueous extract of Gynura procumbens on systolic blood pressure (SBP) and diastolic blood pressure (DBP) in Wistar rats induced to become hypertensive by L-NAME.

PA	Contrôle(G1)	L-NAME (G2)	L-NAME + ED (G3)	L-NAME aqueous extract 250mg/Kg (G4)	L-NAME + aqueous extract 500mg/Kg (G5)	L-NAME + Captopril 50mg/kg (G6)
	m ± ESM	m ± ESM	m ± ESM	m ± ESM	m ± ESM	m ± ESM
SBP (mm Hg)	109,25 ±11,19	149,89 ± 0,30**	134,81 ± 0,67	106,98 ± 8,37ns$^{\#\# +}$	103,91 ± 0,45**$^{\#\#\# +}$	86,97 ±0,62***$^{\#\#\#}$
DBP (mm Hg)	85,84 ± 13,20	140,68 ± 0,34***	105,96 ± 0,54	91,44 ± 6,87ns$^{\#\#+}$	75,99 ± 0,35***$^{\#\#\# NS}$	75,01 ±0,58***$^{\#\#\#}$

Table I: Effects of the aqueous extract of Gynura procumbens on blood pressure

Each value represents the mean ± SEM, n = 3. **p < 0.01; ***p < 0.001, significant difference compared to normotensive rats in Group 1. ns: no significant difference compared to Group 1. ## p < 0.05; ### p <0.001, significant difference compared to G3. + p <0.05, significant difference compared to G6; NS: non-significant difference compared to G6; m: mean; SEM: standard error of the mean; SBP: systolic blood pressure; DBP: diastolic blood pressure. ED : distilled water

Effects of an aqueous extract of Gynura procumbens leaves on heart rate in L-NAME-induced hypertensive rats
Weight changes in rats during the experiment

FC	Contrôle(G1)	L-NAME (G2)	L-NAME + ED (G3)	L-NAME + aqueous extract 250mg/Kg (G4)	L-NAME + aqueous extract 500mg/Kg (G5)	L-NAME + Captopril 50mg/kg (G6)
	m ± ESM	m ± ESM	m ± ESM	m ± ESM	m ± ESM	m ± ESM
FC (BPM)	340,23 ±2,66	420,02 ± 0,31***	388,61 ± 13,06	356,7 ± 19,44ns$^{\#\#\#NS}$	341,35 ± 0,77ns$^{\#\#\#+}$	357,47 ± 0,42ns$^{\#}$

Table II: Effects of an aqueous extract of Gynura procumbens leaves on heart rate (HR) in L-NAME-induced hypertensive rats

Each value represents the mean ± SEM, n = 3. ***p < 0.001, significant difference compared to normotensive rats in Group 1. ns: no significant difference compared to Group 1. # p < 0.05 ; ### p <0.001, significant difference compared to G3; + p <0.05, significant difference compared to hypertensive rats G6; NS: non-significant difference compared to G6. BPM: Beats per minute, HR: Heart rate; m: mean; SEM: standard error of the mean ED : distilled water

MC	Contrôle(G1)	L-NAME (G2)	L-NAME + ED (G3)	L-NAME + Extrait aqueux 250mg/Kg (G4)	L-NAME + Extrait aqueux 500mg/Kg (G5)	L-NAME + Captopril 50mg/kg (G6)
	m ± SEM	m ± SEM	m ± SEM	m ± SEM	m ± SEM	m ± SEM
MC J0 (g)	198,20 ± 3,21	223,20 ± 1,44	187,93 ± 3,06	258,23 ± 0,52	217,90 ± 5,43	180,57 ± 0,69
MC J7 (g)	201,90 ± 0,95	249,87 ± 0,48***	201,33 ± 4,74**	267,70 ± 0,91*	256,67 ± 4,78**	198,53 ± 1,65***
MC J14 (g)	211,27 ± 1,14	-	208,27 ± 1,79 $^{\#}$	269,23 ± 4,54^{NS}	259,47 ± 14,32^{NS}	185,83 ± 0,59$^{\#\#}$

Table (III): Weight Changes in Rats

Each value represents the mean ± SEM, n = 3. *p < 0.05; **p < 0.01; ***p < 0.01, significantdifference compared to the body weight of rats on the first day of the experiment (D0). # p< 0.05; # # p <0.01, significant difference compared to the body weight of rats on the eighth day of the experiment (D7); NS: non-significant difference; SEM: standard error of the mean; BW: body weight.

Discussion

Hypertension induced by the inhibition of nitric oxide synthesis (L-NAME) is a well-established method for simulating hypertension in animals, particularly in Wistar rats (6) (4). This model was used in our study to examine the effects of the aqueous extract of G. procumbens leaves on hypertension in Wistar rats. In our study, chronic administration of L-NAME (20 mg/kg) for one week caused a significant increase in systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) in normotensive Wistar rats. These results corroborate the observations made by Quenum et al., (5), who also found a notable increase in blood pressure and heart rate in rats treated with L-NAME, highlighting the potential of this model for evaluating the therapeutic effects of plant extracts and synthetic molecules. The mechanism of action underlying L-NAME-induced hypertension is primarily based on a reduction in nitric oxide (NO) synthesis, leading to increased vascular tone due to insufficient vasodilator supply (NO) in the face of increased vasoconstrictor input (Angiotensin II, Endothelin-1, and reactive oxygen species), thereby contributing to elevated blood pressure and vascular remodeling (7). Furthermore, at the cardiac level, an increase in norepinephrine release is likely to increase heart rate (positive chronotropic effect) and cardiac contractility (positive inotropic effect) via β1-adrenergic receptors (8). To demonstrate the efficacy of the aqueous extract of G. procumbens, we conducted a comparison with captopril, an angiotensin-converting enzyme (ACE) inhibitor, due to its well documented mechanisms of action and its established clinical use in the management of hypertension. Thus, treatment of hypertensive Wistar rats with the aqueous extract of G. procumbens leaves yielded results comparable to those observed with captopril, with a significant decrease in SBP and DBP. At a dose of 500 mg/kg, the aqueous extract caused a substantial reduction in these parameters, suggesting a significant antihypertensive effect and a possible dose-dependent action of the extract, in accordance with the observations of Hoe and al.(9), who reported a significant decrease in systolic blood pressure and mean arterial pressure in hypertensive rats treated with G. procumbens. Furthermore, the effect of the aqueous extract of G. procumbens on heart rate was also significant, reducing it to a degree comparable to that of captopril, consistent with the findings of Hoe and al., (9);(10), who observed similar effects regarding HR modulation during their studies on G. procumbens. Indeed, they revealed that the G. procumbens extract can lead to a significant reduction in HR, manifested by marked negative chronotropic and inotropic effects on the atria of rats.

Conclusion

This study aimed to evaluate the effects of the aqueous extract of G. procumbens leaves on L-NAME-induced hypertension in Wistar rats using rigorous experimental methods. The results obtained after administration of the aqueous extract, compared with the positive controls, demonstrate a significant improvement in blood pressure parameters. Furthermore, we also observed a decrease in heart rate in the treated rats, suggesting that G. procumbens not onlyexerts an antihypertensive effect but may also beneficially influence heart rate. These observations highlight the potential of this extract in a comprehensive therapeutic approach for the management of hypertension. These results therefore indicate that the leaf extract of G. procumbens possesses significant antihypertensive properties in the context of L-NAME-induced hypertension, with an effect comparable to that of captopril, which justifies its inclusion in the traditional pharmacopoeia. Thus, G. procumbens leaves could prove to be a valuable natural therapeutic alternative for the management of hypertension, contributing to reduced healthcare costs and an improved quality of life for patients.

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