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Hypotensive Effect of an Aqueous Extract of Leaves of *Senna alata* (L.) ROXB (Caesalpiniaceae), a Plant Known to be Antihypertensive, in Rabbits

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Abstract **Original Research Article**

The leaves of *Senna alata* (L.) ROXB (Caesalpiniaceae) are used in traditional African medicine mainly to treat arterial hypertension. The aim of the present study was to evaluate the hypotensive effects of an aqueous extract of leaves of *Senna alata* on arterial hypertension in rabbits. A qualitative phytochemical study was conducted to determine the various secondary compounds contained in this extract. Using a Ludwig manometer, consisting of a U-shaped tube containing mercury surmounted by a writing stylus, the blood pressure of rabbits was recorded on smoked paper wound around a cylinder rotating at constant speed. The doses of test substances are administered to the rabbits via its exposed saphenous vein. At doses ranging from 10 to 25 mg/kg wb, aqueous extract of *Senna alata* leaves (EAqSa) induced a dose-dependent hypotension comparable to that of acetylcholine. The inhibition of this hypotension in the presence of doses of atropine, an antagonist of muscarinic cholinomimetic substances, of between 10^{-6} and 10^{-3}mg/kg bw suggests that EAqSa contains muscarinic cholinomimetic substances. These substances would be responsible for its hypotensive effect and could be alkaloids and/or flavonoids found in this extract. These results support the use of *Senna alata* in the treatment of hypertension in traditional medicine.

Keywords : *Senna alata,* hypotension, cholinomimetic substances.

1. INTRODUCTION

High blood pressure is a chronic cardiovascular disease that is very deadly. This condition causes serious disabilities that can lead to heart and/or kidney damage in more than half of patients. High blood pressure affects more than 30% of the world's population, or approximately 1.4 billion people. It is the most important preventable risk factor for cardiovascular diseases and deaths worldwide [1]. In Africa, it is a public health problem with a population frequency of between 15 and 40% and a hospital frequency of between 30 and 70% [2]. In Côte d'Ivoire, in 2015, the prevalence of high blood pressure was 20.4% [3]. The management of hypertensive patients requires constant monitoring and lifelong, non-curable treatment, based on the use of remedies that are often poorly tolerated and expensive in hospitals. These prohibitive costs, especially for populations in poor countries, who have difficulty accessing so-called modern medicines, direct patients towards traditional remedies. *Senna alata* (Caesalpiniacaeae), is a plant known and appreciated by the Ivorian populations. It is commonly used for its medicinal properties. It is used in the treatment of many diseases including malaria, constipation and especially hypertension. It is a shrubby plant 3 m high, with a blackish stem, from tropical America, introduced in all tropical regions of Africa [4]. The decoction of the roots, mixed with salt or sugar, is taken for a laxative treatment. The infusion or light decoction of the leaves can be used to treat hypertension [5]. The objective of this work is to evaluate the hypotensive effect of the aqueous extract of leaves of *Senna alata* (Caesalpiniacaeae), in rabbits.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1-Plant Material

The plant material consisted of leaves of *Senna alata* (L.) ROXB (Caesalpiniaceae) (Figure 1) collected in Bonoua, south-east Côte d'Ivoire in August 2020. This plant was identified and authenticated at the National Centre of Floristics (CNF) of the Felix Houphouët-Boigny University (Abidjan, Côte d'Ivoire) under the number UCJ009126.

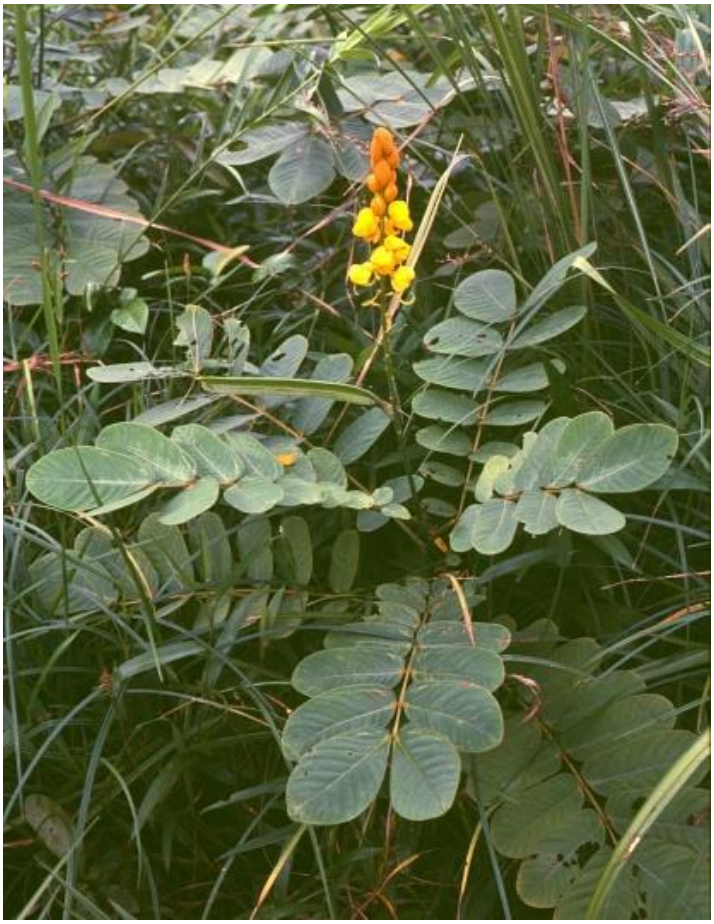

Figure 1: *Senna alata* (L.) Roxb, 1832(Caesalpiniaceae)
https://commons.wikimedia.org/wiki/File:Senna_alata_1.jpg?uselang=fr

2. 1. 2. Animal Material

The animals used in our experiments are rabbits of the species *Oryctolagus cuniculus* (Leporidae) from a breeding farm in Bingerville located east of Abidjan. Before any experiment, these animals are acclimatized to the animal house of the Training and research Biosciences of the Félix Houphouët Boigny University of Abidjan. The room received natural lighting with an average temperature of 25 ± 2 ° C. These animals were placed in cages and had free access to water and food.

2.1. 3. Physiological Solution and Pharmacological Substances

The physiological solution is a 9‰ NaCl solution (Fluka, Germany), used for the study of blood pressure. The chemical substances used during the experiments are Heparin Choay 5000 IU (sanofi-aventis, France) to prevent blood clotting in the cannulas of the Ludwig device, the reference substance is Acetylcholine (SIGMA laboratory St Louis, MO USA, PM: 181.68) with Atropine (prolabo, PM: 289.38) as an antagonist substance and Thiopental 500 mg (neon laboratories limited, India) which was used for the anesthesia of the animals.

2.2. Méthods

2.2.1. Preparation of the Aqueous Extract of Leaves of *Senna alata* (Caesalpiniaceae)

The fresh leaves were dried in the shade at room temperature. One hundred (100) g of crushed dry leaves were placed in two (2) litres of distilled water and boiled for fifteen (15) minutes. The decoctate was double filtered through cotton wool and Wattman paper (3mm). The filtrate is dried in a drying oven at 50°C for 72 hours. The aqueous extract of *Senna alata* leaves (EAqSa) is in powder form.

2.2.2. Qualitative Phytochemical Characterisation of the Aqueous Extract of Leaves of *Senna alata*

This study was carried out at the Pharmacognosy Department of the Pharmaceutical and Biological Sciences Training and Research Unit of the

Université Félix Houphouët Boigny in Abidjan. Qualitative phytochemical screening was carried out using a qualitative method described by Néné Bi *et al.*, (2008) and Abo (2013). It is based on specific chemical reactions that enable the different families of secondary metabolites of pharmacological and therapeutic interest to be identified.

2.2.3. Study of the Effects of the Aqueous Extract of Leaves of Senna alata on the Blood Pressure of Rabbits

2.2.3.1. Experimental Device for Recording Blood Pressure in Rabbits

The device used for recording blood pressure is the Ludwig manometer. It is composed of a U-shaped tube whose two branches contain mercury topped with a writing stylus. This writing stylus is used to transcribe the rabbit's blood pressure onto a cylinder covered with carbon black-coated paper and driven by a constant-speed motor.

2.2.3.2. Recording Technical for Blood Pressure in Rabbits

The animal is anesthetized by intraperitoneal injection of Thiopental at a rate of 1 g/kg bw, its carotid is connected to one of the branches of the Ludwig manometer. The variations in the rabbit's blood pressure are transmitted to the mercury column. Indeed, since the manometer has a uniform section, any variation in the mercury level in each branch containing the writing stylus corresponds to an equal variation, but in the opposite direction, in the other branch. Thus, to determine the exact value of the variations in the rabbit's blood pressure, the pressure variations must be multiplied by two. Given the float and the metal rod of the writing stylus on the paper, there is a certain loss of blood pressure in the measuring device.

2.2.4. Treatment of Results

The recordings made on the smoked paper are varnished in order to fix the carbon black, then scanned before being inverted using MICROSOFT Paint software. The statistical analyses of the values and the graphical representation of the data were carried out using Graph Pad Prism 8 software (San Diego, California, USA). The statistical difference between the results was carried out using the analysis of variances (ANOVA). All values are presented in the form of mean ± SEM (Standard Error of the Mean) and for $P < 0.05$ the observed difference is significant.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Qualitative Phytochemical Composition of the Aqueous Extract of Leaves of *Senna alata*

The phytochemical screening revealed the presence of flavonoids, sterols and polyterpenes, catechic tannins, polyphenols, quinone compounds, saponosides and alkaloids in the aqueous extract of *Senna alata* leaves. Gallic tannins are absent (Table 1).

Table 1: Chemical composition of the aqueous extract of leaves of *Senna alata*

Compounds researched		Tests or reagents	Results
Sterols and polyterpenes		Liebermann	+
Polyphenols		Ferric chloride	+
Flavonoids		Cyanidine	+
Saponosides		Vigorous agitation	+
Quinone compounds		Borntraegen	+
Alkaloids		Dragendorff	+
		Bouchardat	+
Tannins	catechics	Stiasny	+
	Gallic	Hydrochloric acid	-

(+): Presence of the compound **(-)**: Absence of the compound

3.1.2. Effects of the Aqueous Extract of Leaves of *Senna alata* on the Blood Pressure of Rabbits

3.1.2.1. Dose-Response Effects of the Aqueous Extract of Leaves of *Senna alata* on the Blood Pressure of Rabbits

The mean value of the reference blood pressure of rabbits used is 120±1.2 mmHg in our experimental conditions. At doses between 10 and 25 mg/kg bw EAqSa induces a dose-dependent hypotension (Figure 2A). This induced hypotension is reversible at low doses. At 20 mg/kg bw, EAqSa causes a very marked sustained hypotension whose values are 10±0.96 mmHg, corresponding to a decrease of 13.79% of the reference blood pressure. Furthermore, the time to return to normal at this dose is 135 seconds. The mean pressure values obtained (n = 3) made it possible to plot the curve expressing the decrease in blood pressure as a function of the dose of EAqSa (Figure 2B).

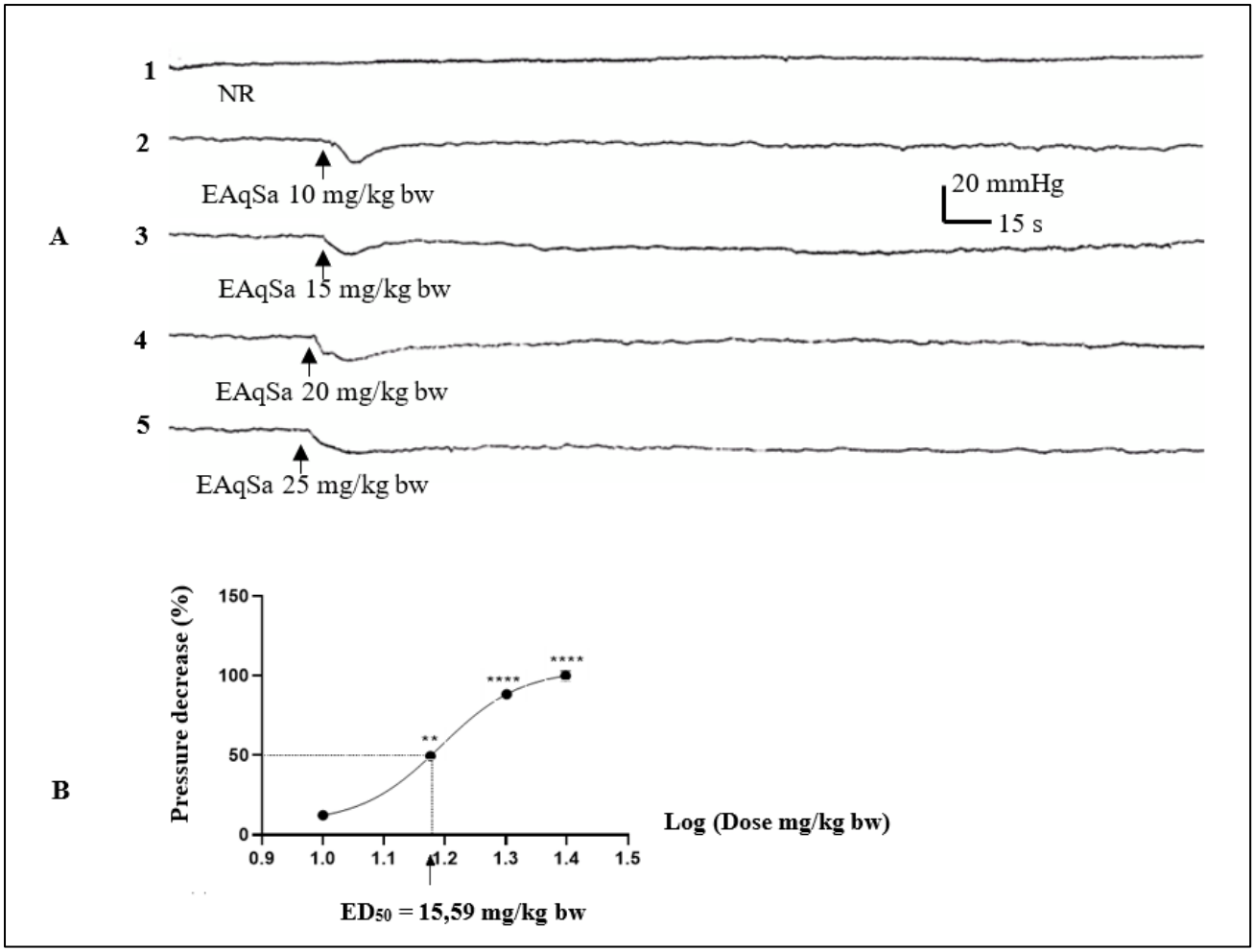

Figure 2: Effect of the aqueous extract of leaves of *Senna alata* on the blood pressure of rabbit

A- Dose-response effects of EAqSa:

NR **(1),** Effects of EAqSa at 10 mg/kg bw **(2)**; 15 mg/kg bw **(3)**; 20 mg/kg bw **(4)** and 25 mg/kg bw **(5)**.

EAqSa induces dose-dependent hypotension

B- Curve of decrease in blood pressure of Rabbit as a function of the dose of EAqSa

This curve allows to determine a fifty percent effective dose (ED_{50}) equal to 15.59 mg/kg bw.

The values express the percentages of maximum decrease in blood pressure compared to the control (Mean ± SEM n = 3; ** $p < 0.01$; **** $p < 0.0001$).

NR: Normal recording; **EAqSa**: Aqueous extract of leaves of *Senna alata*; **SEM**: Standard Error of the Mean

3.1.2.2. Effects of the Aqueous Extract of Senna alata Leaves in the Presence of Increasing Doses of Atropine

The figure 3A shows the effect of 20 mg/kg bw of EAqSa on the blood pressure of the rabbit in the presence of atropine of increasing doses of atropine (ATr). ATr at doses between 10^{-6} and 10^{-3} mg/kg bw, strongly inhibits the hypotension induced by 20 mg/kg bw of EAqSa. The decrease in pressure induced by EAqSa at 20 mg/kg bw is 10%. In the presence of ATr, this hypotension increases to 6.45%, 4.11%, 3% and 1.67% respectively for ATr doses of 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} mg/kg bw.

The mean values obtained for three experiments (n = 3) made it possible to plot the histogram representing the decrease in rabbit blood pressure induced by EAqSa at 20 mg/kg BW in the presence of Atr (Figure 3B). These histograms show that Atr significantly reduces the hypotensive effect of EAqSa.

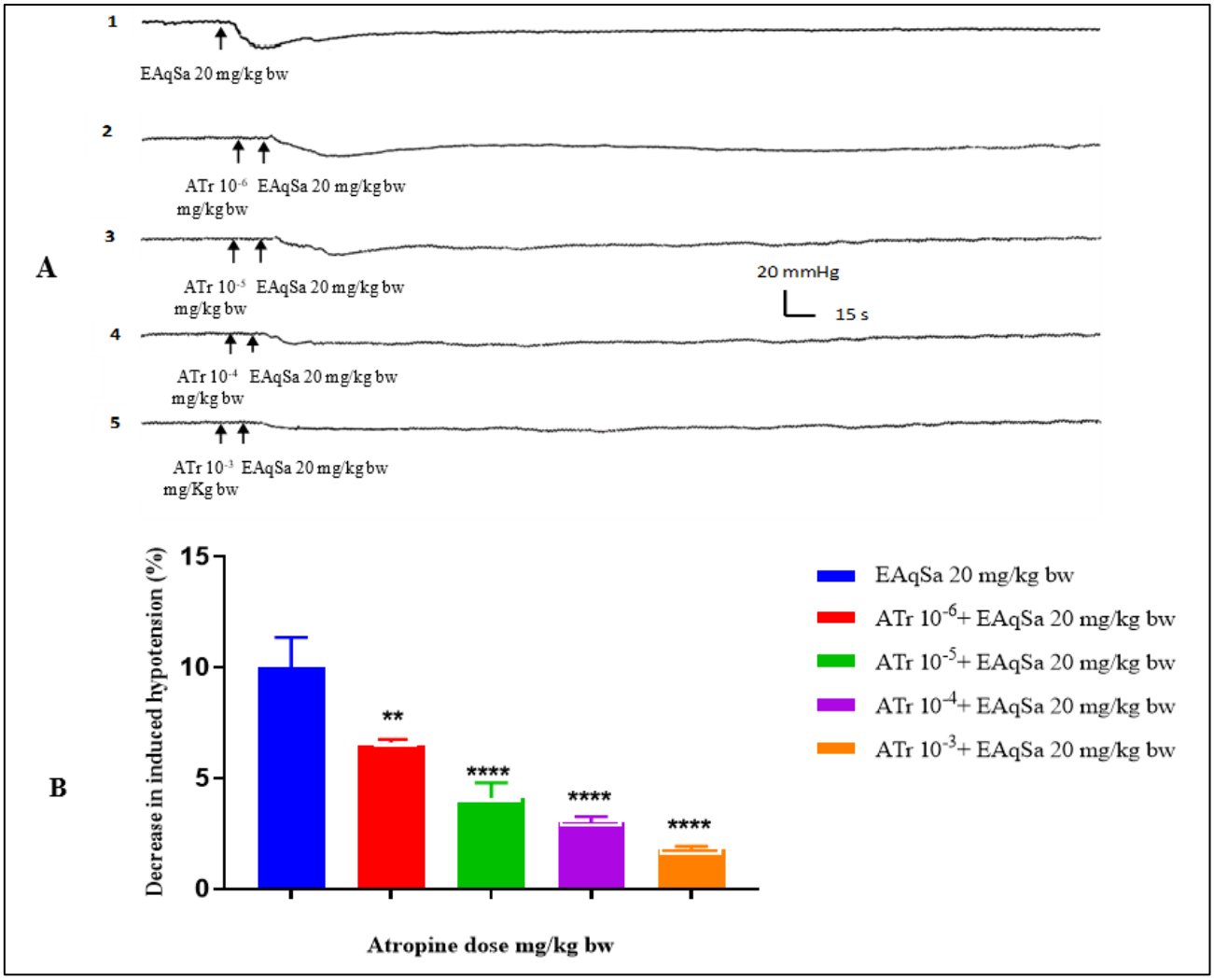

Figure 3: Effects of the aqueous extract of leaves of *Senna alata* on blood pressure in rabbits in the presence of atropine

A-Interaction ATr-EAqSa.

1: Effect of EAqSa at 20 mg/kg bw

2 to 5: Effects of EAqSa at 20 mg/kg bw in the presence of ATr at 10^{-6} **(2)**, 10^{-5} **(3)**; 10^{-4} **(4)** and 10^{-3} mg/kg bw **(5).**

Atropine at doses between 10^{-5} and 10^{-3} mg/kg bw strongly inhibits hypotension induced by EAqSa at 20 mg/kg bw.

B-Histogram of the evolution of hypotension induced by EAqSa in the presence of atropine.

The values express percentages of maximum decrease in blood pressure compared to the control (Mean ± SEM, n = 3; ** $p < 0.01$; **** $p < 0.0001$).

Atr: Atropine, **EAqSa**: Aqueous extract of leaves of *Senna alata*; **SEM**: Standard Error of the Mean

3.1.2.3. Dose-response effects of Acetylcholine on rabbit blood pressure

The figure 4A shows the effects of increasing doses of Acetylcholine (ACh) on rabbit blood pressure. The mean value of the reference blood pressure of rabbits used is 180±8.7 mmHg in our experimental conditions.

ACh at 10^{-6} mg/kg bw causes a transient hypotension whose value is 10±0.79 mmHg corresponding to a decrease in normal blood pressure of 8.33%. At doses of 10^{-5}, 10^{-4} and 10^{-3} mg/kg bw, ACh causes very marked sustained hypotensions with values of 14±0.91 mmHg, 16±1.41 mmHg, and 26±1.23 mmHg, respectively, corresponding to decreases of 11.66%, 13.33% and 21.66% of normal blood pressure.

The mean values obtained for three experiments (n = 3) made it possible to plot the curve of decrease in blood pressure as a function of the ACh dose (Figure 4B). The 50% effective dose (ED_{50}) of ACh obtained from this graph is 5.13 mg/kg bw.

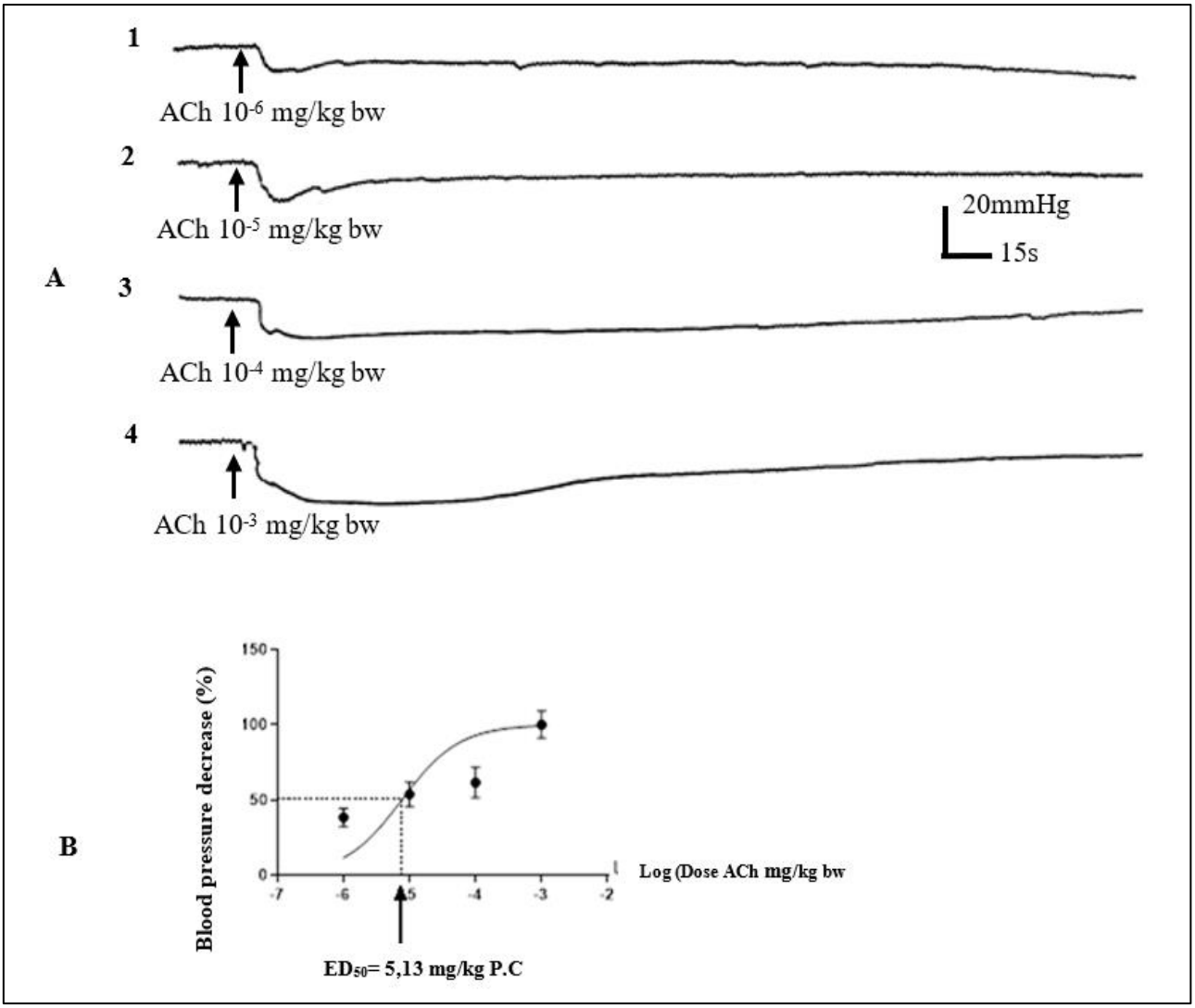

Figure 4: Effect of acetylcholine on blood pressure in rabbits

A- Dose-response effect of ACh

Effects of ACh at 10^{-6} mg/kg bw **(1)**; 10^{-5} mg/kg bw **(2)**; 10^{-4} mg/kg bw **(3)**; 10^{-3} mg/kg bw **(4)**.

ACh induces dose-dependent hypotension

B- Curve of decrease in blood pressure in rabbits as a function of the dose of ACh

This curve allows to determine a fifty percent effective dose (ED_{50}) equal to 5.13 mg/kg bw

The values express percentages of maximum decrease in blood pressure compared to the control (Mean ± SEM, n = 3).

ACh: Acetylcholine; **SEM**: Standard Error of the Mean

3.1.2.4. Effect of Acetylcholine on Rabbit Blood Pressure in the Presence of Atropine

In the presence of increasing doses of atropine ranging from 10^{-6} to 10^{-3} mg/kg bw, the hypotension induced by ACh at 10^{-3} mg/kg bw is progressively suppressed (Figure 5A). It is 26±1.23 mmHg at 10^{-3} mg.kg bw of ACh while in the presence of 10^{-3} mg/kg bw of ATr it is 0.98±1.02 mmHg. These values indicate that the hypotension induced by ACh at 10^{-3} mg/kg bw which is 21.66% is reduced and goes to 1.50% in the presence of ATr.

The mean values obtained after three experiments (n=3) made it possible to plot the histogram representing the decrease in rabbit arterial pressure induced by ACh at 10^{-3} mg/kg bw in the presence of increasing doses of atropine (Figure 5B).

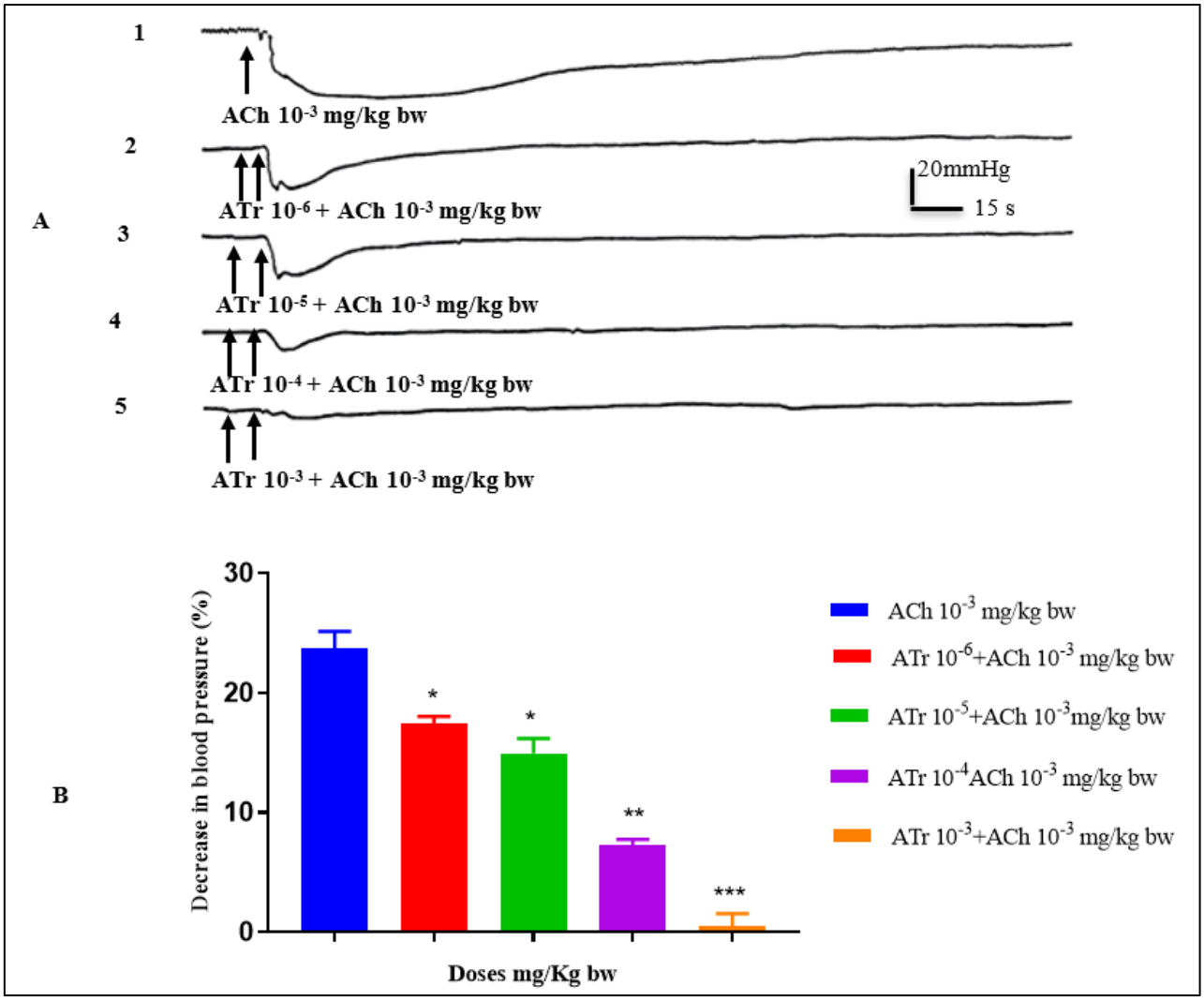

Figure 5: Effects of Acetylcholine on blood pressure in rabbits in the presence of Atropine

A-ATr Interaction.

1-Effect of ACh at 10^{-3} mg/kg bw

2 to 5: Effects of ACh at 10^{-3} mg/kg bw in the presence of 10^{-6} mg/kg bw **(2)**; 10^{-5} mg/kg bw **(3)**; 10^{-4} mg/kg bw **(4)**; 10^{-3} mg/kg bw **(5)** of Atr.

Atropine at doses between 10^{-6} and 10^{-3} mg/kg bw strongly inhibits hypotension induced by ACh at 10^{-3} mg/kg bw

B-Histogram of the evolution of hypotension induced by ACh in the presence of atropine.

The values express percentages of maximum decrease in blood pressure compared to the control (Mean ± SEM, n = 3; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

ACh: Acetylcholine; **ATr**: Atropine; **SEM**: Standard Error of the Mean

3.2. DISCUSSION

The phytochemical screening study, shows that the aqueous extract of leaves of *Senna alata* (Caesalpiniaceae) contains flavonoids, sterols and polyterpenes, catechic tannins, polyphenols, quinonic compounds, saponins and alkaloids. These results are different from those obtained by Khan *et al.*, [8] who highlighted in *Senna alata* the presence of sterols, tannins, flavonoids, anthraquinones, saponins and the absence of polyphenols. This difference can be explained by a variation in the chemical composition of *Senna alata* depending on ecological factors (climatic and edaphic), i.e. the environment in which the plant was collected.

The effects of EAqSa on rabbit blood pressure show that this extract induces dose-dependent hypotension at doses between 10 and 25 mg/kg bw. This hypotensive effect is similar to those of *Passiflora foetida* (Passifloraceae) [9], *Combretum micranthum* (Combretaceae) [10] and *Trema orientalis* [11], plants known for their hypotensive and antihypertensive effects. The hypotensive effects of EAqSa are similar to those of Acetylcholine, a reference substance recognized for its hypotensive effect. Indeed, ACh causes arteriolar vasodilation following the release of nitric oxide (NO) by the endothelium, which leads to the activation of Guanylcyclase and the inhibition of L-type slow calcium channels, which leads to the relaxation of smooth muscle

cells [12, 13]. This endothelium-dependent vasodilation occurs via muscarinic cholinergic receptors M3 [14]. These effects are inhibited by atropine, a non-selective antagonist that blocks all muscarinic receptor subtypes [15]. The study of the EAqSa-atropine interaction shows that the effects of *Senna alata* are also inhibited by ATr. This would suggest the presence of muscarinic cholinomimetic substances in the aqueous extract of leaves of *Senna alata*. These substances could be alkaloids and/or flavonoids, two groups of chemical compounds that have NO-dependent vasorelaxant, hypotensive and antihypertensive effects [16, 17]. These compounds would be responsible for the hypotensive effects induced by the aqueous extract of *Senna alata* and could act by the same mechanism as Acetylcholine. All these observations could explain the use of this plant in the treatment of hypertension.

4. CONCLUSION

The aqueous extract of leaves of *Senna alata* contains polyphenols, sterols and polyterpenes, flavonoids, catechic tannins, saponins, quinonic compounds and alkaloids. An absence of gallic tannins is noted. Flavonoids and alkaloids could be at the basis of its hypotensive property similar to that of acetylcholine induced by muscarinic cholinomimetic receptors. These observations could explain the use of the aqueous extract of leaves of *Senna alata* in traditional medicine in the treatment of arterial hypertension.

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