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Original Research

Determination of the hypotensive effect of aqueous extracts from *Anacardium occidentale* Linn. (Anacardiaceae)

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ABSTRACT:

The phytochemical analysis of the aqueous extract of *Anacardium occidentale* (Family: Anacardiaceae) (ANO) revealed the presence of sterols, polyterpenes, polyphenols, saponins, flavonoids, catechin, gallic tannins, quinones and alkaloids. The presence of all these phyto-chemicals could be responsible for the different therapeutic properties attributed to this herb. At doses between $3.7x10^{-3}$ and $6.2x10^{-2}$ g / kg bw, this aqueous extract induces sustained hypotension (dose-dependent) similar to that induced by acetylcholine (Ach) at $5.6x10^{-7}$ g / kg bw and $5.5x10^{-4}$ g / kg bw. It strongly reduced the pressure induced by adrenaline (Adr) at $2.5x10^{-5}$ g / kg bw. These results indicated that the aqueous extract of *Anacardium occidentale* is hypotensive. The traditional use of this plant to treat high blood pressure was justified. The study of interaction between ANO and atropine (a competitive antagonist of muscarinic cholinergic receptor) showed that this reference molecule does not inhibit the hypotensive effects of this extract. Furthermore, inhibition and hypotensive properties of the extract of *Anacardium occidentale* by chlorpromazine an antipsychotic drug, suggested a central ANO action similar to that of clonidine and alpha-métyldopa. Thus, the aqueous extract of *Anacardium occidentale* contained non- cholinomimetics and hypotensive substances that may have central effects.

Keywords:
Anacardium occidentale, cholinomimetic substances, chlorpromazine, hypotensive effect

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INTRODUCTION

Anacardium occidentale L. (Anacardiaceae), commonly known as "Cajou a pomme" in French or "Cashew" in English is a tree native to the eastern coast of tropical America, introduced into the tropics and cultivated especially for its seed and fruit which posses many uses.

The use of *Anacardium occidentale* for the treatment of various ailments had been already reported by various authors. In Ivory Coast, the decoction of the stem bark of this plant was recommended for usage against hypertension. In Senegal, the abortifacient properties of the leaf extracts of this plant were often reported (Kerharo and Adams, 1974). In Togo, decoction of stems bark was used for treating female infertility. In southern Cameroon, leaf extract was used against diabetes (Sokeng *et al.,* 2001). This fruit had a number of external medical use in the treatment of ulcers, warts, dental disease etc. In Nigeria, the bark of the trunk was used in the treatment of various forms of insomnia (Adjanohoun *et al*., 1989). In the Republic of Congo, the aqueous decoction of the fresh bark was used orally to treat urogenital infections and to calm gastralgia. Walnut oil extracted from this plant is said to be effective against worms, eczema, dermatitis and ulcers in the different populations of West Indies (Boullard, 2001). In South America, the infusion of the bark and leaves was used to treat toothache, it was also used in cases of dysentery, diarrhea, hemorrhoids (Goncalves *et al.,* 2005; Taylor, 2005).

Pharmacological studies have shown that this plant also had fungicidal and vermicidal properties besides anti-protozoal and antimicrobial activity (Goncalves *et al.,* 2005). It was also known for its parasiticide and larvicides property (Kamtchouing *et al*., 1998). The methanol extract of the stem bark had a higher anti-inflammatory activity than diclofenac (Patil *et al.,* 2003) and comparable to that of pentoxisylline and L-NAME in mice (Olajide *et al*. , 2004). The methanol extracts of the leaves of the species of African origin also cause hypoglycemia in rats and made them diabetic by increasing streptozotocin levels (Sokeng *et al.,* 2007) and had an effect on the reproductive function in male rats rendered with diabetic problems (Tedong *et al.,* 2007). Furthermore the anti-hypertensive properties of the bark extract of this plant had also been exposed by Tchicaya *et al*. (2003a). Such a plant with potential therapeutic usage has been taken for the analysis of hypotensive effect as less studies on this regard were elsewhere found on the literature hitherto.

The aim of this study is to evaluate the effect of the aqueous extract of *Anacardium occidentale* (ANO) on the blood pressure of rabbit and to determine the properties of the active principles contained in this extract from using phytochemical and pharmacological studies.

MATERIAL AND METHODS

Plant Material

The plant material used is the bark of *Anacardium occidentale* (Anacardiaceae), purchased from the herbalists of Adjamé market (Abidjan). The plant material was identified and authenticated by Professor Laurent AKE-ASSI, the Plant Biology Laboratory, from the herbarium of the National Centre for Floristic University Félix Houphouët Boigny, where this species is listed and documented in the specimen number 14649 at 18 January, 1979. These sheets of the plant material were dried and preserved at room temperature (28 ± 4°C).

Extraction method of freeze-dried aqueous extract

Fifty grams of the dried bark powder *Anacardium occidentale* was mixed in the two liters of distilled water with magnetic stirring for 24 hours. The solution obtained was filtered through a cotton wool and Whatman paper (3 mm). The filtrate obtained was evaporated under vacuum at 70°C using a Rotavapor evaporator type "Bucchi". The resulting paste was frozen, then lyophilized. The lyophilizate obtained is a water-soluble powder. Its yield was found 19% relative to the

dry plant powder.

Extraction method decoction

Seventy grams of the dried bark powder of this plant were mixed with 800 ml of water. The whole mixture was boiled for 30-45 minutes. The decoction obtained was approximately 500 ml. The decoction was then filtered on Wattman (3 mm) paper and hydrophilic cotton subsequently. The filtrate obtained was evaporated under vacuum at 70°C using a Rotavapor evaporator type "Bucchi". The resulted paste was frozen and lyophilized.

Animal material

The animals used for the experiments were rabbits. They were used to study the action of this extract on blood pressure variation. The rabbits used belong to the species European rabbit (Leporideae) and weighed an average of 2 ± 0.2 kg. They came from different farms around Abidjan. Also they were acclimated for seven days at the pet guage of UFR Biosciences, University Félix Houphouët Boigny, to regulate and harmonize their physiological state before the experiments. The animals were treated according to the principles of scientific ethics committee of Biology, on the use of laboratory animals in experimental tests.

Chemicals and saline

Chemical products:

During this work, chemicals were used viz., Acetylcholine (Ach) (Prolabo, France), Adrenaline (Adr) (Prolabo, France), Atropine (Prolabo, France) and Chlorpromazine (LARGACTIL®, Sanofi Aventis, France). Antagonists substances such as atropine and chlorpromazine were used but at concentrations where these chemicals do not induce specific effect.

Saline

During this study, mammals were fed with normal saline - Mac Ewen at pH 7.4. It contains NaCl 122 mM; KCl 4.9 mM; $CaCl_2$ 2.52 mM; $NaPO_4H_2$ 1.18 mM; $NaHCO_3$ 15.5 mM; $MgCl_2$ 1.2 mM and glucose 5.5 mM.

Characterization of the major chemical compounds

The characterization of the phytochemical constituents of the extract of *Anacardium occidentale* was performed using the methodology followed by Zahoui *et al*. (2012).

The identification of sterols and terpenes were made by the reaction of Liebermann. The characterization of the compounds belonging to the polyphenols group was confirmed by their reaction with ferric chloride. Saponoside identification was done based on the property that aqueous solutions containing saponins used to foam after agitation. The compounds belonging to the group of flavonoids had been identified by their reaction with cyanidin. The compounds belonging to the group of tannins had been identified through the reaction of Stiasny. The free or combined quinone compounds were confirmed through Borntraeger reaction. Alkaloids had been demonstrated using two reagents viz., namely Bouchardat reagent (reagent - iodo-iodide) and the Dragendorff reagent (reagent - potassium iodobismuthate).

Experimental and technical apparatus for recording blood pressure

The experimental device used for recording blood pressure is the Ludwig gauge. The rabbit was anesthetized by éthyluréthane injection intra-peritoneally; dosed at 40% in the ratio of 1 g/kg bw. Its carotid artery was exposed and intubated with a catheter connected to the manometer containing mercury. Changes in the pressure of the rabbit carotid artery were transmitted to the column of mercury. They were transcribed using a stylus listing, on a cylinder-coated paper containing lampblack. It was rotated at a constant speed.

The test substances were dissolved in a solution of Mac Ewen and then injected into the rabbit (saphenous vein) before dissection.

Expression of results

Effective Dose 50% (ED_{50}) was expressed in

grams of lyophilized aqueous extract per kilogram of body weight of the animal (g/kg bw).

Treatment of experimental results

Recordings made on paper were smoky and tend to vanish very soon. To fix the soot it was scanned before being reversed; small corrections were done using Photoeditor software and Microsoft Paint. The statistical analysis of values and graphical representation of data were carried out respectively through GraphPad INSTAT software (Microsoft, San Diego, California, United States) and GraphPad Prism 4 (Microsoft, San Diego, California, United States).

The statistical analysis such as analysis of variance (ANOVA), and multiple comparison Tukey-Kramer test were performed on the obtained data with a level of significance at $P < 0.05$. All values were expressed with the standard error of the mean and presented as means ± SEM.

Place and period of study

These experiments were conducted at the Laboratory of Animal Physiology of the University Boigny Felix Houphouet (Ivory Coast), between March 2008 and October 2008. Additional experiments were performed in April 2010.

Table 1. Comparison of the chemical composition of decoction and lyophilized aqueous extract of Anacardium occidentale (Anacardiaceae)

Chemical groups		Decoction	Freeze-dried aqueous extract
Sterols and polyterpene		±	+
Polyphenols		+	+
Saponosides		-	+
Flavonoids		+	+
Tannins	Gallic	-	+
	Catechic	+	+
Quinones		+	+
Alkaloids		+	+

(+): The presence of the chemical compound
(-): Absence of the chemical compound
(±) limited presence of chemical compound

The decoction and the lyophilized aqueous extract of *Anacardium occidentale* have substantially the same chemical composition

RESULTS

Enumeration of the phytochemical constituents of the bark of *Anacardium occidentale*

The phytochemical study of the freeze-dried aqueous extract of the bark of *Anacardium occidentale* indicated that the extract used in our experiments contains polypterpenes, sterols, polyphenols, flavonoids, quinones, saponins, gallic of tannins, catechin, quinones and alkaloids. Its chemical composition is superficially similar to that of the decoction of the dried bark from the same plant, often used in traditional African medicine (Table 1).

The effects of the aqueous extracts of *Anacardium occidentale* on the blood pressure of rabbits

Dosage of the extracts on the blood pressure of rabbits

Figure 1 shows the effects of aqueous extract of *Anacardium occidentale* (ANO) on blood pressure in the rabbits according to the dose. The rabbit had a normal blood pressure of a value of 128 mm Hg in our experimental conditions. Doses of between $2.5x10^{-4}$ g/kg bw and $6.2x10^{-2}$ g/kg bw of Annacardium extracts caused hypotension and increased blood pressure from 30±1.68 mm Hg to 110±4.77 mm Hg, which corresponds to a decrease in blood pressure of the normal rabbit from 23.43% to 85.93% ($p < 0.001$).

This experiment was performed several times ($n = 3$) and the average values obtained have enabled us to obtain the curve in Figure 1B, reflecting the decrease in blood pressure of the rabbits according to the dose of ANO**.** This allowed a sigmoid graph formulation that determines the effective dose 50% (ED_{50}) Which is equal to $1.91x10^{-2}$ g/kg bw.

Effects of acetylcholine (Ach) on the blood pressure in rabbits (dose dependent).

Figure 2 shows the effects of the administration of increasing doses of Ach on the blood pressure of rabbits. Normal blood pressure of rabbits under our experimental conditions was 120 mm Hg. Ach doses from

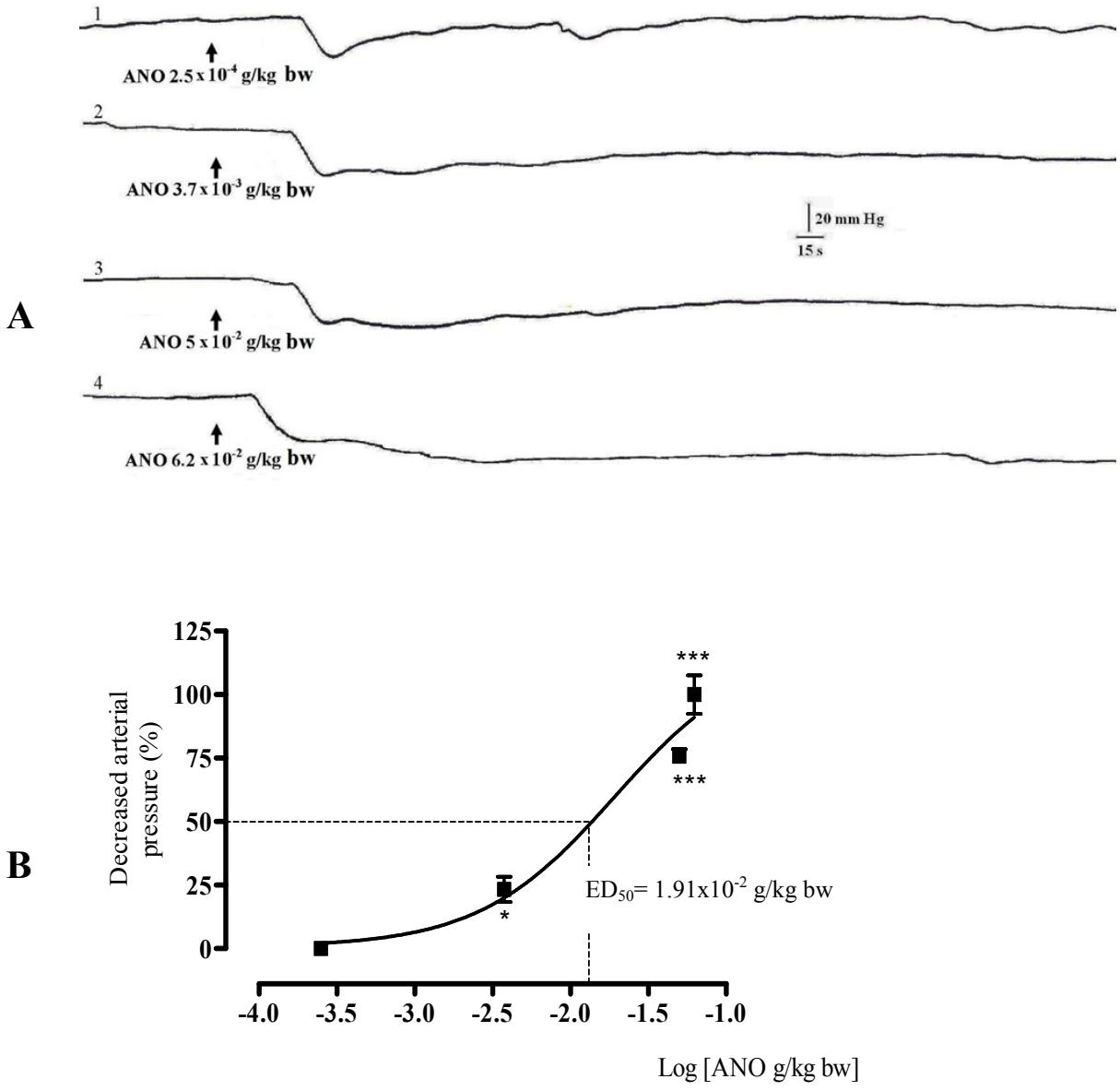

Figure 1. Effect of the aqueous extract of *Anacardium occidentale* on blood pressure of rabbits as a function of the dose

A - Effect of dose response ANO- Effect of ANO in $2.5x10^{-4}$ g / kg bw(1); $3.7x10^{-3}$ g / kg of bw (2); $5x10^{-2}$ g/kg bw (3); $6.2x10^{-2}$ g/kg bw (4). **B - Decreased blood pressure according to the dose of ANO-** The values express the maximum percentage decrease in pressure relative to normal blood pressure (Mean ± SEM; n = 3; * $p < 0.05$, *** $p < 0.001$). ANO causes a dose-dépendent hypotension; $ED_{50} = 1.91x10^{-2}$ g/kg bw.

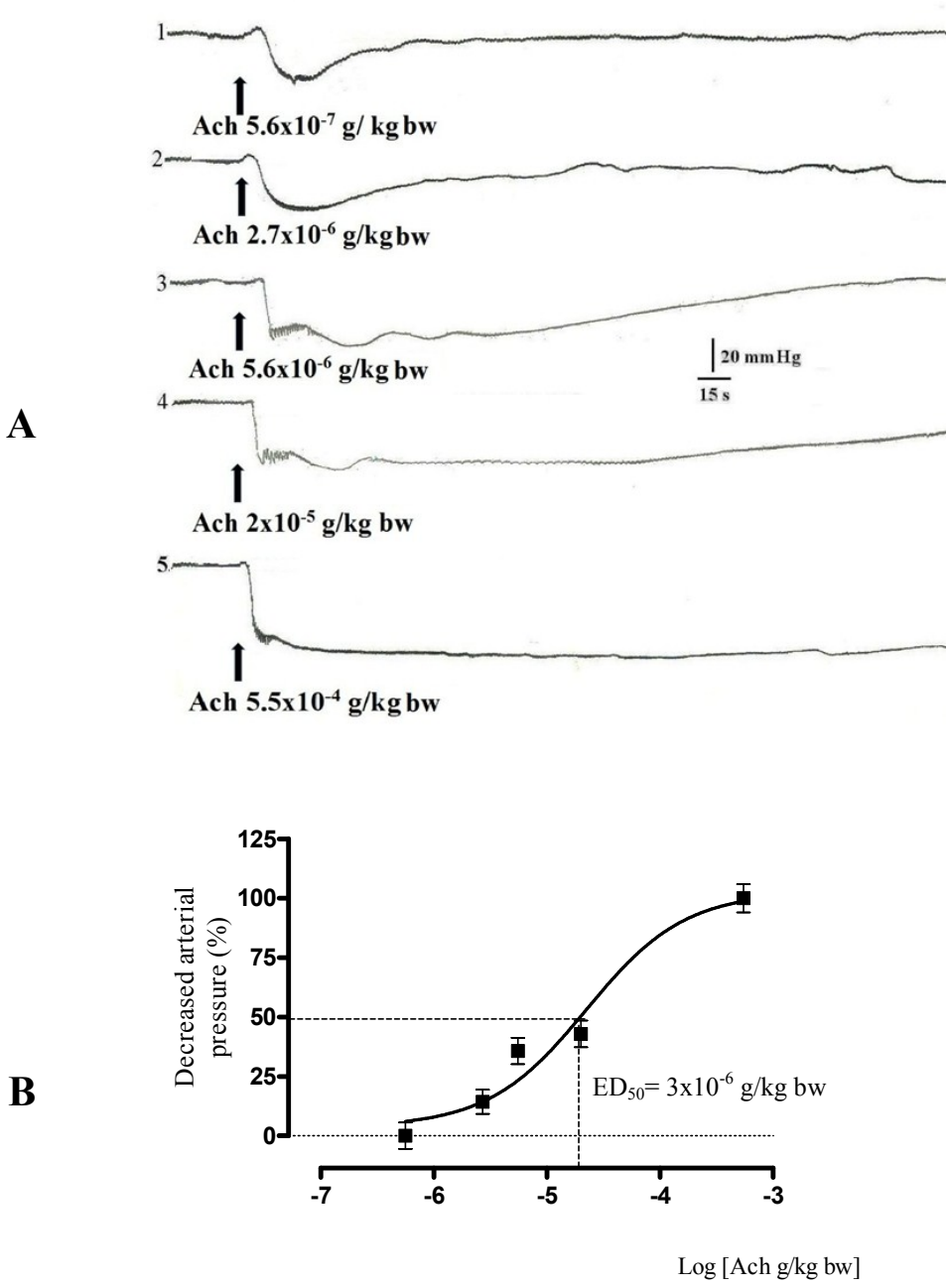

Figure 2. Effects of Ach on blood pressure of rabbits as a function of the dose

A - Effect of Ach depending on the dose- Effect of Ach $5.6x10^{-7}$ g/kg bw (1); $2.7x10^{-6}$ g/kg bw (2); $5.5x10^{-6}$ g/kg bw (3); $2x10^{-5}$ g/kg bw (4); $5.5x10^{-4}$ g/kg bw (5). The Ach lowers blood pressure rabbit between $5.6x10^{-5}$ and $5.5x10^{-4}$ g/ kg bw. **B - Decreased blood pressure rabbit according to the dose of Ach-** The values express the maximum percentage decrease in pressure relative to normal blood pressure (mean ± SEM; n = 4; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). The Ach causes a dose-dependent hypotension, ED_{50} $3x10^{-6}$ g/kg bw.

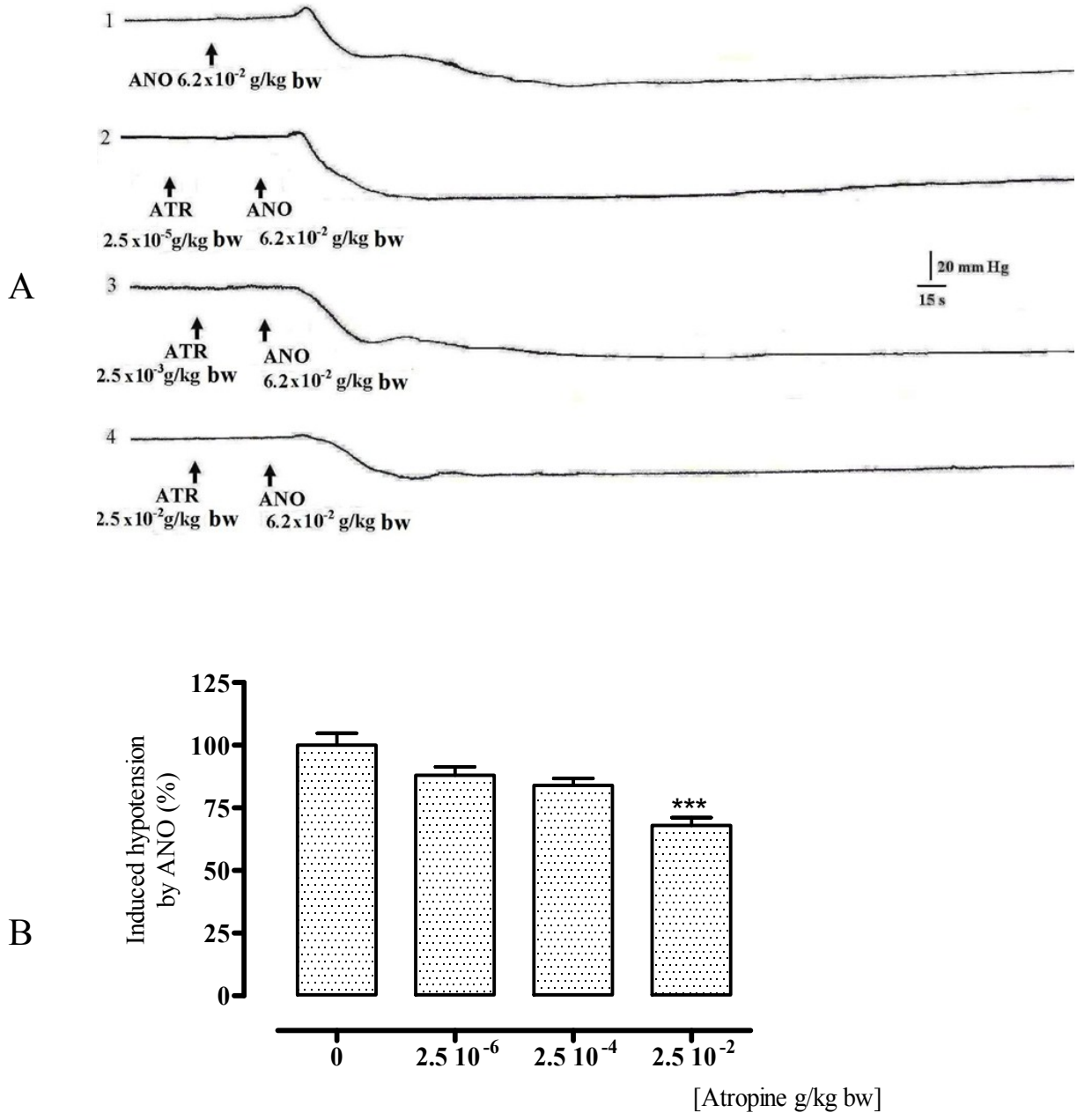

Figure 3. Effects of the aqueous extract of *Anacardium occidentale* on blood pressure of rabbit in the presence of atropine

A - Interaction ATR-ANO- 1- Effect of ANO to $6.2x10^{-2}$ g/kg bw. 2 to 4 - Effect of atropine $2.5x10^{-5}$ g/kg bw (2); $2.5x10^{-3}$ g/kg bw (3); $2.5x10^{-2}$ g/kg bw (4) (first arrow) followed by the injection of ANO to $6.2x10^{-2}$ g/kg bw (second arrow). **B - Histogram of changes induced hypotension in the presence of atropine ANO-** The values express the maximum reduction percentages hypotension compared with the control (Mean ± SEM; n = 3; *** $p < 0.001$). Atropine at doses of between $2.5x10^{-6}$ and $2.5x10^{-2}$ g/kg bw weakly inhibits induced hypotension ANO to $6.2x10^{-2}$ g/kg bw.

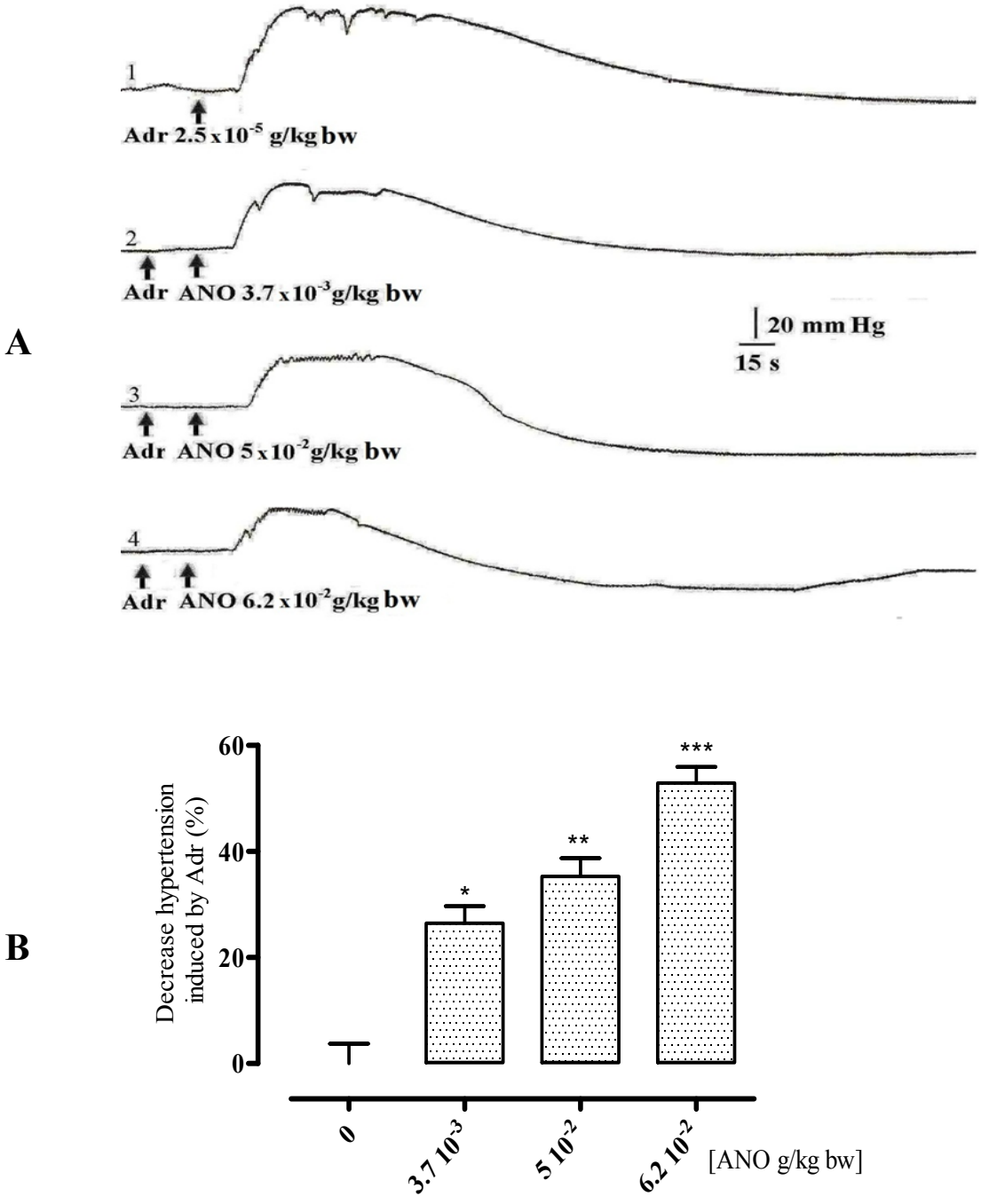

Figure 4. Effects of the aqueous extract of *Anacardium occidentale* on hypertension induced by adrenaline in rabbits.

A - Interaction Adr-ANO- 1- Effect of Adr to $2.5x10^{-5}$ g / kg bw. 2 to 4 - Effect of ANO to $3.7x10^{-3}$ g/kg bw (2); $5x10^{-2}$ g/kg bw (3); $6.2x10^{-2}$ g/kg bw (4) (second arrow) preceded by the effect of the Adr to $2.5x10^{-5}$ g/kg bw (first arrow). **B - Reduction of hypertension induced by adrenaline in function of the dose of *Anacardium occidentale*.** The values express the pressure reduction percentages relative to control (Mean ± SEM; n = 3; * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$). ANO between $2.5x10^{-4}$ and $6.2x10^{-2}$ g/kg bw strongly inhibits hypertension induced by adrenaline.

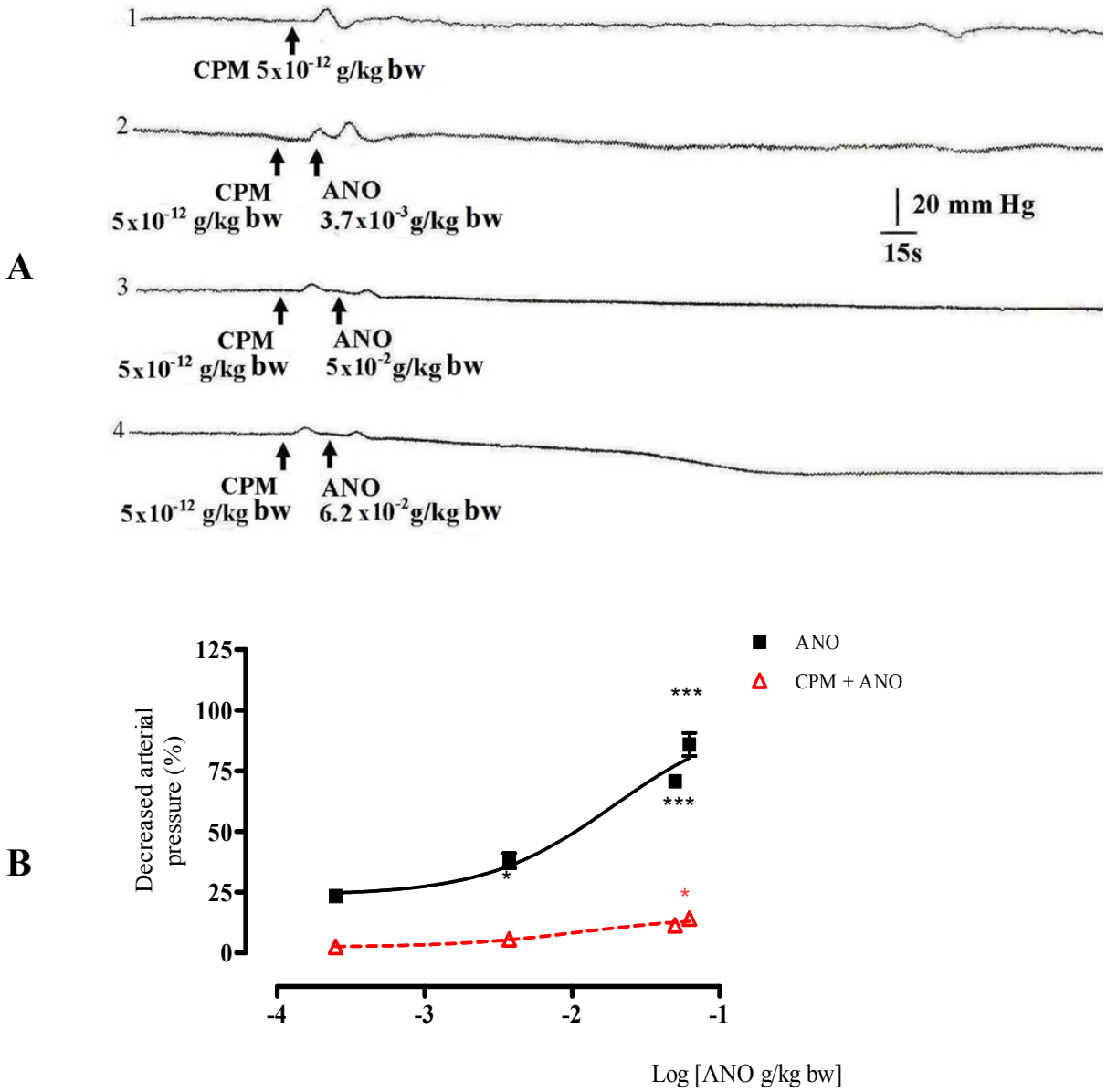

Figure 5. Effects of the aqueous extract of *Anacardium occidentale* on the blood pressure of rabbit according to the dose in the presence of chlorpromazine (CPM)

A - Interaction CPM-ANO- 1- CPM Effect at $5x10^{-12}$ g/kg bw. 2 to 4 - Effect of ANO at $3.7x10^{-3}$ g/kg bw (2); $5x10^{-2}$ g/kg of bw (3); $6.2x10^{-2}$ g/kg bw (4) (second arrow) pre-ceded by the CPM effect $5x10^{-12}$ g/kg bw (first arrow). **B - Decreased blood pressure in the presence of rabbit chlorpromazine (CPM)-**The values express the maximum percentage decrease in pressure relative to control (mean ± SEM; n = 3; * $p < 0.05$, *** $p < 0.001$). CPM almost completely inhibits induced hypotension between $3.7x10^{-3}$ and $6.2x10^{-2}$ g/kg bw.

$5.6x10^{-5}$ g/kg bw to $5.5x10^{-2}$ g/kg bw, caused a sustained dose-dependent hypotension, which varied from 45 ± 3.27 to 115 ± 3.45 mm Hg. This corresponds to a decrease in the normal blood pressure between 37 and 95% ($p < 0.001$). This experiment was carried out several times (n = 3) and the average values obtained, have enabled us to trace the curve of Figure 2B which reflects the reduction in blood pressure of rabbits as a function of the dose of Ach. This sigmoid graph allowed the determination of an ED_{50} which is of $3x10^{-6}$ g/kg bw. The ED_{50} of Ach, which is a reference molecule, that showed approximately 3000 times lower than the ED_{50} of ANO (the lyophilized aqueous extract of *Anacardium occidentale*).

Effects of ANO on blood pressure of rabbits in the presence of atropine

Figure 3 shows the effect of ANO at $6.2x10^{-2}$ g/ kg bw in the presence of atropine on the rabbit's blood pressure. ANO at $6.2x10^{-2}$ g/kg bw caused an sustained hypotension of 110 ± 4.77 mm Hg, which corresponds to 100% of the decrease in the blood pressure. In the presence of atropine doses between $2.5x10^{-6}$ and $2.5x10^{-2}$ g/kg bw and ANO at $6.2x10^{-2}$ g/kg bw, sustained hypotension was induced with a value between 96.8 ± 3 38 mm Hg and 74.8 ± 3.15 mm Hg, which corresponds to the reduction of percentages from 88% to 68% ($p < 0.001$). This experiment was performed several times (n = 4) and the obtained average values were used to draw the histogram (Figure 3), reflecting the decrease in the induced hypotension by ANO, depending on the dose of atropine.

Effects of ANO on hypertension induced by Adrenaline (Adr) in rabbits

Epinephrine was injected at $2.5x10^{-5}$ g/kg bw dosage level to the rabbits and induced a pressure of 68 mm Hg, which represents 100% increase in the initial level of blood pressure (Figure 4). In the presence of ANO to increasing doses of between $3.7x10^{-3}$ g/kg bw and $6.2x10^{-2}$ g/kg bw and adrenaline at the concentration of $2.5x10^{-5}$ g/kg bw (induced hypertension duration and amplitude less significant), ranging from 50 ± 3.25 mm Hg to 32 ± 3.01 mm Hg. This corresponded to 26.47% ($p < 0.01$) and 52.94 % ($p < 0.001$) decrease in the level of hypertension. These hypertensions were followed by hypotension at doses of greater than or equal to $5x10^{-2}$ ANO g/kg bw. The histogram of Figure 4 reflects the average variations (n = 4) in the level of adrenaline-induced hypertension in rabbits according to the dose of ANO.

Interaction of ANO on the blood pressure of rabbit in the presence of Chlorpromazine (CPM)

Injection of Chlorpromazine (CPM) at $5x10^{-12}$ g/ kg of bw had no effect on the blood pressure of rabbit as given in Figure 5. In the presence of CPM at $5x10^{-12}$ g/kg bw, the hypotensive effects of the ANO doses between $3.7x10^{-3}$ g/kg bw and $5x10^{-2}$ g/kg bw, were completely inhibited. Decrease in blood pressure of rabbits were recorded; the values were between 5.67% and 11.35% which corresponds to the normal blood pressure. These variations were not found to be significant ($p > 0.05$). At the doses of ANO equal to $6.2x10^{-2}$ g/kg bw, there seen a significant decrease in the blood pressure. However this decrease was found to be low. Indeed, at this dosage blood pressure of the rabbit moved down to 18.18 ± 2.40 mm Hg representing a decrease of 14.20%, ($p < 0.05$) of the normal blood pressure.

This experiment was carried out several times (n = 4), the obtained average values were used to draw the curves of Figure 5, reflecting the decrease in blood pressure in the presence of CMP. These curves indicate that doses higher than 5.10^{-2} g/kg bw of ANO, induce a small reduction in the blood pressure of the rabbit.

DISCUSSION

The phytochemical study of the freeze-dried aqueous extract of the *Anacardium occidentale,* proved that it contains sterols and polyterpenes, polyphenols, saponins, flavonoids, gallic and catechin tannins, quinones and alkaloids. These results were supported by the work

of several authors. Indeed Satyanarayana *et al*. (2001) highlighted the presence of flavonoids and pro-anthocyanidins (tannins) in this plant. Saponins had also been highlighted in the ethanolic extract of the leaves of *Anacardium occidentale* (Konan *et al.,* 2006), the hexane extract of the leaves of this plant had evidence for containing alkaloids and polyphenols (Tedong *et al*., 2007). The richness of aqueous extract of *Anacardium occidentale,* in all these various compounds may explain its various therapeutic properties. Indeed, it has been proven that flavonoids, tannins, saponins, polyphenols and alkaloids would present various therapeutic properties (Fernandes *et al.,* 2003; Tedong *et al.,* 2007).

The aqueous extract of *Anacardium occidentale* induced a dose-dependent hypotension at doses between $3.7x10^{-3}$ and $6.2x10^{-2}$ g/kg bw. These results confirmed those of Tchicaya *et al*. (2003a) who worked under the same experimental conditions and on the same plant. ANO between $3.7x10^{-3}$ and $6.2x10^{-2}$ g/kg bw reduced hypertension induced by Adrenaline dosed at $2x5.10^{-5}$ g/kg bw demonstrating its anti hypertensive potential.

The traditional use of this plant against high blood pressure is therefore justified. The antihypertensive properties in *Anacardium occidentale* were similar to those of African medicinal plants such as *Parkia biglosa* (Mimosaceae) (Assane *et al*., 1993), *Allium sativum* (Alliaceae) (Elorriga *et al*., 1996) and *Lantana camara* (Verbenaceae) (Belemtougri *et al.,* 2001). These pharmacological effects were comparable to those of acetylcholine, a hypotensive substance (Furchgott and Vanhoute, 1989), which decreases the rate and force of heart contractions by binding to muscarinic cholinergic receptors types (Furchgott, 1981; Stengel *et al.,* 2002). Therefore the competence of ANO was also tested against the competitive antagonist atropine of muscarinic cholinergic receptor types (Gerova *et al*., 2005).

The study of the ANO-ATR interaction on blood pressure of rabbits showed that the reference molecule had no significant effect on the hypotensive properties of ANO. These findings corroborate those of Tchicaya *et al*. (2003b). The hypotensive effects of the aqueous extract of *Anacardium occidentale* between $3.7x10^{-3}$ and $6.2x10^{-2}$ g/kg bw were totally, inhibited by chlorpromazine, an antipsychotic (Colasanti 1994; Bordet, 2004). ANO could also have a central action such as clonidine and alpha-métyldopa (De Cort *et al.,* 2004).

Due to its effects on the central nervous system, this crude extract may inhibit the sympathetic system and cause the whole animal to have cardioinhibition and greater vasodilation caused by the direct effects of ANO on the smooth muscle of the vessels. The aqueous extract of *Anacardium occidentale* thus contain non cholinomimetic hypotensive substances that may have central effects.

CONCLUSION

The phytochemical study of the lyophilized aqueous extract of *Anacardium occidentale* showed that it contained sterols, polyterpenes, polyphenols, quinones, catechols, gallic tannins, alkaloids and flavonoids. This wealth of chemical compounds explain the therapeutic value of this plant. The results of pharmacological studies on the ANO aqueous extract showed that this extract has hypotensive properties comparable to many medicinal plants. The hypotensive effects of this aqueous extract was completely inhibited by chlorpromazine, an antipsychotic, which could have a central action. The aqueous extract of *Anacardium occidentale* contain non cholinomimetics hypotensive substances that may have central effects. According to this study, it is necessary to carry out the fractionation of the lyophilized aqueous extract to determine the most active moiety, for a better characterization of the active ingredients of this plant.

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