

Antihypertensive activity of *Lycopodium cernuum* (Lycopodiaceae) hydro alcoholic extract on rats

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Abstract

The aim of this work was to study the antihypertensive activity of the hydroalcoholic extract of the aerial part of *Lycopodium cernuum* in rats made experimentally hypertensive with a hypersodic diet. Its hypotensive effect was studied in vivo and its vascular effect was studied in vitro on the isolated aorta.

The normal blood pressure of the rats was $103.83 \pm 3.7 / 82.13 \pm 2$ mmHg (systole/diastole). After 21 days on the high-salt diet, their blood pressure rose to $208 \pm 2.2 / 150.06 \pm 2.5$ mmHg. This pressure returned to normal after 22 days in the control lot compared to 6, 10 and 16 days in the animals treated with the extract at 400, 200 and 100 mg/kg of the extract orally administered. In vitro, the extract relaxes the isolated aorta contracted with norepinephrine 10^{-3} M with an EC_{50} equal to 0.65 ± 0.02 mg/ml. These results show that *Lycopodium cernuum* extract has an antihypertensive activity in rats by its vasodilatory effect. This activity could be due to the presence of phenolic compounds and/or alkaloids in the extract.

Keywords: Antihypertensive; Rat; *Lycopodium cernuum*; Diet

1. Introduction

Hypertension affects nearly 20% of the Canadian population [1]. Globally, hypertension is estimated to cause 7.5 million deaths per year. It increases the risk of heart attack, stroke, kidney failure, blindness and other vascular conditions [2]. High blood pressure (HBP) is defined as systolic pressure greater than or equal to 140 mmHg and 90 mmHg for diastolic pressure in an adult between 25 and 65 years old. Blood pressure depends of cardiac output and peripheral vascular resistance (PVR). Growing either of these factors causes a high blood pressure [3]. Cardiac output depends on the myocardial contraction force and heart rate via β_1 adrenergic receptor stimulation [4]. Whereas PVR is elevated if vascular diameter decreases or circulating blood volume increases.

Antihypertensive drugs decrease cardiac output and cardiac contraction by inhibiting β_1 adrenergic receptor. Whereas those acting on peripheral resistance increase vascular diameter by inhibiting angiotensin II and calcium ion receptors or by increasing β_2 adrenergic action and nitric oxide concentration in the vascular endothelium [5].

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Many medicinal plants are also used in traditional medicine for the management of high blood pressure. Some examples, Malagasy people use are the leaves of *Adansonia madagascariensis* (vern. baobab) (BOMBACACEAE), the aerial part of *Bidens pilosa* (vern. tsipolitra) (ASTERACEAE), the leaves of *Passiflora edulis* (PASSIFLORACEAE) and the bulb of *Allium sativum* (garlic) (LILIACEAE) [6,7]. *Cajanius indicus* (vern. Amberivatry) (FABACEAE), *Cassia alata* (vern. four pin) (CAESALPINIACEAE), and *Vernonia pectoralis* (vern. Sakatavilotra) (ASTERACEAE), are also plants used to treat this disease [8,9].

The decoction preparation of the aerial part of *Lycopodium cernuum* is used in traditional Malagasy medicine of Itasy region against dizziness and headaches that we considered as symptoms of arterial hypertension. Therefore, we set the objective of this study to verify this hypothesis.

2. Materials and methods

2.1. Plant material and extraction

The aerial part of *Lycopodium cernuum* (LYCOPODIACEES) was harvested in the Itasy region (Madagascar) in January month. It was dried in the shade. The powder obtained was macerated in a mixture of ethanol-water (60v:40v) during 3 days at the room temperature. The macerate was filtered with Wattman paper (n°1) and then evaporated to dryness to obtain the hydroalcoholic extract. This extract was subject of phytochemical screening and used in the biological tests.

2.2. Study of the activity of *Lycopodium cernuum* extract on blood pressure

The antihypertensive activity of the extract was studied in the Wistar rat. The protocol used follow the guidelines of the ethics committee of Sciences Faculty, University of Antananarivo, Madagascar.

Male rats weighing 250 to 270 g bred in the animal house of General Pharmacology and Cosmetology laboratory at the University of Antananarivo, Madagascar, were used. To induce arterial hypertension, they were fed with high-salt diet in a proportion of (NaCl:provend) (8:100) for 21 days [10]. During this period, the blood pressure variation was measured daily with a tail cuff sphygmomanometer and recorder [11]. After the high-salt diet, animals presenting high blood pressure were used for the continuation of the test. They were fasted for 18 hours, divided into 4 groups of 6 rats, 1 control and 3 treated with the extract. Animals of control group received 10 ml/kg of distilled water by oral route and those in the 3 groups received orally the extract at doses 100, 200 and 400 mg/kg [12,13]. The blood pressure of these animals was measured every day before the administration until it returned to its normal value.

2.3. Study of the activity of *Lycopodium cernuum* extract on isolated aorta

The rats were sacrificed by decapitation, and the thoracic aorta was isolated and cleaned. A 3 mm length ring of these aorta was suspended between two parallel stainless-steel hooks, in an organ bath contained 5 ml of Krebs solution (NaCl 118 mM, KCl 4.07 mM, MgSO₄ 1.1 mM, KH₂PO₄ 1.2 mM, CaCl₂ 1.5 mM, NaHCO₃ 25 mM and glucose 10 mM) maintained at a temperature of 37 °C, and bubbled with a carbogène (95% O₂ and 5% CO₂). The organ was subjected to a basic tension of 2 g. The contraction force of the isolated aorta was connected and measured with a Statham Gould® isometric transducer and recorded with physiography (Signal Monitor®) [14].

The organ was allowed to stabilize for 45 min. Then, norepinephrine was injected in a cumulative manner into the bath in a until maximum contraction was achieved. And finally, *Lycopodium cernuum* extract was injected into the bath in a cumulative manner from 0.25 mg/ml until the organ was fully relaxed and concentration responsible for 50 % of the relaxation (EC₅₀) were determined.

2.4. Statistical analysis

The results were presented as mean ± SEM. The data obtained was analyzed with analysis variance ANOVA followed by Student t-test. The difference between the means were significant with the p-value < 0.05.

3. Results

3.1. Result of phytochemical screening

Phytochemical screening shows that the hydro alcoholic extract of *Lycopodium cernuum* contains a several secondary metabolites, such as alkaloids, tannins, phenolic compounds, anthocyanins, steroids and triterpenes.

3.2. Effect of *Lycopodium cernuum* extract on blood pressure

The normal systolic and diastolic blood pressure of rats were 103.83 ± 3.7 mm Hg and 82.13 ± 2 mm Hg, respectively. After the high-salt diet, systolic blood pressure increases to 208 ± 2.2 mm Hg and diastolic blood pressure to 150.06 ± 2.5 mm Hg. *Lycopodium cernuum* extract decreases blood pressure in experimentally hypertensive animals. This decrease depends on the dose of the extract administered. The blood pressure returns to its normal value after 22 days for the control animals. Those of animals treated with the extract at doses of 400, 200 and 100 mg/kg return to their normal value after 6, 10 and 16 days of treatment ($P < 0.05$). This result shows that *Lycopodium cernuum* hydroalcoholic extract exhibits antihypertensive activity.

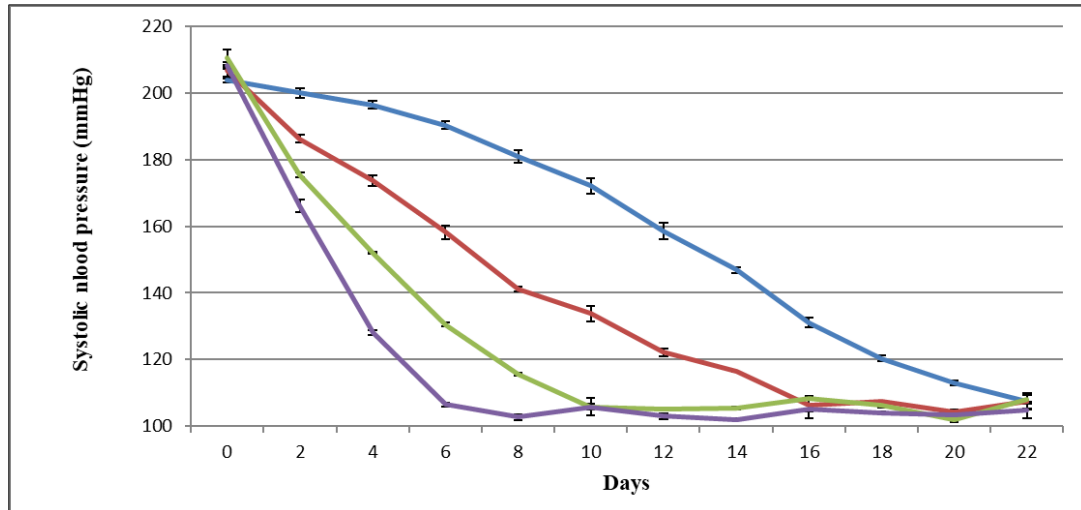


Figure 1 Systolic blood pressure variation of the control animals (—) and those of animals treated with the extract at doses of 100 (—), 200 (—) and 400 (—) mg/kg once daily, administered orally, after high salt diet ($\bar{m} \pm \sigma$, $n = 6$, $P < 0.05$)

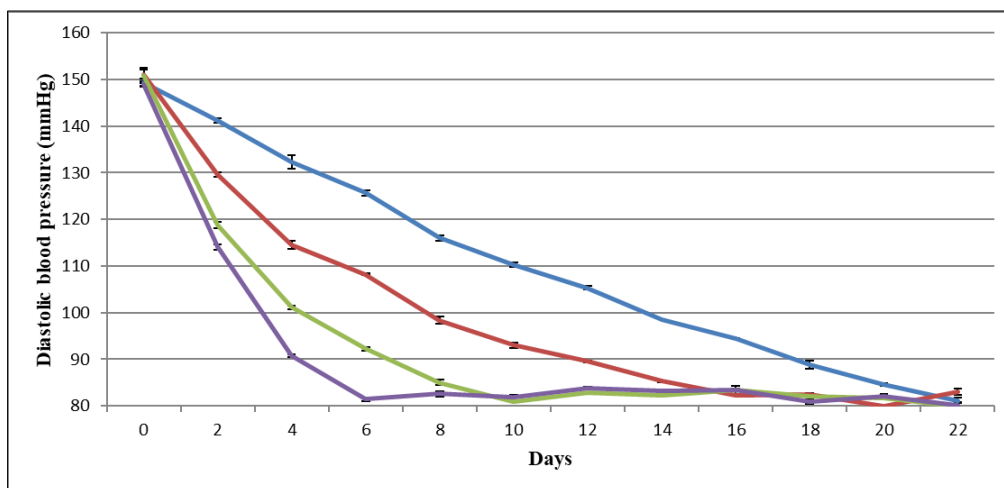


Figure 2 Diastolic blood pressure variation of the control animals (—) and those of animals treated with the extract at doses of 100 (—), 200 (—) and 400 (—) mg/kg once daily, administered orally, after high salt diet ($\bar{m} \pm \sigma$, $n = 6$, $P < 0.05$)

3.3. Effect of *Lycopodium cernuum* extract on the isolated aorta

Norepinephrine injected into the bath contracts the isolated aorta. The maximal of this contraction is equal to 0.71 ± 0.05 g, which is considered as 100%. Injection in a cumulative manner of the *Lycopodium cernuum* extract into the bath completely relaxes the isolated organ precontracted with norepinephrine, with CE_{50} of 0.65 ± 0.02 mg/ml ($P < 0.05$) (Figure 3). This result shows the vasorelaxant activity of *Lycopodium cernuum* hydroalcoholic extract.

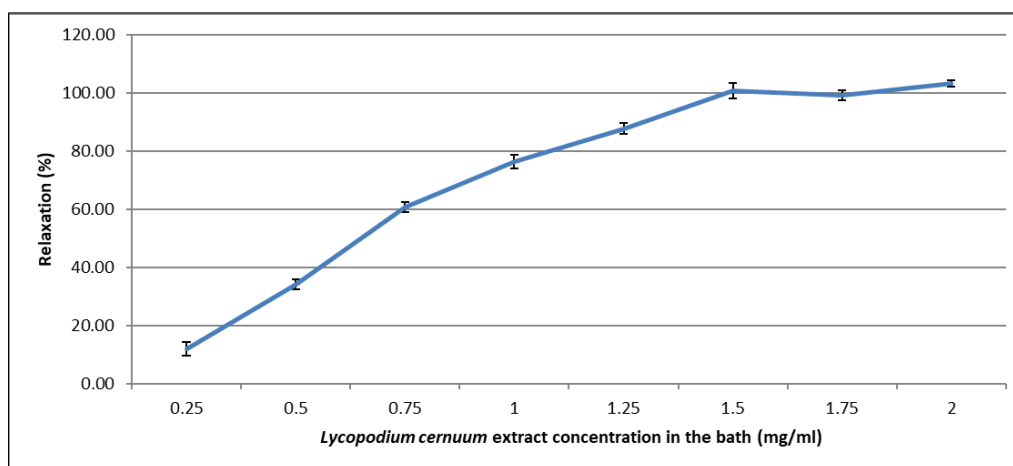


Figure 3 Variation of the relaxation of isolated aorta precontracted with norepinephrine in the presence of *Lycopodium cernuum* extract injected in the bath in a cumulative manner ($\bar{m} \pm \sigma$, $n = 6$, $P < 0.05$)

4. Discussion

Arterial hypertension is defined as persistent systemic high blood pressure caused by increased cardiac output and/or elevated peripheral vascular resistance. This pathology involves the interaction of several organs and results from numerous independent or interdependent pathophysiological mechanisms. Its treatment is based on the use of vasodilator drugs, beta-blockers or diuretics. However, several medicinal plants are also used for the management of this disease in traditional medicine [15]. The objective of this work is to study the anti-hypertensive activity of the extract of the aerial part of *Lycopodium cernuum*, a plant used in traditional Malagasy medicine to alleviate the symptoms of high blood pressure. To achieve this objective, rats made experimentally hypertensive with a high-salt diet were used. Excess of sodium strengthen vascular tone and increases blood volume [16].

The result of in vivo tests reports that the oral administration of the hydroalcoholic extract of *Lycopodium cernuum* decreases the blood pressure of rats. This activity approves the empirical use of the plant. This decrease could be due to a decrease in cardiac output or a decrease in peripheral vascular resistance [17]. on this, its activity on vascular diameter has been studied because vasorelaxation is an important mechanism for lowering blood pressure by decreasing systemic vascular resistance [28].

In vitro, the results report that the hydroalcoholic extract of *Lycopodium cernuum* causes a significant concentration-dependent relaxation on the isolated rat aorta precontracted with norepinephrine. This would lead to the decrease of systemic vascular resistance, which is the cause of the decrease of blood pressure [19].

Hypotheses could be put forward to explain the probable mechanisms of action of this vasodilator activity. This action could be due to the phenolic compounds present in the extract. This secondary metabolite activates the enzyme responsible for the synthesis of nitric oxide (NOS) in the vascular endothelium according to studies carried out on the effect of *Vitis vinifera* against high blood pressure [20]. Previous studies on *Nauclea officinalis* also show that this effect could be due to the presence of alkaloids into the plant. Alkaloids decrease intracellular calcium concentration. Either they inhibit the calcium channel in the vessel wall or they reduce the release of calcium from the sarcoplasmic reticulum [21]. As long as this chemical family is present in *Lycopodium cernuum*, it is possible that one of the molecules of this family is at the origin of the relaxation of the isolated aorta precontracted with norepinephrine. Since the extract used in this study contains several chemical families, further research is needed to identify the active molecule in the plant and to study its precise mechanism of action against high blood pressure.

5. Conclusion

The results showed that hydroalcoholic extract of *Lycopodium cernuum* has an antihypertensive effect. They provide insight to explain the use of *Lycopodium cernuum* against high blood pressure in traditional Malagasy medicine. This activity could be due to the phenolic compounds and alkaloids it contains. Further studies are needed to determine the mechanism of action.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The experiments were conducted following the guidelines of the ethic committee of the Sciences Faculty, University of Antananarivo, Madagascar (Ref: 17 / 2021).

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