ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 12, pp.42727-42733, December, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

THE EFFECT OF AN AQUEOUS EXTRACT OF BARK OF *ANARCADIUM OCCIDENTALE* L. (ANACARDIACEAE) ON ARTERIAL BLOOD PRESSURE AND RESPIRATORY MOVEMENTS IN THE RABBIT

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ARTICLE INFO

Article History:
Received 25th September, 2016
Received in revised form
10th October, 2016
Accepted 22nd November, 2016
Published online 30th December, 2016

Key words:

Anacardium,
Hypotensive, Antihypertensive,
Adrenaline, Atropine.

ABSTRACT

An aqueous extract of Anacardium occidentale (AEAo) with a concentration from 1 mg / kg b.w. to 50 mg / kg b.w. induces dose-dependent hypotension on rabbit arterial blood pressure ($p < 0.01$). The respiratory movements of the animal grow in amplitude and frequencies. High doses of 40 and 50 mg / kg b.w. lead to severe hypotension followed by respiratory gasps. AEAo reduces hypertension induced by adrenaline to 5x10-3 mg / kg p.c. Injected to the animal before or after the injection of adrenaline, AEAo reduces hypertension. The greatest reduction occurs when AEAo is injected before the adrenaline injection ($p < 0.01$). Hypertension induced by adrenaline results in a decrease in the amplitude of respiratory movements ($p < 0.05$). Injection of AEAo rapidly restores these depressed respiratory movements ($p < 0.01$). Atropine significantly reduces hypotension induced by AEAo. This suggests that this hypotension is due to muscarinic cholinergic substances contained in the plant. The presence of atropine abolishes the observed gasp respiratory effect. Respiratory movements under these conditions are not significantly modified ($p < 0.05$).

Citation: Mea Arsene, Abo, K. J. C., Kassi, Y.T. and Irie Bi, J. S. 2016. "The effect of an Aqueous Extract of Bark of *Anarcadium occidentale* L. (Anacardiaceae) on Arterial Blood Pressure and Respiratory Movements in the Rabbit", *International Journal of Current Research*, 8, (12), 42727-42733.

INTRODUCTION

Côte d'Ivoire is one of the world's largest producers of *Anacardium occidentale* nuts. Village plantations are mostly located in the northern part of the country. New plantations have been established in the center and southeast for several years. The multinationals operate in the sector for its commercialization. The *Anacardium occidentale* also called cashew in Asia is a plant of which several parts are used in the traditional African and Asian pharmacopoeia. Cashew nut is considered a source of energy, having antitoxidant, anti-enteric and antidiuretic properties (F.A.O. 1998). Thus, bark, gum, nuts, roots, leaves and hull oil are used in pharmacopoeias to treat different diseases in humans and animals (Ramiakajato *et al.,* 2001; Aderiye *et al.,* 2015). Bark, leaves, gum, nuts and roots are used for their antimicrobial effects (Omojasola and Awe 2004; Agedach *et al.,* 2010; Ifesan *et al.,* 2013). An antidiabetic, anti-inflammatory, anti-ulcerogenic and anti-carcinogenic effect of the extract of bark, roots, leaves and gum is then known (Akiinpelu 2001; Kubo and Lee 1999; Arekemase *et al.,* 2011). The fruits apple and leaf extract are used against nausea and diarrhea (WHO and ISH 2003).

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The nut and apple of *Anacardium occidentale* would have aphrodisiac effects for some authors (Baszy *et al.,* 2012; Sing and Sing 2012). On the cardiovascular system, extracts of leaves, bark and roots are used to lower blood pressure. Scientific work has proved beneficial effects on hypertensive subjects (Akhlaghni and Bandy 2009: Xu *et al.,* 2011). On the respiratory system, the extract of the bark is used to fight against cold, congestion (Mahaderappa *et al.,* 2011) and cough (Chung and Payord 2008). In this work, we studied the hypotensive and antihypertensive effect of an aqueous extract of bark of Anacardium occidentale, the form used in traditional medicine. Then, we recorded concomitant changes in the respiratory activity of the normal animal during the various tests.

MATERIALS AND METHODS

Biological material

Plant material

The fresh bark is taken from trees in a plantation in Abengourou (250 km from Abidjan). It is allowed to dry for three days in the sun. After being thoroughly cleaned, it is crushed with porcelain mortar and macerated at 40 ° C. for 10

minutes. The mixture is filtered first on hydrophilic cotton and then on Whatman filters paper. The filtrate is dried at 40° c and then lyophilized. The powder obtained constitutes the aqueous extract of *Anacardium occidentale.*

Animal material

Rabbits of the species *Oryctolagus cuniculus* (Leporidae) are used for the experiments. They are raised in a farm in Bingerville (south of Abidjan) and acclimatized for one week at the Laboratory of Animal Physiology of the Department of Biosciences (University Felix Houphouet Boigny). They are kept at a constant temperature of 24 ± 2 ° C with 50-55% moisture and in a photoperiod of 12 hours of day and 12 hours of night. They are fed ad libitum in pellets (Ivograin, Abidjan, Côte d'Ivoire) and water. They are treated and healthy during the period of acclimatization and experimentation. All procedures were conducted in accordance with the guidelines for the care and use of laboratory animals published by the National Institutes of Health.

Recording blood pressure of the rabbit

The recording of the blood pressure of the rabbit is obtained thanks to the mercury manometer of Ludwig. Before the intubation of the carotid of the animal, an overpressure is carried out according to the method described by Abo *et al.,* (200). In accordance with the National Government regulation of Côte d'Ivoire, rabbits are anesthetized by intraperitoneal injection of ethylcarbamate 40% at the dose of 1 g / kg b.w. The carotid is exposed and intubated in the direction of the heart by a tube in polyvinyl bonded to the manometer. This allows direct recording of intra-carotid pressure. The saphenous vein is intubated by a catheter connected to a syringe. The syringe allows the animal to be injected with the pharmacodynamic substances and the aqueous extract of *Anacardium occidentale*. Our method of recording arterial blood pressure is similar to that described by Abo *et al.,* (2016).

Recording respiratory movements

The breathing movements are recorded thanks to the Benedict apparatus. It is composed of a vase surmounted by a spirometer. In this vessel, a tracheal catheter and a polyvinyl tube are connected, which is connected to the Marey capsule, which is itself surmounted by a writing stylet in contact with a recording cylinder rotating at constant speed. The method has been described by Leandre *et al.* (2007).

Pharmacodynamic substances

Adrenaline (L-adrenaline) originates from the Fluka laboratories (Germany) and the Atropine brand Sigma-Aldrich (France).

Statistical analysis of the results

GraphPadInstat software (San Diego CA, USA) is used for statistical analysis of results. Values are given with the mean followed by standard errors on the mean (SEM). The difference between two values is determined by the Student-Newmann-Keuls comparison test. It is considered significant for $p < 0.05$. Graphs are plotted using GraphPad 6 software (San Diego CA, USA). The sigmoid curve is obtained after conversion of the values of the abscissa X to the decimal logarithm and the ordinate Y as a percentage of the fall.

RESULTS

Dose-response effect of an aqueous extract of *Anacardium occidentale* on the rabbit blood pressure

In this study, increasing doses of AEAo are injected to the rabbit at 15 minute intervals after normal recording (Figure 1A) and after the effect of each dose. The normal blood pressure of the rabbit is measured at 90 ± 5.5 mmHg. It is determined by measuring the pressure drop after the depression of the recording device (figure 1A). AEAo concentrations between 1 and 5 mg / kg b.w. cause hypotension between 5 and 20 mmHg, a drop of 5 to 22%. The mean doses of 10 and 20 mg / kg b.w. result in hypotension of 25 and 30 mg / kg b.w., a hypotension of 27 and 33%. Strong doses of 40 and 50 mg / kg b.w. result in invariable and reversible hypotension of 35%. Figures 1B, 1C, 1D, 1E and 1F show the effects of doses of 5 mg / kg b.w., 20 mg / kg b.w., 40 mg / kg b.w. and 50 mg / kg b.w. Doses above 50 mg / kg b.w. cause irreversible hypotension of 38 mg / kg b.w. and are lethal to the animal. They are therefore non-physiological. The curve of figure 2 shows the regression percentages of the blood pressure of the rabbit compared to the normal pressure compared on five experiments. This sigmoid curve makes it possible to determine the efficacy dose at 50% or ED50 AEAo which is 11.04 mg / kg p.c. The doses of AEAo ranging from 1 mg / kg b.w. to 20 mg / kg b.w., result in a small increase in amplitude and frequency of the respiratory, none significant (ns, $p>0.05$) is maintained throughout the experiment. The dose of 40 mg / kg b.w. leads to an apnea arrest followed by an acceleration of the respiratory rate ($p <0.05$) (Figure 1E). At 50 mg / kg b.w., an apnea stop followed by an acceleration of the respiratory rate and a decrease in amplitude ($p <0.05$) (figure 1F). The effect-dose curve obtained is sigmoid. Then AEAo causes dose-dependent hypotension on the rabbit.

Effects of AEAo on hypertension induced by adrenaline

The aim of this experiment is to evaluate the effects of AEAo on a hypertensive animal. In this manipulation hypertension is induced in animals by an intravenous injection of adrenaline. Injection of adrenaline at 10^{-3} mg / kg b.w. increases the blood pressure of the rabbit by 55.25 ± 5.15 mmHg or 61.66%. In a first series of experiments the adrenaline is first injected to the animal then AEAo is injected 10 s later at the dose of 20 mg / kg p.c. (Figure 3B). The same method is used with the same concentration of adrenaline but with AEAo at a dose of 40 mg / kg b.w. (Figure 3C). Injection of adrenaline followed by doses of AEAo decreased induced hypertension by 20 ± 2.3 mmHg, or 36.36% at the dose of 20 mg / kg b.w. While the 40 mg / kg b.w. dose decreased hypertension of 30 mmHg, a drop of 54.54%. In the second series AEAo is first injected into the animal at the dose of 20 mg / kg b.w. then the adrenaline at the dose of $5x10^{-3}$ mg / kg b.w. (figure 3D) and the same procedure is carried out with AEAo to 40 mg / kg b.w. and then adrenaline to $5x10^{-3}$ mg / kg b.w. (Figure 3E). Injection of AEAo at a dose of 20 mg / kg b.w. followed by that of adrenaline decreases induced hypertension by 30 mmHg, of 54.54%. While the AEAo dose of 40 mg / kg pc reduced it by 45 mmHg to 90.81%. The histograms of figure 3 show the hypertension induced by adrenaline and the reductions that the different concentrations of AEAo entail.

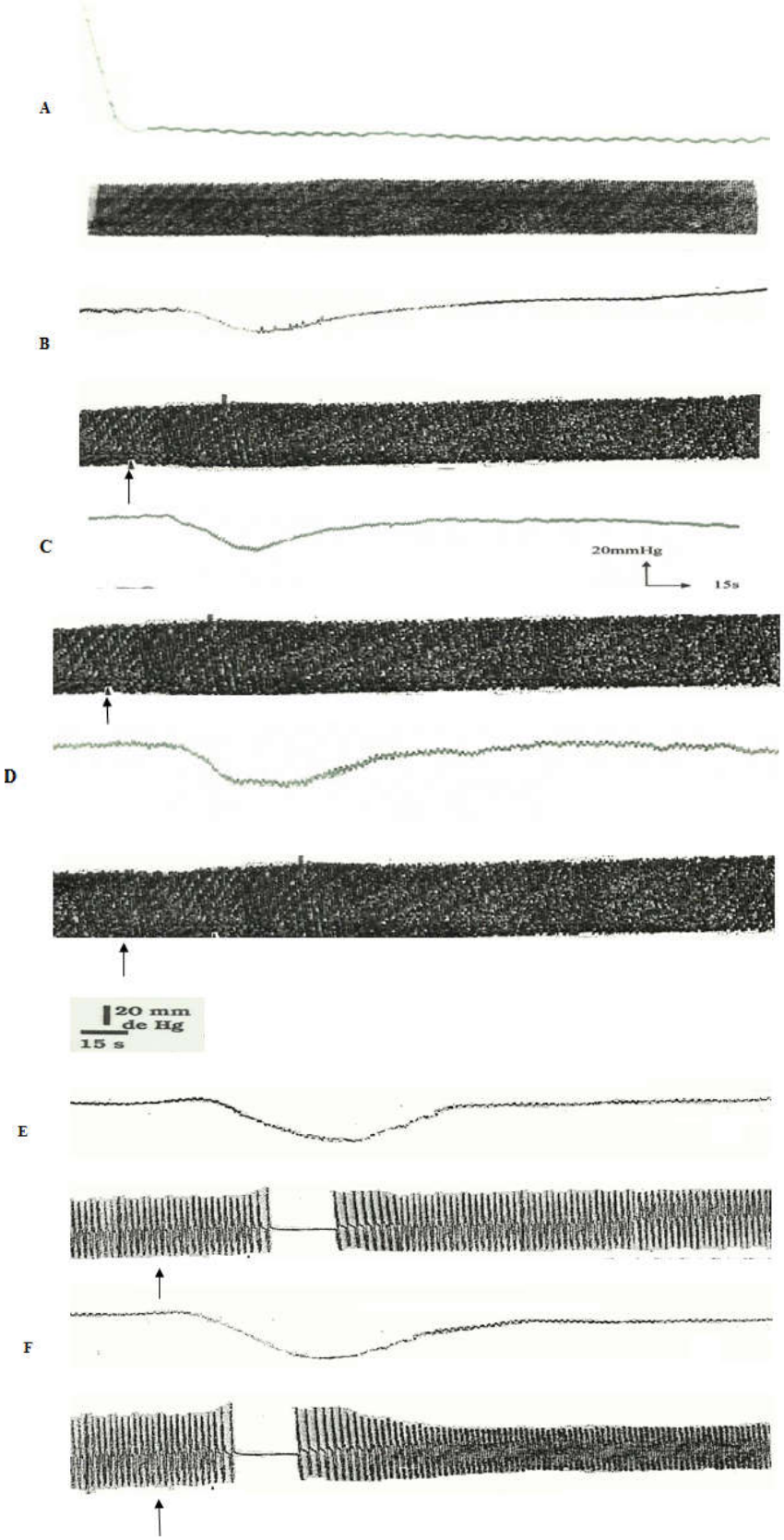

Figure 1. AEAo effect on rabbit blood pressure and respiratory

A: recording of depression followed by recording of normal pressure and respiration
B: normal recording before the arrow followed by the effect of AEAo at 5 mg / kg b.w.
C: normal recording before the arrow followed by the AEAo effect at 10 mg / kg b.w.
D: normal recording before the arrow followed by the AEAo effect at 20 mg / kg b.w.
E: normal recording before the arrow followed by the effect of AEAo at 40 mg / kg b.w.
F: normal recording before the arrow followed by the AEAo effect at 50 mg / kg b.w.

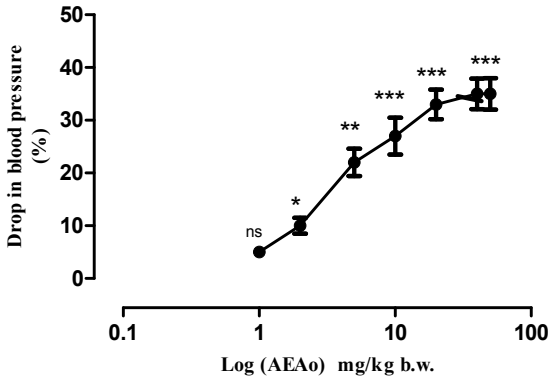

Figure 2. Curve of decrease of the pressure as a function of the logarithm of the doses administered. n = 5; ns (not significant); p>0.05 * p <0.05, ** p <0.01, *** p <0.001

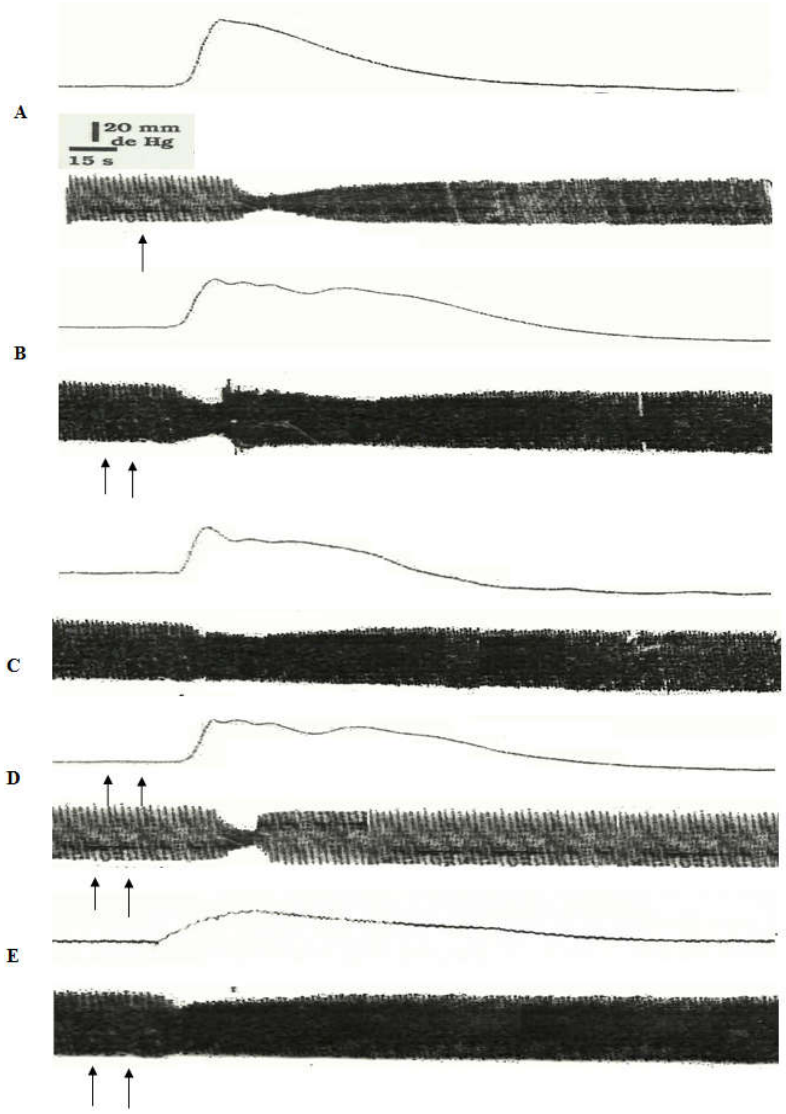

Figure 3. Effect of AEAo on hypertension induced by adrenaline

A: effect of adrenaline at the dose of 5.10^{-3} mg / kg p.c.
B: normal recording followed by the effect of adrenaline at 5.10^{-3} mg / kg b.w. followed by AEAo injection at 20 mg / kg b.w.
C: normal recording followed by the effect of adrenaline at 5.10^{-3} mg / kg b.w. followed by AEAo injection at 40 mg / kg b.w.
D: normal recording followed by the effect of AEAo injection at 20 mg / kg b.w. then adrenaline at $5x10^{-3}$ mg / kg bw.
E: normal recording followed by the effect of AEAo injection at 40 mg / kg b.w. and then adrenaline at $5x10^{-3}$ mg / kg b.w.

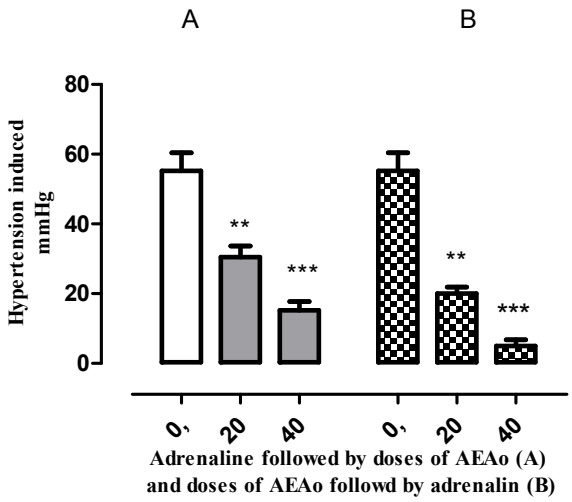

Figure 4. Effects of AEAo on the hypertension induced by adrenaline

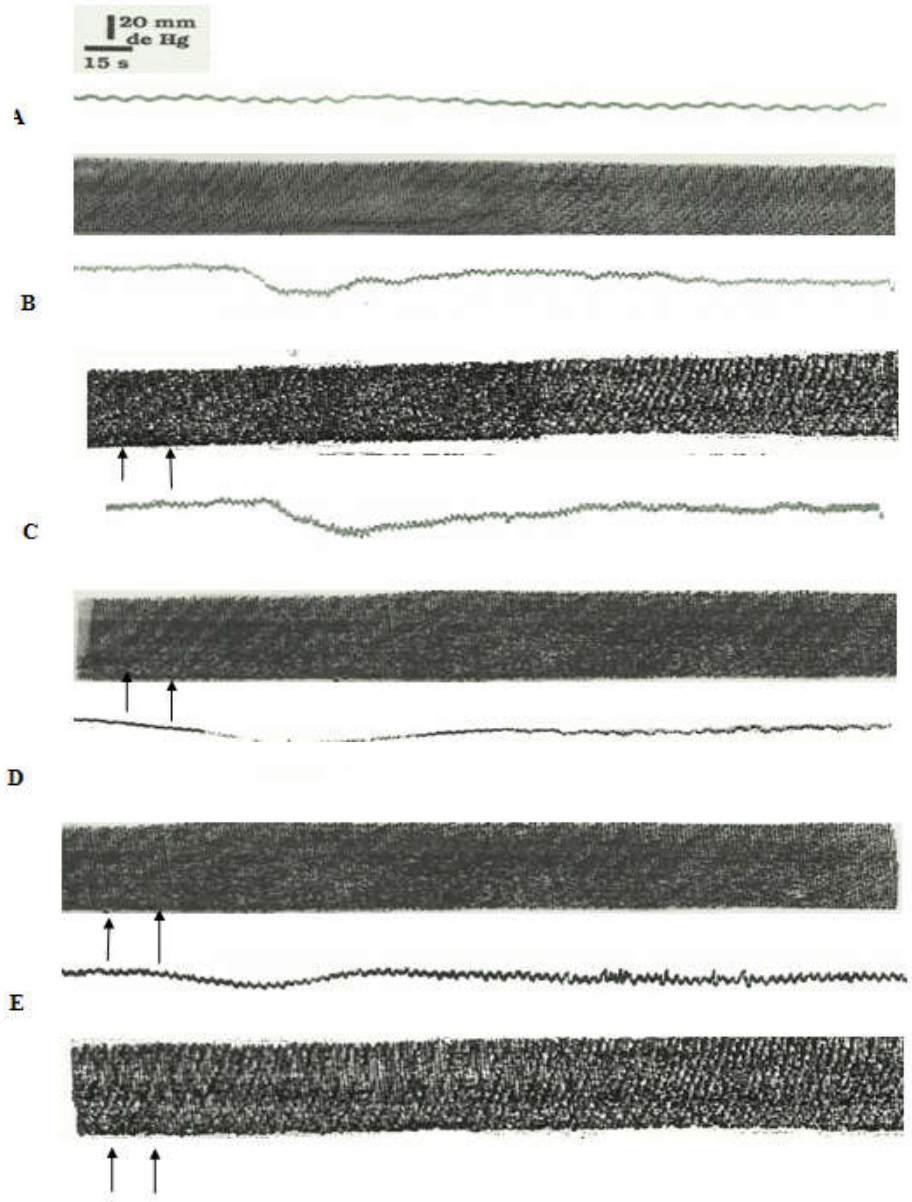

Figure 5. Effects of atropine injected after and before AEAo on the hypotension

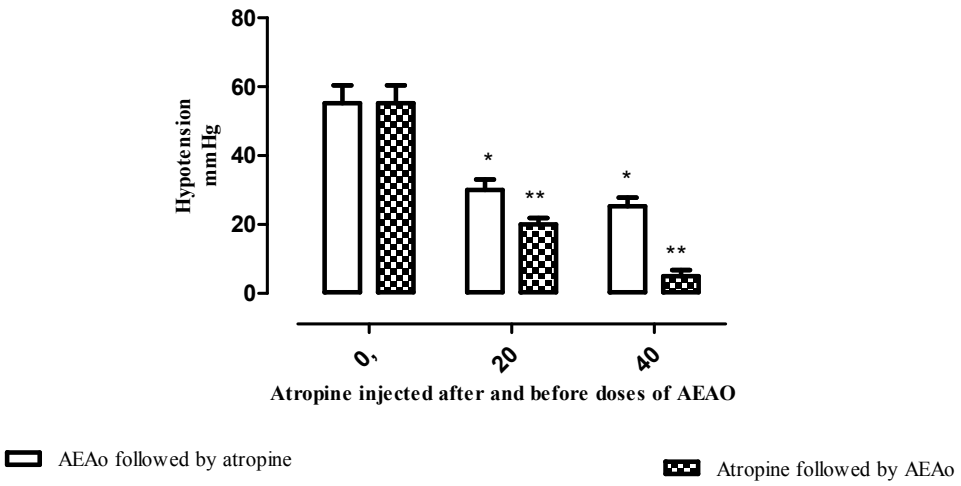

Figure 6. Effect of atropine on the hypotension induces by AEAo

Thus, figure 4A indicates these different modifications when the adrenaline is injected 10 s before the injection of AEAo. While figure 4B shows those of the injection of AEAo 10 s before the injection of adrenaline. In both cases, we observed a temporary decrease in the magnitude of ventilatory activity at the peak of hypertension followed by gasps. Surrounding 10 to 15 s after, there is a resumption of ventilatory activity characterized by an increase in frequency and progressively amplitude.

Effects of atropine on the hypotension induced by AEAo

In this study, it is necessary to check the effect of atropine on the hypotension induced by the intravenous injection of AEAo. As previously, in a first series of experiments AEAo is first administered to the animal, then atropine is injected at a single dose and effective but having no inherent effect (figure 5A).
In the first series, atropine reduced the hypotension to 30 and 20% at the doses of 20 and 40 mg/kg b.w. (figure 5B and 5C).
In the second series, atropine is first administered to the animal followed by the injection of doses of AEAo. AEAo at the dose of 20 mg / kg b.w. leads to hypotension of 70%. The blood pressure of the animal increases from 90 ± 5.5 mmHg to 60 ± 3.5 mmHg (Figure 5D). The dose of 40 mg / kg p.c. results in a maximum hypotension which is 58 ± 2.5 mmHg (figure 5E). This hypotension is reduced to 95%. Injection of atropine after AEAo reduces partially the hypotension. Atropine injection before AEAo reduces the hypotension more significantly (by 95%) as it is showed by the figure 6.

DISCUSSION

The total aqueous extract of *Anacardium occidentale* bark at doses between 1 mg / kg bw and 50 mg / kg bw induces dose-dependent hypotension. This extract greatly reduces adrenaline-induced hypertension at 5.10^{-3} mg / kg b.w. As a result, the aqueous extract of *Anacardium occidentale* exerts a hypotensive and antihypertensive activity. Indeed, the leaves of this plant contain phenolic compounds and flavonoids that have antihypertensive properties. The fresh extract also contains tannins and polyphenols that reduce arterial blood pressure (Cunfuegos-Jovellanos *et al.,* 2009). For some authors, this extract also inhibited the strength and velocity of atrial contractions in isolated guinea pig atria and relaxed contractions induced by phenylephrine and k + in the environment without Ca2 + (Khan and Gilani 2008). Several mechanisms that can contribute to this antihypertensive effect include inhibition of phosphodiesterase, reduction of intracellular ca2 +, induction of nitric oxide (NO) in smooth muscle (Torres-Piedra *et al.,* 2011). Among them, the induction of NO by the vascular endothelium resulting from the antioxidant activity of the flavonoid and phenolic compounds has an important contribution. These compounds are often used in the treatment of arterial hypertension because it retains vascular function (WHO and ISH 2003; Xu *et al.,* 2011; Torres-Piedra 2011). Atropine partially inhibits the hypotension induced by the aqueous extract of *Anacardium occidentale* bark. This presages the presence of muscarinic cholinergic compounds. The activation of these receptors (Rc M2) induces a decrease in atrio-ventricular conduction and a decrease in the contractile force. NO via the EDRF (endothelium releasing factors) releases the intact vessels (Serge *et al.,* 1997). On the respiratory activity, the stop observed could be due either to a dilatation of the diaphragm or to a compensatory reflex due to the temporary variation of the arterial pressure. Because hypotension leads to a lack of oxygenation (hypoxemia) giving rise to apnea (Alajmi *et al.,* 2007). While hypertension leads to a decrease in CO2 evacuation (hypercapnia) giving rise to hypopnea (Peppard *et al.,* 2000).

Conclusion

This work allowed showing the hypotensive and antihypertensive activity of the aqueous extract of bark of *Anacardium occidentale*. This justifies its use in traditional pharmacopoeia in the treatment of high blood pressure.

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