Hypotensive and Antihypertensive Effects of Total Aqueous Extract of Justicia secunda Vahl M. (Acanthaceae) in Rabbits

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Abstract: The total aqueous extract of the leaves of Justicia secunda (Acanthaceae) from 1.25 to 75 mg/kg of body weight (BW) induced a dose-dependent hypotension in rabbits. The hypertension induced by adrenaline at 5.10⁴ mg/kg of BW is reduced by AJse administered at doses ranging from 10 to 30 mg/kg BW in dose-dependent manner (P < 0.01). AJse, at 40 mg/kg BW, cancels completely the hypertension induced by adrenaline at 5.10⁴ mg/kg BW and provokes in consequence a hypotension. Additionally, AJse at 10-30 mg/kg BW significantly reduces hypertension induced by the electrical stimulation of the sympathetic nerve fibers, and even cancel completely at 30 mg/kg BW or superior doses. This aqueous extract of leaves of Justicia secunda is thus hypotensive and antihypertensive in rabbits. The antihypertensive effect induced by AJse extract in the presence of adrenaline, or upon his release following stimulation of the sympathetic nerve fibers, indicates that this extract would act by inhibiting β-adrenergic receptors. The total aqueous extract of Justicia secunda leaves therefore contains substances acting as beta-blockers. Hypotensive and antihypertensive properties of the total aqueous extract of Justicia secunda (Acanthaceae) justify the use of this plant in traditional medicine to treat high blood pressure.

Keywords: Justicia secunda, β-adrenergic receptor, hypotension, hypertension, antihypertensive.

1. Introduction

Cardiovascular diseases are a major cause of death worldwide and hypertension is the most common of these diseases (Waebber et al., 1999). WHO considers cardiovascular disease in general and hypertension in particular as a public health problem in the world. In order to counter the rising cases of hypertension studies are undertaken to seek cost effective remedies against the disease. Several antihypertensive herbal recipe known and recognized for their therapeutic effects, are offered by traditional medicine for the treatment of this disease (Tra-Bi et al., 2008; WHO, 2012).

Justicia secunda M. Vahl (Acanthaceae) is a plant used in traditional medicine in Côte d'Ivoire (Tra-Bi et al., 2008) and the Caribbean (Etifissier-Chalone, 2005) for its therapeutic properties in the treatment of hypertension. Some studies have shown that this plant is involved in reproduction (Lans, 2007). It has antimicrobial (Herrera et al., 2002; Rojas et al., 2006), abortive (Grenand et al., 1987) and anti-anemia (Chifundera, 2001; Moswa et al., 2008; Tossou et al., 2008; N’guessan et al., 2010) properties.

This study aims to verify the antihypertensive properties of aqueous leaf extract Justicia secunda which the decoction of leaves, in traditional medicine, is deemed effective in treating the high blood pressure.

2. Materials and Methods

2.1 Biological material

2.1.1 Plant material

Our study used fresh leaves of Justicia secunda (Acanthaceae) collected in Abidjan (Côte d'Ivoire). This plant was identified and authenticated by Mr. AKE-ASSI Laurent, Professor Emeritus of Botany at the University Felix Houphouet Boigny (Côte d'Ivoire). A specimen is deposited under the number 21160 in the herbarium of the National Centre of Floristic (CNF) of this University.

2.1.2 Preparation of total aqueous extract of Justicia secunda (AJse)

Fifty grams (50 g) of fresh leaves of Justicia secunda (Acanthaceae) are dropped in one liter (1 L) of distilled water. The mixture is boiled for ten minutes (10 min). The decoction obtained is filtered three times on cotton wool and Whatman filter paper. The filtrate was dried in an oven at 50 °C and then lyophilized. The powder obtained is the total aqueous extract of Justicia secunda (AJse).

2.1.3 Animals

Rabbits of the specie Oryctolagus cuniculus (Leporidae), were used for these experiments. Specimens used weigh between 2 and 2.5 kg. These animals were brought from different farms located in the suburbs of Abidjan (Côte d'Ivoire). Before the experiments, they were acclimated to laboratory conditions in the Department of Biosciences of the University Felix Houphouet Boigny for one week to regulate and harmonize their physiological states. Thus, they were kept at constant temperature (24 ± 2 °C) with 50-55 %
of humidity and a photoperiod of 12 hours of daylight and 12 hours of darkness. They are fed ad libitum with pellet (Iov groin, Abidjan, Côte d’Ivoire) and water. They were carefully screened and confirmed to be healthy during the period of acclimatization and experimentation. All the procedures were conducted in accordance with the guidelines for Care and Use of Laboratory Animals published by the National Institutes of Health.

2.2 Recording arterial blood pressure of Rabbit

The recording of arterial blood pressure of rabbits was performed with a Lud wig mercury manometer. Before intubation of the carotid artery of the animal, a pressure gauge is made in the manometer according to the method described by Abo et al. (2000). In accordance with the National Government rules of Côte-d’Ivoire, rabbit was anesthetized through intraperitoneal injection of ethyl carbamate dose at 40 %, 1g/kg BW. His carotid artery was exposed and intubated and connected to the heart through a polyvinyl tube and the manometer, this allowed us to obtain directly the intracarotid pressure which was recorded on a recording paper. Our method of recording arterial blood pressure by the bloody method is similar to that described by Abo et al. (2000). The saphenous vein was intubated with a catheter connected to a syringe for the administration of pharmacodynamic substances and the extract. A sympathetic nerve is identified at the neck of the animal. It was stimulated with exciting electrodes connected to an electrical stimulator (JEULIN, Réf : 554 035).

2.3 Substance pharmacodynamic

The pharmacological substance used in this study is adrenaline (L-adrenaline) from FLUKA (Germany).

2.4 Statistical analysis of results

The computer program GraphPad InStat (San Diego CA, USA) was used for statistical analysis of results. Values are given as mean followed by the standard error of the mean. The difference between two values was determined by Student-Newman-Keuls comparison test. It was considered significant for p < 0.05. The computer program GraphPad Prism 5 (San Diego CA) was used for plotting graphs. Sigmoid curve was drawn after transformation of values of x-axis as decimal logarithm and as a percentage for the y-axis.

3. Results

3.1 Dose-response effect of total aqueous extract of Justicia secunda (AJs e) on normal blood pressure

In this study, increasing doses of AJs e were injected to rabbits, in time intervals of 15 minutes after the effect of each dose. AJs e administrated in increasing doses from 1.25 to 75 mg/kg BW induced a drop in blood pressure. This hypotension was dose-dependent. AJs e doses below 1.25 mg/kg BW had no effect on blood pressure. The figure 1-a shows a typical recordings of the effects of AJs e on blood pressure of rabbit. In this first series of experiments to study, the average of normal blood pressure of rabbits used was 95 ± 6 mm Hg (n = 4). The injection of AJs e gave three types of results according to the doses of the extract.

- The extract of AJs e, between 1.25 and 40 mg/kg BW, induced hypotension ranging from 5 to 28 mm Hg, resulting a drop in blood pressure ranging from 5.21 % to 29.17 % compared to the reference blood pressure of rabbit used. At these doses, the AJs e effects are completely reversible.
- However, doses of 50-60 mg/kg BW induced a hypotension partially reversible. The decrease in the blood pressure varied from 33 to 36 mm Hg. The reduction of the blood pressure ranged from 34.35 % to 37.5 % compared to the reference blood pressure of rabbits used.
- For the doses of AJs e superior or equal to 75 mg/kg BW, the induced hypotension, greater or equal to 38 mm Hg, was irreversible and became lethal from 100 mg/kg BW.

The curve in Figure 1-b shows the percentages of the decrease of arterial blood pressures recorded compared to the reference used rabbits in the presence of increasing doses of AJs e for 4 experiments. This sigmoidal curve allowed us to determine the value of the 50 % effective dose (ED50) of AJs e which is 15.17 mg/kg body weight.

3.2 Effects of AJs e on induced-hypertension in rabbit

3.2.1 Effects of AJs e on the hypertension induced by adrenaline

The purpose of this study was to measure the effects of AJs e on the hypertension induced by the administration of adrenaline. Thus, in a first series of experiments, the injection of different doses of AJs e was preceded by the injection of a single dose of adrenaline. The figure 2-a shows a typical recordings of the results of this series of experiments. Adrenaline, at 5.10^-4 mg/kg BW, increased blood pressure of 52.75 ± 4.25 mm Hg (Figure 2-a-A). When the injection of the same dose of adrenaline (5.10^-4 mg/kg BW) was followed, after 10 s, by the injection of AJs e in the concentration range of 10 to 30 mg/kg BW, adrenaline induced hypertension is reduced. This decrease in the blood pressure is dose-dependent and varied from 4.74 % to 51.18 %. When the injection of adrenaline at 5.10^-4 mg/kg BW was followed by AJs e at 40 mg/kg BW, it appeared a transient hypertension of 8.25 ± 2.18 mm Hg, followed by a hypotension of 6.5 ± 2.04 mm Hg. Thus, AJs e was preceded by the injection of 5.10^-4 mg/kg BW and provoked hypotension. The columns in Figure 2-b shows the effects of AJs e on hypertension induced by adrenaline for 4 series of experiments.

In the second series of experiments, adrenaline at 5.10^-4 mg/kg body weight was associated with increasing doses of AJs e and administrated to rabbits. The figure 3-a shows a typical recordings of the results of this second series of experiments. Injected alone to rabbits, adrenaline at 5.10^-4 mg / kg BW induced hypertension of 50.75 ± 3.25 mm Hg (n = 4). When the adrenaline was associated with different doses of AJs e, hypertension was reduced proportionally to the dose of the extract. Indeed, with a dose of adrenaline of 5.10^-3 mg/kg BW associated with AJs e ranging from 5 to 30 mg/kg BW, the increased of the blood pressure, which
initially were 50.75 ± 3, 25 mm Hg in the presence of adrenaline alone decreased. For these AJs extracts doses, the values of the hypertension induced by adrenaline varied between 38.75 ± 2.95 mm Hg and 4 mm Hg ± 1.91, which corresponded to a reduction in blood pressure ranging from 23.65% to 92.12%. At 40 mg/kg BW AJs totally cancelled the hypertension induced by the adrenaline at 5.10^-3 mg/kg BW because the two compounds associated at these doses didn’t provoke hypertension. It was rather observed a hypotension of 4.5 ± 2.65 mm Hg. The columns in Figure 3-b shows the hypertension induced by adrenaline in the presence of AJs for 4 series of experiments.

### 3.2.2 Effects of AJs on hypertension induced by sympathetic nerve stimulation in rabbit

In this series of experiments, we determined the effects of AJs on the hypertension induced by the stimulation of the peripheral nervous system. Thus, stimulation of the sympathetic nerve of the rabbit was immediately followed by the injection of AJs at different doses. Figure 4-a shows a typical recording of the results of this series of experiments. Electrical stimulation of the sympathetic nerve of rabbits induced an increased blood pressure of 15 ± 2.82 mm Hg throughout the duration of the stimulation (45 s) (Figure 4-a-A). When stimulation of the sympathetic nerve is followed after 3 s, by the administration of AJs at 10 mg/kg BW, the increased of blood pressure obtained was 11.25 ± 3.82 mm Hg; resulting 25% of drop in blood pressure, while stimulation was maintained for 1 min. AJs administered at a dose of 20 mg/kg BW, after sympathetic nerve stimulation induced a transient decrease in the blood pressure of 6.76 ± 3.86 mm Hg, followed by a return to a normal high blood pressure despite the continued stimulation of the nerve (for 1 min). The hypertension induced by stimulation of the sympathetic nerve disappeared immediately after the administration of AJs at 30 mg/kg BW and a hypotension of 4.5 ± 2.66 mm Hg appeared.

Thus, AJs significantly reduced and even canceled hypertension induced by stimulation of the sympathetic nerve. The results obtained from the four series of experiments enable us to plot the columns in Figure 4-b showing the effects of AJs on hypertension induced by stimulation of the sympathetic nerve.

### 4. Discussion

The total aqueous extract of Justicia secunda (AJs) at doses ranging from 1.25 to 75 mg/kg BW induced in rabbit dose-dependent hypertension. This extract reduced and even canceled hypertension induced by adrenaline at 5.10^-4 mg/kg BW or electrical stimulation of the sympathetic nerve fibers of the rabbit. AJs is therefore a hypotensive and antihypertensive substance. The hypotensive properties of Justicia secunda were also noted by Manda et al. (2011).

Stimulation of orthosympathetic fibers causes the release of adrenaline from the nerve endings of these fibers. To act on the cardiovascular system, the substance binds to β-adrenergic receptors (Castaigne, 1988). According to Lands et al. (1967 and 1976), there are two sub-types of receptor β: β1 and β2 adrenergic receptors. In the myocardial cells, β1 receptors are predominant. The binding of a β1 adrenergic agonist to its specific receptor leads to an increase in cardiac output, increased cardiac contractility leading to an increase in blood ejection volume (Witchitz, 1994, Brown, 1995; Westfall and Westfall, 2006). Classically, vascular β-adrenergic receptors are of type β2 and their stimulation causes vasoconstriction (Berdeaux and Edward, 1997; Guimarães and Moura, 2001). Cardioactivates and vasoconstrictor effects of adrenaline cause rise in blood pressure (Castaigne, 1988; Berdeaux and Edward, 1997).

The inhibition of hypertension induced by adrenaline administered or released after stimulation of sympathetic nerve fibers of rabbit, by AJs indicates that this extract would act by inhibiting the effects of activation of β-adrenergic receptors. The total aqueous extract of Justicia secunda therefore contains substances acting as beta-blockers. Indeed, beta blockers or beta-adrenergic or antiadrenergic beta are competitive inhibitors of the effects of catecholamines on β-adrenergic receptors (Castaigne, 1988; Witchitz, 1994; Brown, 1995; Westfall and Westfall, 2006). Beta-blockers inhibit specifically and competitively peripheral and also central beta-adrenergic receptors if they pass the blood-brain barrier. They have common effects essentially on cardiovascular system. They act primarily by reducing the activity of catecholamines on the heart and blood vessels and also decrease blood pressure (Bradley et al., 2006; Heusser et al., 2007; Wiysonge et al., 2007).

### 5. Conclusion

This study shows that the total Justicia secunda aqueous extract contains hypotensive and antihypertensive substances. These properties of this extract of Justicia secunda justify the use of this plant in traditional medicine to treat high blood pressure.

### References

6. Chifundera K. 2001. Contribution to the inventory of medicinal plants from the Bushi area, South Kivu
Figure 1: dose-response effects of an aqueous extract of *Justicia secunda* total (AJse) on arterial blood pressure of rabbit

**a** – Recording result

A to J - Effects of AJse (after the arrow) at 0.5 (A), 1.25 (B), 2.5 (C), 5 (D), 10 (E), 20 (F), 30 (G), 40 (H), 50 (I) and 75 (J) mg/kg body weight (BW)

**b** - Drop in blood pressure according to the dose of AJse Administered

n = 4, ns P > 0.05, * P < 0.05, ** P < 0.01, *** P < 0.001.

AJse causes a dose-dependent hypotension in rabbit.

The curve showing the effect of AJse on blood pressure is a sigmoid.
Figure 2: Effects of a total aqueous extract of *Justicia secunda* (AJse) on hypertension induced by adrenaline administration in rabbit

**a** – Recording result

- **A** - Effects of adrenaline (ADR) at $5 \times 10^{-4}$ mg/kg body weight (BW)
- **B to E** - Effects of ADR at $5 \times 10^{-4}$ mg/kg body weight, followed by injection of AJse at 10 (B), 20 (C), 30 (D) and 40 (E) mg/kg BW

**b** – Decrease in hypertension induced by adrenaline by AJse

$\text{n} = 4$, ns $P > 0.05$, ** $P < 0.01$, *** $P < 0.001$.

*Hypertension induced by administration of adrenaline was significantly reduced in the presence of AJse.*
Figure 3: Cumulative effects of total aqueous extract of Justicia secunda (AJse) and adrenaline on the arterial blood pressure of rabbit

A - Recording result
B to F - Effects of ADR at 5.10^4 mg/kg BW + AJse at 5 (B), 10 (C), 20 (D), 30 (E) and 40 (F) mg/kg BW

AJse significantly reduced and even canceled the hypertension induced by the administration of Adrenaline to the rabbit.
Figure 4: Effects of total aqueous extract of *Justicia secunda* (AJse) on hypertension induced by sympathetic nerve stimulation of rabbit.

A – Recording result

A: Effects of sympathetic nerve stimulation
(Intensity = 6 mV, duration = 0.6 ms, frequency = 25 Hz)

B to D: Effects of sympathetic nerve stimulation and injection of AJse at 10 (B), 20 (C) and 30 (D) mg/kg body weight (BW)

b – Reduction of hypertension induced by stimulation of sympathetic nerves by AJse

(n = 4, * P < 0.05, ** P < 0.01, *** P < 0.001)

AJse significantly reduced and even canceled hypertension induced by stimulation of the sympathetic nerve of the rabbit.