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Hypotensive and cardio-inhibitor effects of *Cajanus cajan* harms (fabaceae) aqueous leaf extract in anesthetized normotensive rabbits

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Abstract
Cajanus cajan (Fabaceae) is a plant traditionally used in Cote d'Ivoire to treat many illnesses such as sickle-cell anemia, measles and diabetes. To our knowledge, no research works were undertaken on its probable effect against cardiovascular diseases. Therefore, the present study aims to assess the hypotensive and cardio-inhibitor effects of *C. cajan* leaves extract (AqECc) through the arterial blood pressure (BP) and the electrocardiogram (ECG) recording in anesthetized rabbits. The carotid artery was intubated using a catheter connected to Ludwig's mercury manometer for BP measurement. The recording of the ECG was performed using an electrocardiograph. Varied *C. cajan* macerate doses (0.5 to 50 mg/kg bw) were administered to anesthetized rabbits intravenously. Phytochemical analysis was carried out in order to determine the phytoconstituents contained in the extract.
Results showed that AqECc induced a dose dependent hypotension in normotensive rabbits with a fifty percent effective dose (ED_{50}) of 9.33 mg/kg b.w. The hypotension induced by AqECc was significantly reduced by atropine (a muscarinic cholinoreceptor antagonist). On rabbits' electrocardiogram, AqECc decreased the amplitudes of P-wave, QRS complex, T-wave and the heart rate. As for the duration of PQ interval, it was significantly ($p< 0.05$) increased. However, the extract had no significant effect on the QT interval. Phytochemical screening results of the extract revealed the presence of sterols and polyterpenes, phenols, flavonoids, catechic tannins, alkaloids and saponins which are known for their hypotensive effects could explain the use of this plant by traditional healers to treat hypertension.

Keywords: *Cajanus cajan*, hypotension, cardio-inhibition, rabbit, phytoconstituents

1. Introduction
Blood pressure (BP) is created by the force exerted by the blood on the walls of blood vessels as it is pumped by the heart [1]. Classified as a non-communicable disease, high blood pressure is a devastating medical condition in which the blood vessels have persistently raised pressure [2]. Arterial hypertension is the most widespread cardiovascular disease in black Africa population [3]. It is characterized by increase in systolic and/or diastolic pressures higher than 160 mmHg and 95 mmHg respectively, it constitutes a public health problem with very serious consequences including about 5% of cases of cerebrovascular disease worldwide [4].
Approximately one-quarter of the global adult population is hypertensive and this proportion is expected to reach 29.2% (about 1.6 billion people) by 2025. Out of total 58.8 million deaths worldwide in year 2004, high blood pressure was responsible for 12.8% (7.5 million deaths) [5]. In sub-Saharan Africa, this condition currently affects approximately 27-28% of the adult population of age 20 and above. The World Health Organization (WHO) in 2005 estimated the overall prevalence of arterial hypertension in Côte d'Ivoire to be 21.7% and this was significantly more important in rural areas with 29.6% against 21% in urban areas [6].
The prevalence of hypertension has increased, especially in low-income countries. Despite the increasing prevalence, the proportions of hypertension awareness, treatment and BP control are low, particularly in low-income countries, and few comprehensive assessments of the economic impact of hypertension exist [7]. Various anti-hypertensive drugs have been used in the treatment of hypertension. Though most of these medicines have been found helpful, they nevertheless come with several problems such as side effects and high cost, which limit their extensive use [8]. Hence studies are warranted on implementation of novel strategies for hypertension prevention, treatment and control.

Indeed, it is well known that plants, the main therapeutic means in Africa, are used by more than 80% of the population [9]. 50,000 species of vascularized plants used in the treatment of various conditions have been identified in Africa [10]. In Côte d'Ivoire, 1421 species of medicinal plants and 761 medicinal recipes have been listed [11]. In this context, several traditional healers mentioned that the decoction of *Cajanus cajan* is used in beverage for the treatment of hypertension. *C. cajan* is found out from Senegal to Cameroon and Egypt and also in Côte d'Ivoire. It is called "*pois d'Angole"*, "*pois d'ambrevade*" or "*pois pigeon*" in French. It's a shrub of 1 to 4 m tall, with composite leaves alternate. The green leaflets are oblong-elliptic. The inflorescences with 5 or 10 yellow flowers are on the on leaves axils. The stem bark surface is smooth and grayish. *C. cajan* is used in traditional medicine to treat several illnesses. For instance, the decoction of the fresh leaves is orally administered against cardiovascular diseases. The fresh leaves pasta is also applied on the skin against varicella. However, scientific evidence for its traditional use in cardiovascular conditions is not yet elucidated. Thus, the objectives of this work are to evaluate the effects of the leaves aqueous extract of *C. cajan* on anesthetized rabbits' blood pressure and electrocardiogram and also to screen the phytoconstituents responsible for the pharmacological effects of the extract.

2. Material and Methods

2.1 Animals

The experiments were carried out on rabbits, *Oryctolagus cuniculus* (Leporidae). They were fed with standard granules for rodents and water ad libitum and kept in the animal facility of the Laboratory of Physiology, Pharmacology and Pharmacopoeia of the Nangui Abrogoua University (Côte d'Ivoire) for two weeks according to the principles for the care and use of laboratory animals of the Ethical Committee of the University and also in accordance with the protocols for the protection of experimental animals of the European Council of Legislation 87/609/EEC [12]. The average weight of the animals was 2±0.1 kg.

2.2 Plant

Fresh leaves of *Cajanus cajan* were collected in Abidjan. Taxonomical identification of the leaves was established by the National floristic Centre of the University of Felix Houphouet-Boigny (Abidjan, Côte d'Ivoire).

2.3 Chemicals and drugs

Ethyl urethane and atropine were purchased from Prolabo (France) and used uses as chemicals and drugs.

2.4 Plant extraction

One hundred grams (100 g) of fresh leaves of *C. cajan* were decocted in two liters (2 L) distilled water for 45 minutes. The solution was carefully and subsequently filtered three times on cotton wool and Whatman (n° 1) filter paper. The filtrate was dried using an oven calibrated at 45 °C. The dry extract of the aqueous extract of *C. cajan* (AqECc) were weighed, labeled and stored at 4°C in airtight bottles until ready for use.

2.5 Invasive arterial blood pressure measurement in rabbits

The method was as previously described by Kouakou *et al*. [13]. The rabbits were anaesthetized using ethyl urethane (40%) at a dose of 1 g/kg b.w. The saphenous vein was cannulated with heparinized polyvinyl tubing for intravenous injection of the extract and drugs. The left common carotid artery was cannulated and connected to a Ludwig mercury manometer kymograph. Thus, the variations of the carotid blood pressure were transmitted to the mercury and recorded by a stylet on paper.

2.6 Registration of the global electrical activity (ECG) of the rabbit

The method was as previously described by Traoré *et al*. [14]. The electrocardiogram of the rabbit was recorded using the technique of external electrodes used in the human practices and adapted to the rabbit. Briefly, the saphenous vein of the anesthetized rabbit was intubated in order to administer the plant extract and the drugs. The armpits of the anterior limbs and the groin of the posterior limbs were shaved and cleaned with 90% ethanol. After applying electrolytic dough, four electrodes were put and bound to the four sockets of the registration cable connected to the electrocardiograph (CARDIOFAX ECG-6851K, Nihon Kohden, Japan). Parameters such as waves (P, QRS, T), intervals (PQ, QT) and heart rate were recorded from the DIII derivation of the standards or bipolar Einthoven derivations on thermo sensitive paper, at constant speed (25 mm/s). AqECc was dissolved in Mac Ewen solution prepared from the following chemicals (mM): NaCl (130); KCl (2.5); $CaCl_2$ (2.4); NaH_2PO_4 (1.18); CO_3NaH (11.9); $MgCl_2$ (0.24); $C_6H_{12}O_6$ (2.2) at a pH adjusted at 7.4.

2.7 Phytochemical screening

The leaf decoction of *Cajanus cajan* was screened for the presence of polyphenols, tannins, flavonoids, saponins, alkaloids, sterols and ployterpens, and quinones. Detection of these constituents was carried out as described by Bekro *et al***.** [15].

2.8 Data analysis

All values were expressed as mean ± standard error on the mean (M ± SEM). Statistical analysis and graphics were carried out using the software Graph Pad Prism 5.01 (San Diego California, USA). The significance of the differences between the doses was achieved by analysis of variances (ANOVA) of the multiple tests of comparison of Tukey-Kramer. Differences between concentrations were considered statistically significant when $p< 0.05$.

3. Results

3.1 Dose response effect of the aqueous extract of *Cajanus cajan* (AqECc) on rabbits' arterial blood pressure

The varied doses of AqECc (from 0.5 to 50 mg/kg bw) caused a significant ($p< 0.05$) dose-dependent hypotension in rabbits from 9.84 ± 1.31 to 43.86 ± 6.6% compared to reference blood pressures in rabbits. A 50% effective dose (ED_{50}) of 9.33 mg/kg bw was found (Figure 1).

3.2 Effect of AqECc on rabbits' arterial blood pressure in presence of atropine

To investigate a possible involvement of cholinoceptors in the hypotension induced by AqECc, atropine, a muscarinic antagonist of cholinoceptors, was used. Control value of hypotension induced by AqECc at 30 mg/kg b.w. was 41.14 ± 5.02%. Increasing doses of atropine ranging from 5.10^{-8} to

5.10^{-2} mg/kg b.w. significantly ($p< 0.05$) attenuated the hypotension elicited by AqECc at 30 mg/kg b.w. Indeed, the control value of hypotension was reduced from 38.22 ± 7.46% (Atr 5.10^{-8} mg/kg b.w.) to 23.08 ± 4.2% (Atr 5.10^{-2} mg/kg b.w.). The action of atropine on hypotension induced by AqECc is illustrated in Figure 2.

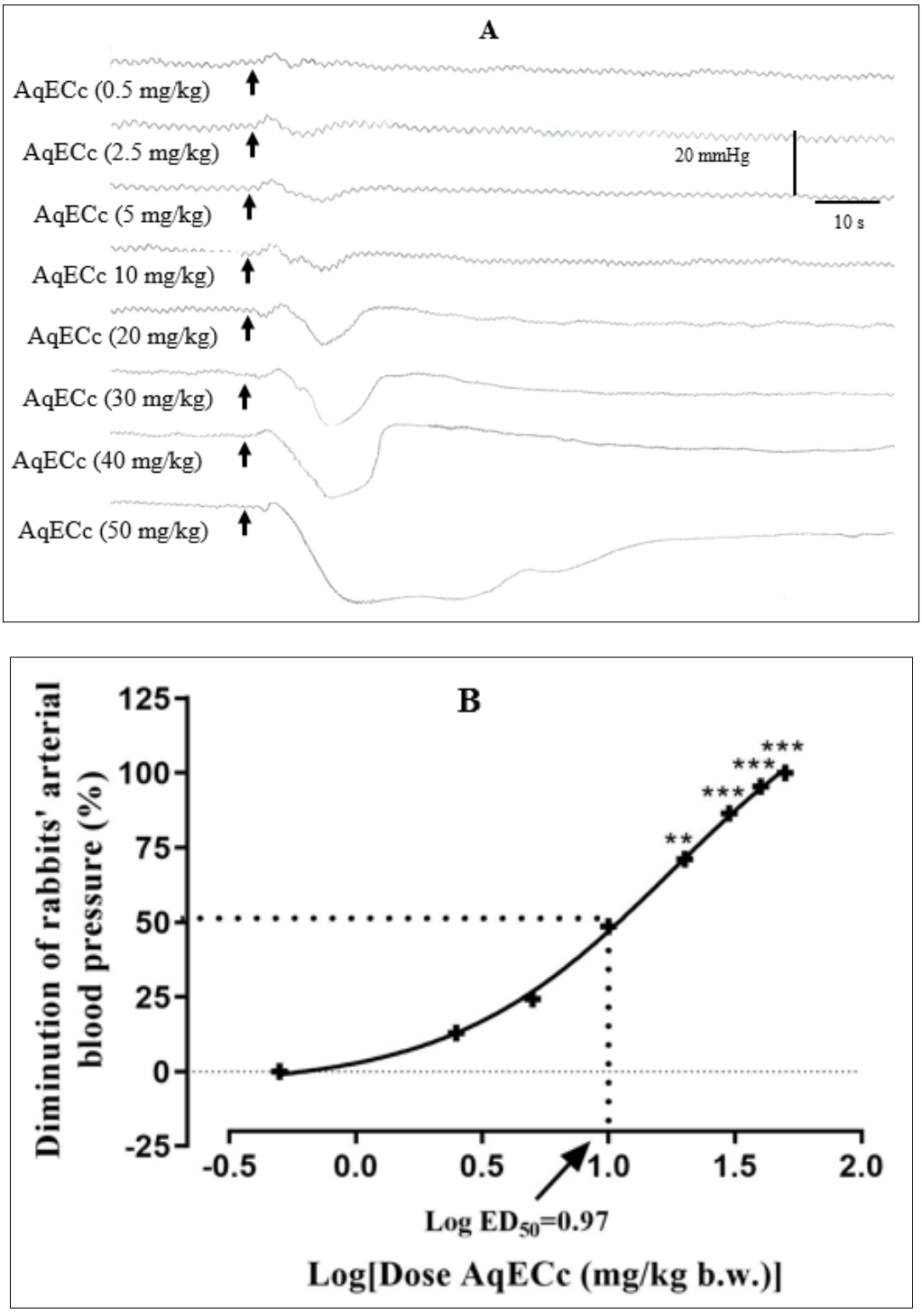

Fig 1: Dose response effect of AqECc on rabbits' arterial blood pressure

A: Dose response effect; B: Effective dose (ED_{50}) determination

AqECc induced significant dose dependent decrease of rabbits' arterial blood pressure. The fifty percent effective dose (ED_{50}) determined graphically was 9.33 mg/kg b.w., n=5; $**p< 0.01$; $***p< 0.001$: Significant difference when compared to the reference blood pressure. AqECc: aqueous extract of *Cajanus cajan*.

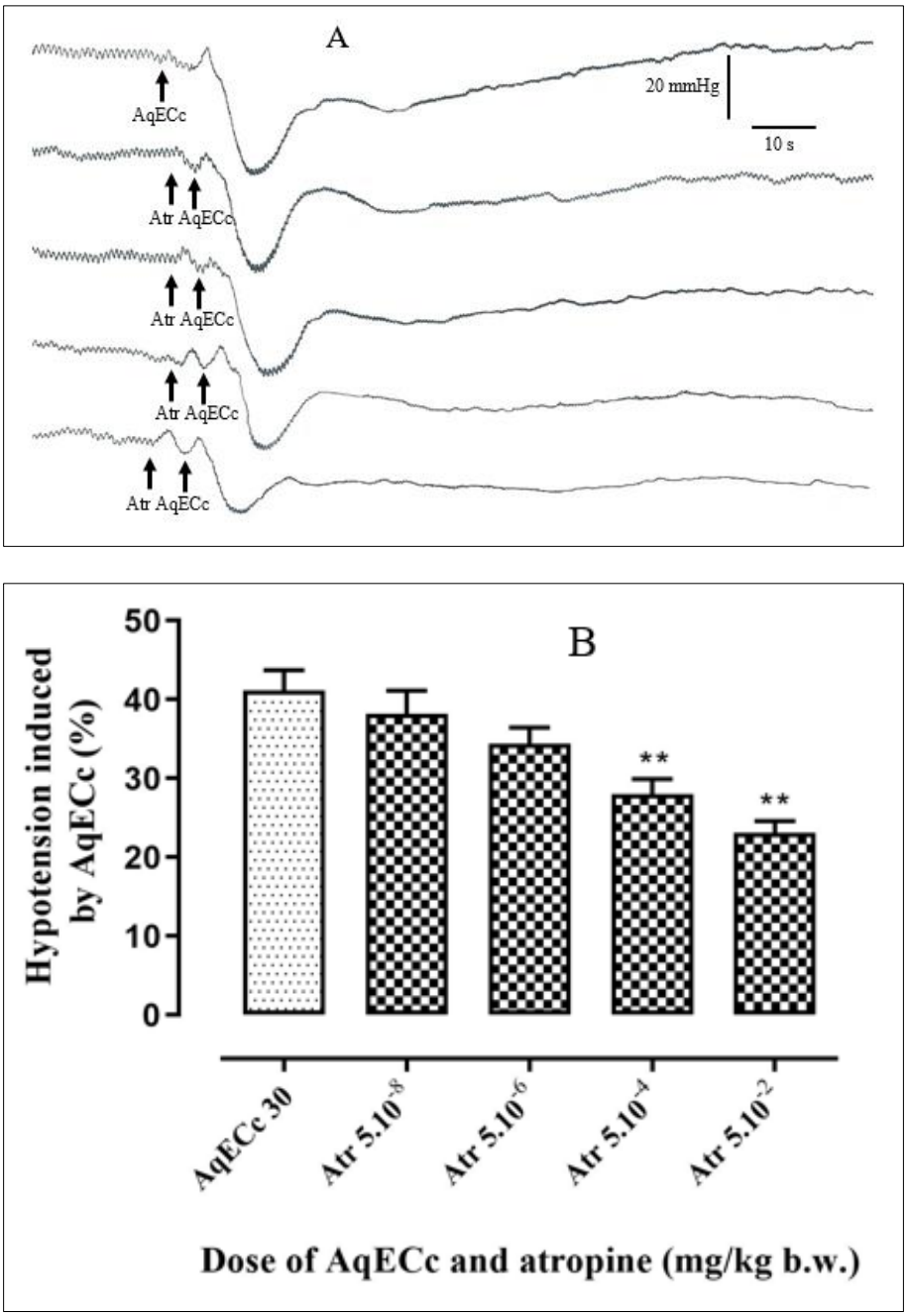

Fig 2: Effect of AqECc on the rabbits' blood pressure in presence of atropine

A: Dose-response effect of AqECc in presence of increasing doses of atropine
B: Hypotension induced by AqECc in presence of increasing doses of atropine; n=4
**p< 0,01 values were significantly different when compared to that of AqECc at 30 mg/kg b.w.
AqECc: aqueous extract of *Cajanus cajan*; Atr: atropine

3.3 Dose response effect of AqECc on rabbits' electrocardiogram (ECG)

As shown in Table 1, the dose response effect of AqECc was investigated on rabbits electrocardiogram. The results showed that the extract (0.5 to 50 mg/kg b.w.) influenced the different parameters of rabbits' ECG. The normal ECG values recorded (control) was significantly ($p< 0.05$) decreased for the T-wave and the heart rate. Indeed, the T-wave diminished from 112 ± 21.5 to 58.67 ± 3.76 µV while the heart rate dropped from 234.33 ± 28.70 to 140.67 ± 10.90 Cycles/min. The P wave, QRS complex and QT interval which control values were 143.33 ± 6.61 µV, 786.67 ± 69.30 µV and 52.67 ± 3.53 ms respectively non-significantly dropped and reached 113.00 ± 9.07 µV, 687.67 ± 134.0 µV and 173.87 ± 13.9 ms. Only the PQ interval was significantly ($p< 0.05$) increased from 52.67 ± 3.53 ms to 62.13 ± 3.14 ms.

3.4 Phytochemical screening of AqECc

Phytochemical screening of AqECc, as shown in Table 2, revealed the presence of sterols and polyterpens, polyphenols, flavonoids, catechin tannins, alkaloids and saponins.

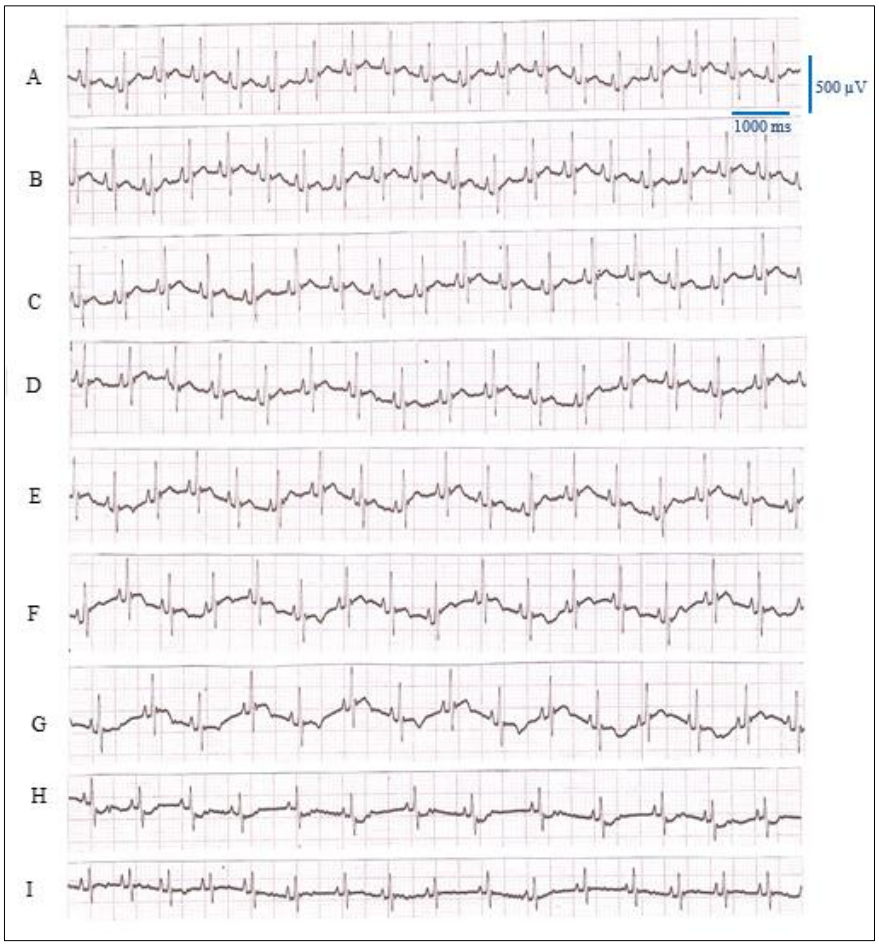

Fig 3: Dose response effects of AqECc on rabbits' electrocardiogram

A to I: Effects of 0 (A), 0.5 (B), 2.5 (C), 5 (D), 10 (E), 20 (F), 30 (G), 40 (H) and 50 (I) mg/kg b.w. of AqECc on the rabbits electrocardiogram

Table 1: Dose response effect of AqECc on rabbits electrocardiogram parameters

AqECc (mg/kg b.w.)	P wave (µV)	QRS complex (µV)	T wave (µV)	PQ interval (ms)	QT interval (ms)	Heart rate (Cycles/min)
0 (Control)	143.33 ± 6.61	786.67 ± 69.3	112 ± 21.5	52.67 ± 3.53	177.93 ± 12.0	234.33 ± 28.7
0.5	139.83 ± 6.1	803.5 ± 77.00	116.67 ± 23.6	53.60 ± 3.19	180.13 ± 11.7	234.33 ± 28.7
2.5	136.33 ± 4.81	808.67 ± 81.7	120.33 ± 20.9	55.27 ± 3.53	183.73 ± 11.4	224.67 ± 27.8
5	134.33 ± 6.36	818.67 ± 85,1	129 ± 25	57.30 ± 2.48	186.80 ± 11.4	214.33 ± 27.7
10	131.50 ± 5.97	780.00 ± 58.8	117.67 ± 23.4	58.00 ± 2.57	186.37 ± 11.8	209.33 ± 27.2
20	129.00 ± 5.51	745.83 ± 24.8	111.67 ± 21.2	58.53 ± 2.54	18490 ± 12.8	206.67 ± 27.8
30	126.57 ± 7.55	751,83 ± 31.6	102.83 ± 18.7	58.87 ± 2.48	183.67 ± 13.6	200.0 ± 29.6
40	122.33 ± 7.06	688.00 ± 104.0	94.5 ± 16.8 *	60.37 ± 2.72 *	177.0 ± 12.9	184.00 ± 28.2 *
50	113.00 ± 9.07	687.67 ± 134.0	58.67 ± 3.76 *	62.13 ± 3.14 *	173.87 ± 13.9	140.67 ± 10.9 *

T-wave and heart rate were significantly dropped by increasing doses of AqECc. PQ interval was augmented in presence of AqECc. P-wave, QRS complex and QT interval were not significantly affected by the extract. n=5; * $p< 0.05$: Significant difference when compared to the control.

Table 2: Phytochemical screening of AqECc

Constituents	Tests	AqECc
Polyphenols	$FeCl_3$	+
Tannins	Stiasny	+
	$FeCl_3$	-
Flavonoids	Cyanidine	+
Quinones	Borntraëger	-
Alkaloids	Bouchardât	+
	Dragendorff	+
Saponins	Frothing	+
Sterols and polyterpenes	Liebermann	+

-: absence; +: presence

4. Discussion

The present study was design to evaluate the hypotensive and cardio-inhibitor effects of the fresh leaves' decoction of *Cajanus cajan* (Fabaceae) in anesthetized rabbits. The results showed a significant dose dependent hypotension for the extract doses ranging from 0.5 to 50 mg/kg b.w. Similar results were obtained with extracts of herbs such *Mimosa invisa* [16, 17] and *Justicia secunda*.

To investigate a possible involvement of acetylcholine receptors in the induction of hypotension of AqECc, atropine pathway was hypothesized since it's a muscarinic cholinoreceptor antagonist. Thus, rabbits were administered

with 30 mg/kg bw of AqECc prior the administrations of increasing doses of atropine. Results exhibited a significant inhibition of the hypotension induced by AqECc. This suggests that this extract contained cholinomimetic substances acting via muscarinic receptors. The same conclusion was highlighted by some authors in the interaction of plant extracts with atropine study. Indeed, Ghayur and Gilani [18] showed that the hypotensive effect induced by an extract of *Raphanus sativus* in rats was inhibited by atropine. Gilani *et al*. [19], reported that dried flowers 70% aqueous methanol extract from *Lavandula stoechas* produced a drop in blood pressure in anesthetized normotensive rats which was abolished by atropine. The presence of acetylcholine-like phytoconstituents in the alcoholic extract of *Sesamum indicum* seeds was indicated by Nakano *et al*. [20]. The actions of acetylcholine on blood pressure are well known. According to Supple and Powell [21], the intravenous injection of acetylcholine in humans or animals leads to an immediate and transient drop in blood pressure resulting from cardiac slowing and vasodilatation. The decrease in the force of contractions followed by hypotension is due to a reduction in Ca^{2+} entry caused by an inhibition of adenylate cyclase and also a reduction of Ca^{2+} release from sarcoplasmic stores [22]. The assessment of AqECc on rabbits' electrocardiogram revealed that the T-wave and heart rate were significantly reduced. The extract didn't significantly affect the P-wave, QRS complex and QT-interval. The PQ-interval was significantly increased. Therefore, the general outcome of the action on the global electrical activity on rabbit heart was inhibition. These effects could be due to the presence of cholinomimetic substances present in AqECc which action was found to produce a decrease of cardiac activity. Similar findings were obtained with an aqueous leaf extract *of Sesamum radiatum* and a chromatographic fraction from the aqueous leaf extract of *Bidens Pilosa* [23, 13]. These authors suggested that the inhibitory effects of their extracts were attributable to acetylcholine-like actions resulting in a depressive effect on the sinusal node and thus reducing global depolarization of other cardiac tissues. QT interval increased slightly but not significantly. This is interesting because as stated by certain authors, a prolongation of QT-interval could be source of arrhythmias [24]. A phytochemical screening implemented with AqECc showed the presence of sterols and polyterpenes, polyphenols, flavonoids, saponins, catechin tannins and alkaloids. These substances may be responsible or involved in the hypotensive and cardio-inhibitor of the aqueous extract of *C. cajan*. Indeed, many researchers highlighted their beneficial effects on the cardiovascular system. For instance, the hypoglycemic and Hypotensive effects of polyphenols, flavonoids [25].

5. Conclusion

The present study revealed that the leaf extract of *C. cajan* (AqECc) possess hypotensive and cardio-inhibitor effects probably due to cholinomimetic substances such as polyphenols and flavonoids present. These results could justify the use of this plant in traditional medicine to treat hypertension.

6. Conflict of interest: The authors declare no conflict of interest

7. Funding: None

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