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Effects of crude aqueous extracts of *Heliotropium indicum* Linn (Boraginaceae) on blood pressure in Wistar rats

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Abstract

The present work was carried out to investigate the effects of crude aqueous extracts of the stem with leaves and roots of *Heliotropium indicum* Linn on blood pressure in Wistar rats. Crude aqueous extracts were obtained by decoction of *Heliotropium indicum* stem with leaves and roots. A phytochemical screening of extracts has been made by thin layer chromatography. The pharmacological effect of the extracts was assessed by administration to L-NAME-induced hypertensive Wistar rats. Blood pressures were measured by carotid catheterization. The chemical screening revealed the presence of many secondary metabolites such as coumarins, flavonoids, anthocyanics, lignans, saponins, tannins, terpenes and triterpenes in both extract. Alkaloid were detected only in the root extract.7-days administration of L-NAME induced an increase is of rat mean arterial pressure (MAP) from 106±3 mmHg to 134±2 mmHg. Administration of both extracts significantly decreased the MAP from 134±2 mmHg to respectively to 108 ±1 mmHg for the stem with leaf extract and to 121±2 mmHg for the root extract**.** These results provide a support for the use of this plant in traditional medicine.
Keywords: *Heliotropium indicum* Linn, Hypertension, Wistars rats, L-NAME.

1. Introduction

Heliotropium indicum Linn is a plant commonly used in pharmacopoeia in many countries [1]. In addition, several pharmacological activities of this plant have been reported by many authors. The ethanolic extracts of the whole plant and the aqueous extracts of the leaves have shown an anticancer activity due to an alkaloid so called indicine-N-oxide [2]. The leaves extracts of *Heliotropium indicum* possesses a pharmacological activity against leukemia Schwart, in wound healing, anti-inflammatory and antimicrobial [3-7]. The essential oil extracted from the aerial parts of this plant showed high biological activity against tuberculosis [8]. The aqueous extract from the leaves of *Heliotropium indicum* Linn have shown also histo-gastroprotective and an antimicrobial activities [9].

It has been shown that the leaves of *Heliotropium inducum* Linn increase stimulation of system [10]. *In-vitro* cytotoxic activities of the extracts of the plant have been observed on Hela cell lines [11].

On rat cardiovascular system, *heliotropium indicum* extract has been shown to induce aorta vasorelaxation[12,13]. However, the large uses of the plant against cardiovascular diseases were common, particularly against arterial hypertension; very few *in vivo* studies have been conducted to evaluate the pharmacological effect on induced-hypertension. Then the long-term effects of this plant on hypertension were not described.

This work aims to study the long term pharmacological effects of *Heliotropium indicum* Linn crude extracts on arterial hypertension induced in Wistar rats.

2. Material and method

2.1 Material

2.1.1 Plant material

The stem with leaves and the roots of *Heliotropium indicum* L. (Boraginaceae) were harvested in the middle area of Benin (Savalou) and authenticated by the Herbier National du Bénin (reference: N° 6481AA/HNB).

2.1.2 Chemicals reagents.

N (G) esternitro-L-arginine-methyl (L-NAME) was purchased from Sigma-Aldrich (Germany)

Thiopental sodium, purchased from Dev Life Corporation (India)

Captopril (Pharmaquick Benin), Physiological serum (Fresenius Kabi France)

2.2 Methods

2.2.1 Extracts preparation.

After washing and drying, the plant materials were ground into powder using a blender (MIKACHI-MK 1861 AP). 200g of the powder obtained from each plant materiel were boiled with1.4L of distilled water for one hour in a flask of 2.5L. The decoction was filtered using Whatman paper N° 1 (Whatman international Ltd; Maidstone, England) and then concentrated at 60°C under reduced pressure using a rotary evaporator Rotavapor Buchi R-II (Sigma-Aldrich, Germany). The crude aqueous extract obtained was protected against light and humidity and was stored in a refrigerator at 4°C.

2.2.2 Phytochemical Analysis

Phytochemical analysis of the two extracts was performed using the thin layer chromatography as described by Wagner and Bladt[14].

2.2.3 *In vivo* experimental protocol

Twelve to fifteen weeks old male wistar rats weighing 200 g - 250 g, were used. They were maintained in standard environmental conditions (22 to 25°C, 12h dark/light cycle) and had free access to food and water. All the experimental procedures using these animals have been performed in accordance with institutional ethical recommendations.

The pharmacological effect of the extracts on blood pressure was evaluated *in vivo* using the model of hypertension induced by L-NAME.

2.2.4 Design

Rats were assigned to five (5) groups of seven (7) animals as follows:

Control group:

Control group of rats which received distilled water from day 1 to day 14.

L-NAME group:

Rats were treated with L-NAME (20mg/kg) from day 1 to day 7 and received distilled water from day 8 to day 14.

Two (2) treated groups:

Rats of each group were treated with one of the extract (500mg/Kg of body weight) for 7 days after 7-days-administration of L-NAME.

Captopril group:

Animals in this group were treated with Captopril at 100mg/kg of body weight from day 8 to day 14 after L-NAME administration from day 1 to day 7.

All substances (L-NAME, captopril and extracts) have been administrated orally (gavage) to rats.

2.2.5 Blood pressure measurement

Rats were anesthetized by intra peritoneal injection of thiopental (40mg/kg of body weight). Left carotid artery was catheterized and blood pressure was measured by invasive method as previously described [15].

2.3 Statistical Analysis

Blood pressure data were presented as Mean ± SEM (standard error of mean). Data were analyzed using GraphPad Prism V7 software. Analysis of Variance (ANOVA) followed by Dunnett's multiple comparisons test was used for comparison between groups. Statistical significance was set at $p < 0.05$.

3. Results

3.1 Extraction efficiency (rate)

From 200g of plant powder, respectively 32.76g and 24.76g of aqueous extracts were obtained for respectively *Heliotropium indicum* stem with leaves and *Heliotropium indicum* roots. The extraction rate was thus 16.38% for the stem with leaves extract and 12.38% for roots extract.

Table 1: Extraction yield of the tested plants

(Extract)	Stem with leaves of *Heliotropium indicum*	Root of *Heliotropium indicum*
Material weight	200g	200g
Weight of the Extract obtained	32.764g	24.76g
Yield	**16.38%**	**12.38%**

3.2 Phytochemical Analysis

Coumarin, flavonoid, anthocyanics, lignans, saponins, tannins, terpenes glycosiled and triterpenes were detected in both extract whereas the alkaloids were found only in the root extract. Naphtoquinones, anthracenes, glycosides, terpenes and sesquiterpenes were not detected in any of the extracts. (Table 2)

Table 2: Phytochemical analysis of *Heliotropium indicum* extracts

Chemical family	HI Leaf and stem extract	HI Roots extract
Alkaloids	-	+
Coumarins	+	+
Flavonoids	++	++
Naphtoquinones	-	-
Anthocyanics	+	++
Lignans	++	++
Saponins	++	++
Anthracenes	-	-
Terpenesand sesquiterpenes	-	-
Tannins	++	++
Terpenes glycosiled	++	++
Triterpenes	+	++
Glycosides	-	-

HI: *Heliotropium indicum* (+) detected, (++) abundantly detected, (-) absent

3.3 Antihypertensive Effects

Figure 1 shows the effects of the both extracts on rat blood pressure after hypertension induction.7-days administration of L-NAME induced an increase of mean arterial pressure from 106±2.9 mmHg to134±1.6mmHg. Administration of extracts decreased significantly the mean arterial pressure respectively to 108±1.2 mmHg for the stem with leaf extract and to 121±2.4 mmHg for the root extract.

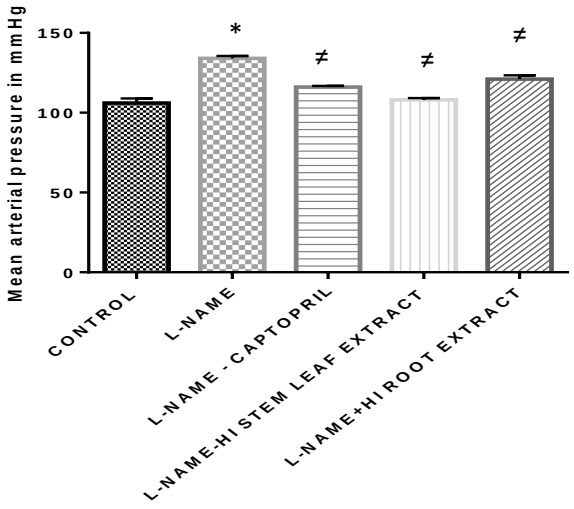

Figure 1: Effect of extracts on the arterial blood pressure

Values of MAP are means ±SEM; n= 7/group.
*: different from control value, p<0.05.
≠: different from L-NAME group value, p<0.05.
L-NAME group vs Negative Control group value, p<0.0001
L-NAME group vs L-NAME Captopril group value, p<0.0001
L-NAME group vs L-NAME –Stem Leaf Extract group value, p<0.0001
L-NAME group vs L-NAME –Root Extract group value, p<0.0002

4. Discussion

4.1 Yields and phytochemical screening

The yields obtained are respectively of 16.38 % and 12.38 % for the stems with leaves and roots of *Heliotropium indicum*. These results are similar to those of Arama[16] which generated a return of 20% for the decoction from the plant's aerial parts.

The phytochemical screening revealed in both extracts, the presence of glycosylated coumarins, glycosylated flavonoids, anthocyanin pigments, lignans, saponins, tannins and glycosylated terpenes and triterpenes. However, the aqueous extract from the roots of *H. indicum* showed the presence of alkaloids, which are absent in the aqueous extract from the stems with leaves. These results are consistent with those of reports [9,12]. However, our results differ from those of Traore *et al.*[17] which highlighted presence of cardiac glycosides from the aerial parts of *H. indicum*. Similarly, alkaloids components have been found in the aerial parts of the same plant [18,19]. These differences may be due not only to the method of phytochemical screening used, but also to the time and location, where the plant samples were harvested. In addition, tannins and flavonoids were found in moderate amount in the stems with leaves but in high amount in the roots of *H. indicum*.

4.2 Antihypertensive Effects

In the present work, we show that aqueous extracts of *Heliotropium indicum* induced significant reduction of blood pressure in an animal model of hypertension. Although it has been reported that *Heliotropium indicum* is one of the medicinal plant used in the treatment of hypertension, there is a few published data on its effects on blood pressure in human or animal model of susbtained hypertension.

Hypertension has been induced in wistar rat by administration of L-NAME at the dose of 20 mg/kg of body weight. This dose has been previously reported effective to induce hypertension [20,21]. As it has also been shown, 7-days administration of L-NAME was sufficient to induce a high blood pressure which persisted for at least 7 additional days [22].

Both aqueous extracts of *Heliotropium indicum* induced a significant decrease of blood pressure in hypertensive rat; but, at 500mg/kg, only the aerial part (leaves and stem) extract has normalized the blood pressure. These data are very interesting since them not only evidenced the highest blood pressure lowering effect of the aerial part (instead of the root) of the plant but also they contribute in the plant preservation.

In view of the hypertension model used, one of the mechanisms of action of the antihypertensive effect observed could be a relaxation of vascular smooth muscle.

Indeed, an endothelium-dependent vasorelaxation activity of *Heliotropium indicum* leaf extract on rabbit aorta has been reported. Negative inotrope and chronotropic effects on heart have also been suggested as antihypertensive mechanism action of *Heliotropium indicum* extracts [12].

Phytochemical analysis of the extracts allowed to the detection of chemical groups of substances which be involved in the antihypertensive effect observed. Flavonoids, tannins, anthocyanins were detected in both extracts. It has been shown that flavonoids induced vasorelaxation and increased nitric oxide (NO) production by endothelial cells [22, 23].

Some alkaloids were known to have muscarinic activity [24]. It has also been documented that saponins have some hypotensive activity [25, 26]

In addition, the antihypertensive effect could be explained by the antioxidant and radical-scavenging activities of *HI* due to the presence of phenolic and flavonoid compounds; their actions on oxidative stress thereby reducing the production of free radicals on the vascular vessel. Moreover it has been also noted a diuretic effect of the methanolic roots extract of *Heliotropium indicum* Linn that may also explain the antihypertensive effect [19].

5. Conclusion

In conclusion, the results of this study seem to support the traditional claim that the stem with leaves and the root of *Heliotropium indicum* have blood pressure lowering effect and this is due to their chemical components. The use of the aerial part but not the whole plant or the roots will contribute to the preservation of plant species.

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