Evaluation of the antihypertensive activity of total aqueous extract of *Justicia secunda* Valh (Acanthaceae)

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Accepted 28 September, 2011

Our study aim at contributing to establish the scientific basis for the use of *Justicia secunda* Vahl. (Acanthaceae) in the traditional treatment of hypertension in Côte d'Ivoire. We used a total aqueous extract of leaves of *J. secunda* (TAEJS). The experimental device used for recording blood pressure in the rabbit is based on the principle of Ludwig mercury manometer. TAEJS between 5.55 and 55.55 mg/kg causes reversible dose-dependent hypotension. TAEJS caused drops in blood pressure ranging between 7.93 ± 3.02 and -8.41 ± 5.2% compared to normal blood pressure of rabbits. Fifty percent effective dose of TAEJS is 26.18 mg/kg. Just like hypotension induced by acetylcholine, that caused by TAEJS at dose of 26.18 mg/kg in rabbit is progressively inhibited by atropine dosed between 3 × 10⁻⁵ and 3 × 10⁻¹ mg/kg. However, for a dose of atropine as high as 3 × 10⁻¹ mg/kg, this inhibition is not total. Hypotension induced by TAEJS dosed at 26.18 mg/kg was 58 ± 2.1% in the absence of atropine, and decreased from 45.3 ± 2.11 to 5.09 ± 0.96% with doses of atropine between 3 × 10⁻⁵ and 3 × 10⁻¹ mg/kg. The increasing doses of TAEJS, between 22.22 and 55.55 mg/kg reduced significantly the hypotension induced by adrenaline dosed at 5.78 × 10⁻⁴ mg/kg from 60.8 ± 1.29% to -5.91 ± -1.22%. Pharmacological studies of TAEJS on blood pressure of rabbits showed that extract of our plant has a potential antihypertensive effect which is due to the presence of several active substances whose nature remains to be determined.

Key words: *Justicia secunda*, total aqueous extract, antihypertensive activity.

INTRODUCTION

More than a quarter of the world adult populations are hypertensive, this percentage would increase in coming years and sub-Saharan Africa is no exception to this trend. In year 2000 about 80 million patients suffered from high blood pressure (hypertension), and according to epidemiological projections this figure will increase to 150 million in 2025 (Fourcade et al., 2007). The current emergence of chronic diseases such as hypertension is therefore a public health problem in Africa and particularly in Côte d'Ivoire. The prevalence of hypertension in Côte d'Ivoire is estimated about 8 to 12% in year 2000 about 13% of the population of which 21% in Abidjan alone (Koffi, 2007). The modern treatment of hypertension is expensive for many people in developing countries. Treatment and medical expenses to them is additional economic problem. As alternative therapy this population now relies on medicinal plants for the treatment of various ailments, good enough African forest is well endowed (Adjouonou, 1990). Indeed, many medicinal plants are used by this alternative medicine for the treatment of various ailments. Ethnobotanical surveys conducted in the markets of Abidjan (Cote d'Ivoire) have enable us to establish an inventory of 27 (46.55%) of the plants often...
used specifically in the treatment of hypertension among 58 species of medicinal plants commercialized (Tra et al., 2008). Ignorance of the real effects these plants can cause discomfort in some cases and can lead to dramatic complications (Bleyere et al., 2010). In light of this problem, several projects are being undertaken for the development of traditional medicine through the establishment of scientific databases. This research work has led to the production of, in some cases, phyto-drugs used in primary health care (OMS, 2003). Indeed the World Health Organization (WHO) encourages the development of regional strategies on traditional medicine to undertake research on medicinal plants and promote their optimal use in the health care delivery system. Many plants such as *Stephania tetrandra* (Menispermaceae) (Yu et al., 2004), *Berberis vulgaris* L. (Berberidaceae) (Fatehi-Hassanabad et al., 2005), and *Morinda Monnindoides* (Nguessan et al., 2004) have been evaluated experimentally to justify their anti-hypertensive effect. An ethnobotanical survey directed us to a plant in the Ivorian pharmacopoeia, traditionally used by peoples of southern forest of Côte d’Ivoire to treat high blood pressure: *Justicia secunda* Vahl. Acanthaceae (Fournet, 1978). The decoction of this ornamental plant is used as antihypertensive and blood purifier. Our study is a contribution to the promotion of the pharmacopoeia in Côte d’Ivoire, aims to establish the scientific basis for the use of *J. secunda* in hypertension treatment. Indeed, we assessed the effects of total aqueous extract (TAEJS) of leaves of *J. secunda* on blood pressure of rabbits.

**MATERIALS AND METHODS**

**Biological materials**

**Preparation of total aqueous extract of *J. secunda* (TAEJS)**

The biological material used consists of plant leaves of *J. secunda* (Acanthaceae). These leaves were collected in Alepe, a city in southern Côte d’Ivoire, between January and March 2008. The leaves were then washed and dried under shade at room temperature (25 to 30°C). Once dried, these leaves were made into fine powder. The method used to prepare the TAEJS has been described by several authors (Guede-Guina et al., 1993; Zirihi et al., 2007; Moroh et al., 2008). Several test solutions at different concentrations were prepared with the extract.

**Animals**

The experiments were performed on rabbits of the species *Oryctolagus cuniculus* (Leporidae). These animals were obtained from a poultry farm on the outskirts of the city of Abidjan. They were acclimated for one month prior to the commencement of the experiment in the Toxicology Laboratory of the Faculty of Pharmaceutical and Biological Sciences at the University of Cocody-Abidjan. The specimens used weighed between 1.9 and 2.1 kg.

**Pharmacodynamic substances**

The reference substances used in this study were: atropine (ATR), acetylcholine (ACH), adrenaline (ADR) from laboratory Prolabo (France). These substances were dissolved in NaCl 9%.

**Experimental setup and recording technique**

The experimental device used for recording blood pressure in the rabbit is based on the principle of the mercury manometer of Ludwig. The rabbits were anesthetized by intraperitoneal injection of ethyl-urethane 40% dosed at 1 g/kg of body weight (bw). His carotid artery was exposed and intubated using a catheter connected to a U-tube manometer, which collects directly the intracarotid. This method measures the level of reference pressure in rabbits. Changes in the carotid pressure, transmitted to the mercury column of the device are recorded with a pen that translates the movements of mercury on the smoked paper placed on a cylinder driven at constant speed. Pharmacodynamic substances, and the total aqueous extract extract of *J. secunda* (TAEJS) are injected into the animal through the saphenous vein.

**Pharmacological tests**

**Dose-response hypotensive effect of acetylcholine and TAEJS**

Once the normal blood pressure recorded at the equilibrium point, increasing doses of total aqueous extract of *J. secunda* (5.55 to 55.5 mg/kg) injected through the saphenous vein of rabbits previously anesthetized with ethyl-urethane 40%. Variations in arterial blood pressure under the effect of different doses of TAEJS are observed and recorded for plotting the dose-response curve to determine the effective doses 50 (ED₅₀). This operation was repeated in other rabbits prepared under the same conditions with acetylcholine (3×10⁻⁵ to 3×10⁻⁴ mg/kg), injection of different doses occurs every 30 min. With the different ED₅₀ obtained, we perform the different interactions.

**Interactive effects of ATR- TAEJS, ADR- TAEJS on variation of blood pressure**

From hypotension state induced by TAEJS (fixed concentration), we injected increasing concentrations of atropine. The aim of this experiment is to find out whether the hypotension observed in the presence of a fixed concentration of TAEJS could be reduced by atropine (3×10⁻⁵ to 3×10⁻⁴ mg/kg) compared to the effect observed with acetylcholine (fixed concentration) following administration of The same increasing concentrations of atropine. The injection of ACh or TAEJS was preceded to that of atropine.

From hypertension induced by adrenaline (fixed concentration), we injected increasing concentrations of TAEJS. We are studying the anti-hypertensive effect of TAEJS (22.22 to 55.55 mg/kg) following injection of a fixed concentration of adrenaline.

**Statistical analysis**

Statistical analysis and graphs were made using the software graph pad prism 5.01 (San Diego, California, USA). The results were arithmetic averages of individual values assigned to the standard error of the mean (m ± SE). The significance of differences observed between the percentage of drop value compared to the reference pressure was obtained by analysis of variance (ANOVA 1) and test of multiple comparisons Tukey-Kramer. In this test, when p <0.05, p <0.01 and p <0.001, respectively, the differences are significant, very significant and highly significant. The ED₅₀ could be calculated with this software.
Figure 1 (A and B). Dose response effect of acetylcholine and TAEJS on blood pressure of rabbits. 1A: Dose-response effects of ACh: A1 to A7: Effect of Ach of $3 \times 10^{-8}$ (A1), $3 \times 10^{-7}$ (A2), $3 \times 10^{-6}$ (A3), $3 \times 10^{-5}$ (A4), $3 \times 10^{-4}$ (A5), $3 \times 10^{-3}$ (A6) and $3 \times 10^{-2}$ (A7) mg/kg bw. Arrows indicate the time of injection of ACh. 1B: Dose-response effects of TAEJS: B1: before administration of TAEJS, B2 to B11: Effect of TAEJS of 5.55 and 55.55 mg/kg bw. Arrows indicate the time of injection of TAEJS.

RESULTS

Comparative study of dose-response effects of acetylcholine and TAEJS

Figure 1 (A and B) show a recorded type of dose-dependent hypotension induced by TAEJS and acetylcholine in the rabbit. TAEJS between 5.55 and 55.55 mg/kg bw and acetylcholine between $3 \times 10^{-8}$ and $3 \times 10^{-2}$ mg/kg bw cause dose dependent reversible hypotension. TAEJS cause drops in blood pressure ranging between 7.93 ± 3.02 and -8.41 ± 5.2% while the acetylcholine induced hypotension between 0 and 63.20 ± 8.2% compared to normal blood pressure of rabbits. The curves of Figures 2 and 3 were used to determine 50% effective doses (ED$_{50}$) of ETAJS and acetylcholine which were respectively 26.18 mg/kg(bw) and 3.74 .10$^{-4}$ mg/kg(bw).

Effects of acetylcholine (Ach) in the presence of atropine (ATR)

In the presence of increasing concentrations of atropine...
***P <0.001: significant difference when compared to the percentage decrease from reference blood pressure (n = 3). The 50(E50) is 3.74.10^(-4) mg/kg bw. **% effective dose.

dosed between 3.10^{-5} and 3.10^{-1} mg/kg, hypotension induced by ACh of 3.74.10^4 mg/kg was progressively suppressed (Figure 4A). It vanishes completely in the presence of atropine dosed at 3.10^{-1} mg/kg. Indeed, the effectiveness of hypotension induced by ACh of 3.74.10^4 mg/kg (bw), was 44.9 ± 2 mmHg in the absence of ATR with a decrease of 58.80 ± 1.4% compared to the normal pressure recorded. In the presence of atropine with increasing doses of 3.10^{-5} to 3.10^{-1} mg/kg bw percentagess of drop in blood pressure recorded ranged from 43.5 ± 1.78% to 0.133 ± 0.03% (n = 3). This hypotension vanishes with 3.10^{-1} mg/kg bw of ATR (Figure 5). Thus 3.74.10^{-4} mg/kg of ACh was 100% inhibited by 3.10^{-1} mg/kg bw of ATR.

Effects of TAEJS in the presence of atropine

Just like hypotension induced by ACh also hypotension caused by 26.18 mg/kg bw of TAEJS in rabbit was progressively inhibited by ATR dosed between 3.10^{-5} to 3.10^{-1} mg/kg (Figure 4B). However, for a dose of ATR as high as 3.10^{-1} mg/kg, this inhibition is not total. Hypotension induced by TAEJS with 26.18 mg/kg bw which was 58 ± 2.1% in the absence of ATR, decreased 45.3 ± 2.11 to 5.09 ± 0.96% in the presence of ATR dosed between 3.10^{-5} and 3.10^{-1} mg/kg (Figure 6).

Antihypertensive effects of TAEJS

Figure 7 is a recorded type of the antihypertensive effect of TAEJS on blood pressure of rabbit. The increasing doses of TAEJS, between 22.22 and 55.55 mg/kg bw significantly reduced the hypertension induced by adrenaline (ADR) 5.78.10^4 mg/kg bw. The results obtained were used to sketch the reduction of hypotension induced by adrenaline, depending on the dose of TAEJS (Figure 8). The value of the normal average blood pressure in this series of experiments is 92 ± 1 mmHg. Adrenaline injected at a dose of 5.78.10^4 mg/kg bw induced hypertension with a value of 137 ± 1 mmHg. The injection of increasing doses of TAEJS, ranging from 22.22 to 55.55 mg/kg bw, decreased hypertension created by adrenaline dosed at 5.78.10^{-3} mg/kg bw from 60.8 ± 1.29% to -5.91 ± -1.22%.

DISCUSSION

Our results show that TAEJS, from 5.55 to 55.55 mg/kg bw have a dose-dependent hypotensive effects in rabbits similar to those of many herbal extracts such as Mareya micrantha (Benth.) Mull. Arg. (Euphorbiaceae) (Abo, 1996; Abo et al., 2000), Daucus carota L. (Apoaceae) (Gilani et al., 2000), Swartzia madagascariensis Desv. (Fabaceae) (Soro et al., 2004), Heliotropium indicum L. (boroginate) (Traoré et al., 2002) and Gossypium barbadense L. (Malvaceae) (Hasrat et al., 2004), Bidens pilosa (Asteraceae) (Kouakou et al., 2008). Hypotension induced by this extract in rabbits is reversible at low doses and irreversible at high doses. These results are comparable to those obtained by other authors with extract of Mareya micrantha (Euphorbiaceae) (Abo et al., 2000) and Musanga cecropioides R.Br. (Urticaceae) (Kamanyi et al., 1995). The regulation of blood pressure is primarily dependent on cardiac activity and vascular resistance, the hypotensive effect of TAEJS could be exercised, either on one or the other of these two factors or the two at a time. The hypotensive action induced by total aqueous extract of J. secunda is similar to the well-known one of acetylcholine on blood pressure. Consequently, these effects require the presence of cholinomimetic substances in the total extract. To test this hypothesis, we undertook a comparative study of antagonisms ATR- TAEJS and ATR-ACh. The results obtained showed that the hypotensive effects of ACh were completely inhibited by ATR, in contrast to the effects of TAEJS that are only partially inhibited by ATR. This study suggests the existence of muscarinic type of cholinomimetic substances in TAEJS. The partial inhibition effect of TAEJS by ATR suggests the presence of hypotensive active ingredient non-cholinomimetic in addition to muscarinic type of cholinomimetic substances in this total extract. These substances may act through the same mechanism as acetylcholine. Indeed, intravenous injection of acetylcholine in humans or animals causes an immediate drop in blood pressure from the slowing of the heart rate and vasodilation (Supple and Powell, 1981; Abo, 1996; Nguesan et al., 2004). This injection causes sinus bradycardia, decreased atrioventricular conduction and decrease the strength of ventricular
Figure 4A and 4B. Effects of ACh and TAEJS in the presence of ATR on blood pressure of rabbit. 4A: Effect of ACh in the presence of ATR: A1: before administration of ACh; Effects of ATR $3 \times 10^{-5}$ (A2), $3 \times 10^{-4}$ (A3) $3 \times 10^{-3}$ (A4) $3 \times 10^{-2}$ (A5) $3 \times 10^{-1}$ (A6) mg/kg bw followed by the effect of ACh $3.74 \times 10^{-4}$ mg/kg bw. Arrows indicate the time of injection of different substances. 4B: Effects of TAEJS in the presence of ATR: B1: before administration of ACh; Effects of ATR $3 \times 10^{-5}$ (B2) $3 \times 10^{-4}$ (B3) $3 \times 10^{-3}$ (B4) $3 \times 10^{-2}$ (B5) $3 \times 10^{-1}$ (B6) mg/kg bw followed by the effect of TAEJS of 26.18 mg/kg bw. Arrows indicate the time of injection of different substances.

Figure 5. Changes in blood pressure of rabbits depending on the dose of atropine with ACh. *P <0.05, ** p <0.01, *** p <0.001: significant difference compared to the percentage decrease from reference blood pressure (n = 3).

Figure 6. Change in blood pressure of rabbits depending on the dose of atropine with TAEJS. *P <0.05, **p <0.01, ***p <0.001: significant difference compared to the percentage decrease from reference blood pressure (n = 3).
Figure 7. Antihypertensive effect of TAEJS on blood pressure of rabbits. A: Effect of TAEJS on hypertension induced by adrenaline in a dose-dependent. A1: before administration of ADR; Effect of TAEJS from 22.22 (A2) to 55.55 (A8) mg/kg preceded by the effect of ADR $5.78 \times 10^{-4}$ mg/kg. Arrows indicate the time of injection of different substances.

Figure 8. Antihypertensive effect of TAEJS on blood pressure of rabbit. *P <0.05, **p <0.01, ***p <0.001: significant difference compared to the percentage decrease from reference blood pressure (n = 3).
ventricular contraction. The cardiac slowing is due partly to the cell hyperpolarization following the opening of potassium channels that are directly related to G proteins (Zakarov and Harvey, 1997; Kurachi and Ishii, 2003; Feischmann et al., 2004). The reduction in force of contraction was due to lower entry of calcium into the cell, probably by inhibiting adenylate cyclase (Hartzell, 1988; Hanf et al., 1993; Jurevièius and Fischmeister, 1996; Han et al., 1998). The injection of acetylcholine causes vasodilation pressure following the release by the endothelium of a vasodilator, nitric oxide (NO) or a hyperpolarizing factor (endothelium derived hyperpolarizing factor or EDHF) (Furchgott and Zawadzki, 1980; Damas, 2002; Stuart-Smith, 2002; Takano et al., 2004). Our results also showed that adrenaline, adrenergic receptor agonist, dosed 5.78.10⁻⁴ mg/kg of body weight induced hypertension in rabbits. Interactions adrenaline– ETAJS showed a significant reduction of hypertension by ETAJS. This antihypertensive effect obtained with our extract is similar to that observed with extracts of S. tetrandra (Menispermaceae) (Yu et al., 2004) and B. vulgaris (Berberidaceae) (Fatehi Hassanabad et al., 2005). The inhibition of adrenoceptors causes a reduction in adenylyl cyclase activity, a decrease in the concentration of cAMP in the cell and reduced the concentration of cytosolic calcium (Witchitz, 1994; Guimaraes and Moura, 2001). Finally, the hypertension induced by adrenaline (ADR) is blocked by ETAJS, suggesting an interference of the extract with certain classes of adrenergic receptors. But the antihypertensive action goes beyond the normalization of blood pressure with a hypotensive effect that follows at higher doses. We suggest that the ETAJS extract could have a more expanded action in the adrenergic receptors that is to say an antagonistic action in both the Beta 1 receptor and the heart (whose stimulation leads to contraction myocardial fibers), also in the alpha 1 receptors located in blood vessels (whose stimulation causes vasoconstriction). Accordingly, hypotensive non cholinomimetic substances in the ETAJS could act like alpha and Beta blockers can rapidly induce hypotension. The hypotension that follows the hypertension inhibition of pressor effect of adrenaline, with the administration of increasing doses of ETAJS could also confirm a nonspecific action on the adrenergic system by our sample by its effect on the cholinergic system. As extracts of Swartzia madagascariensis Desv. (Fabaceae) (Soro et al., 2004), Mareya mirantha (Benth.) Mull. Arg. (Euphorbiaceae) (Abo et al., 2000), Morinda morindoides (Nguessan et al., 2004), Bidens pilosa (Asteraceae) (Kouakou et al., 2008), could contain two antihypertensive factors: muscarinic type of cholinomimetic factors whose effects are inhibited by atropine and adrenolytic type of non cholinomimetic factors. Indeed ETAJS appears to induce more lasting hypotension which does not depend only on the interference with the cholinergic system. However the hypotheses regarding the possible mechanisms underlying the antihypertensive action of ETAJS must be confirmed by more elaborate studies. Indeed, pharmacologically, taking into account interactions ETAJS - ATR and ETAJS-ADR, it is not excluded that our sample interferes with only one system (cholinergic or adrenergic). Thus the antihypertensive action observed in the interaction with ATR or ADR does come as a functional type of antagonism. A pharmacological screening following the isolation of several active fractions of ETAJS is needed to better clarify the possible mechanisms behind the antihypertensive activities.

Conclusion

Pharmacological studies of ETAJS on blood pressure of rabbits revealed that this extract has potential antihypertensive effect which is due to the presence of several active fractions whose nature remains to be determined. Active ingredient of various actions on the functional regulation of blood pressure could justify the traditional use of this plant in curing hypertension in Côte d'Ivoire.

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