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Full Length Research Paper

Muscarinic activity of aqueous leaves extract of *Excoecaria grahamii* Stapf 1913 on rabbit blood pressure and toad isolated perfused heart

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***Excoecaria grahamii*, African shrub, is used in many West African countries traditional medicine against different diseases as powerful purgative and biopesticide. There is little work about pharmacological effects of this plant because of its high toxicity. The effects of the aqueous extract of the plant was investigated on rabbit blood pressure and toad isolated and perfused heart activity. *E. grahamii* extracts (EGE) injection exhibited a dose dependent blood pressure decrease like acetylcholine effect. This activity was inhibited by atropine, suggesting muscarinic effect. Same results (inhibition effects) were found on both electrical and mechanical actions of toad isolated and perfused heart. Previous results obtained with aqueous extracts on smooth intestine muscle exhibit a muscarinic effect (hypotensive principle) then alcoholic extracts showed hypertensive and cardiotonic effects on rabbit. Our results confirm existence of muscarinic activity on rabbit blood pressure and toad heart activity.**

Key words: *Excoecaria grahamii*, rabbit blood pressure, toad heart, electrocardiogram.

INTRODUCTION

Excoecaria grahamii Stapf synonym *Sapium grahamii* (Stapf) Prain (1913) is an African small shrub, glabrous and not branched, 60 to 90 cm tall of Euphorbiaceae family (Figure 1). It contains milky and sticky latex and has deep creeping rhizomes. The inflorescence is a thin terminal spike, reaching 50 mm long, mostly numerous male flowers for basal one or two female flowers. The fruit is a three lobed, few rare times four lobed capsule of 20 to 25 mm diameter, with explosive dehiscence. Each lobe contains one globose and brown to yellow seed of approximately 5 mm long (Schmelzer, 2007).

Its local therapeutic uses are reported in Benin, Burkina Faso, Ivory coast, Niger and Nigeria (Curasson, 1931; Ouédraogo et al., 1976; Belemtougri et al., 1995). Local uses concern whole plant (decoction) 6, latex and pounded leaves (direct applications) or decoction, latex

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or smoke of burnt of roots. Situations or illness where *E. grahamii* is used are skin affections, oedema, leprosy, ascites, guinea worms, hallucinations and abortions. Whole plant decoction is known, in Burkina Faso, to have violent purgative effects and is used in small quantities against constipation. Also, roots are used as an ingredient of arrow poison and its latex is used for ritual scarifications and tattooing (Schmelzer, 2007), and as insecticide for crops protection. Leaves and roots are used against dysentery (Ouédraogo et al., 1976).

There is no much works about *E. grahamii*, mainly because of its high toxicity that explains its limited uses in local medicine and in pharmacological researches. Schmelzer (2007) thinks that “It would be interesting to evaluate its pharmacological properties as other *Excoecaria* spp. yield compounds with interesting anti-HIV activities”. Then, Ouédraogo (1976, 1977) and Belemtougri et al. (1995) reported intestinal smooth muscle contractile activity of total aqueous extracts of leaves and roots, a hypertensive activity in rabbits of alcoholic extracts. These studies suggest existence of two opposing active principles in extracts of the plant: inhibitor and stimulating principles of the tone of smooth muscle (rabbit duodenum and ileum of guinea pig). Since then, this issue has not been documented and clarified by new research data. Traoré et al. (2014) came to highlight and evaluate anthelminthic activity of leaf extracts of *E. grahamii* on worm *Haemonchus contortus* presumably by nicotinic receptors.

In this work, we attempt to explore acute toxicity and muscarinic activity on rabbit arterial pressure of aqueous leaves extracts.

MATERIALS AND METHODS

Plant collection

E. grahamii (EG) was collected at Kombissiri, province of Bazèga south east of Burkina Faso about 80 km of Ouagadougou (position 11° 55’ 33.8’’ North; 01° 17’ 10’’ East) in dry season and identified by Biodiversity Center Herbarium of Université de Ouagadougou, where the voucher specimen (n° ID: 16703 sample n°: 6786) has been deposited.

Preparation of the plant extract

Aqueous extract was prepared from the shade dried leaves of *E. grahamii.* Leaves powder (100 g) were macerated in 1 L of deionized water for 24 h at room temperature and then filter and freeze-dried.

Animals

Naval Medical Research Institute (NMRI) adult mice (30 to 36 g), local adult rabbits (1.4 to 2.2 kg) and common toad *Amietophrynus regularis* (43 to 91 g) were used in these experiments. Mice and rabbits were fed with standard diet and were kept in our animal house at 22 ± 5°C and submitted to a 12 h light/dark cycle with food and tap water *ad libitum*. The toads were kept in plastic pots for a week in the laboratory with free access to dechlorinated water but not fed. They are then anesthetized with MS 222 bath (1 g/L) before being sacrificed. All animals’ procedures were strictly within respect for the ethics of scientific research, the treatments of laboratory animals’ standards described in the "Guide for the Care and Use of Laboratory Animals" of the National Academy of Sciences of the United States and Burkina Faso “Code de l’Environnement”. Efforts have been made to minimize the number of animals used and their suffering.

Acute toxicity study

Mice were used in this experiment according to Bayala et al. (2005). Mice were fasted for 24 h. They had free access to water during first 14 h and deprived of water during resting for 10 h. A subcutaneous single-dose study was conducted in mice to evaluate the potential toxicity of *E. grahamii* extract (EGE). Sixty six (66) healthy mice were used in two groups. The first group of thirty (30) mice were administered 10, 25, 50, 75, 100, 150, 250, 500, 1000 and 2500 mg of EGE per kg body weight in order to determine the highest dose without mortality and the lowest dose with 100% lethality. According to those data, the second group of thirty six (36) mice were administered 75, 110, 140, 170, 200 and 250 mg of EGE per kg body weight in order to determine lethal doses which induce 1% (LD_1), 50% (LD_{50}) and 99% (LD_{99}) of mortality. Mice were observed for mortality and changes in behaviour after treatment at 1, 24, 48, and 72 h and were conserved for 14 days.

Blood pressure measurement

The study of the activity of the plant extract on blood pressure was carried out according to Belemtougri et al. (2001; 2007). Rabbits were anaesthetized with urethane 40% at dose of 1 g/kg by intraperitoneal injection. The saphen vein and the left carotid artery were dissected and cannulated with catheters filled with heparin saline solution (125 UI/mL) for further injection and blood pressure recording, respectively. Blood pressure from carotid was recorded using an Elcomatic EM 750 SER n° 2203 transducer, connected to blood pressure amplifier FC 137 Palmer Bioscience, connected to a Palmer Bioscience 400 MD 4Roscillograph. The animals were allowed to stabilize for 25 min and the baseline blood pressure was recorded before injection of any substance.

Toad perfused heart

The toad was pithed and pinned and placed in supine position. The chest was opened and the pericardium was carefully removed to expose the heart. A small cut was made in the inferior vena cava and a cannula was inserted towards the heart and tied. The heart was then removed from the animal and the cannula was connected to a tank containing the Ringer by a plastic heating coil immersed in a water bath maintained at 37°C. A valve allows us to stabilize the perfusion flow of the heart.

There was an opening in the cannula through which drugs could be injected by pushing a capillary tube attached the syringe through an injection needle. Aorta was intubated to collect the perfusion fluid that flowed freely in a container. The heart was connected to an oscillograph Palmer Bioscience 400 MD 4R through two couplers and two sensors.

Table 1. Results of the toxicity study.

Observation time (h)	Lethal doses (mg/kg of body weight)			Security index		
	LD_{99}	LD_{50}	LD_1	LD_{50}/LD_1	LD_{99}/DL_{50}	LD_{99}/LD_1
72	221.19	141.49	90.51	1.56	1.56	2.44
48	260.05	146.17	82,16	1.78	1.78	3.17

LD_{50} is 141.49 mg/kg of body weight with a lower limit of 118.34 and an upper limit of 161.85. The security index is 3.17 at 48 h and 2.44 at 72 h.

A sensor of isotonic contraction is connected to a FC 124 Palmer Bioscience Coupler for the registration of the mecanocardiogram and ECG was recorded by using an Elcomtatic ECG transducer attached to FC 142 coupler.

Substances used

Acetylcholine chloride, atropine sulfate, sodium chloride, urethane were provided from Sigma (Sigma Chemical Company, USA). All chemical solutions were freshly prepared.

Statistical analysis

Results were expressed as mean ± standard error of mean (SEM). Student's t test was used to compare test values and control values; p values less than 0.05 is considered statistically significant.

RESULTS

Acute toxicity

The maximum non-lethal (LD_0) or the highest dose which caused no mortality is 75 mg/kg of body weight, and the lowest dose causing 100% mortality is 250 mg/kg of body weight. Lethal doses at various rates are given in Table 1.

Blood pressure decrease by EGE

E. grahamii extracts (EGE) injection exhibited a dose dependent blood pressure decrease, starting at 3 and 10 s after injection and during 10 to more than 40 s, according to the injected doses. Typical recording of the action of EGE was given in Figure 2. Graphic synthesis of all results was given on Figure 3. There is no significant difference between decrease of systolic pressure (SP) and diastolic pressure (DP). Acetylcholine injection decreased blood pressure in dose dependent manner as shown in Figure 4 (typical recording) and Figure 5 that presents the synthesis for all results in SP and SD decrease. Partial inhibition of 10 mg/kg EGE effect is observed with low doses of Atr (0.3 to 1.5 µg/kg) and total inhibition with higher doses (3.0 to 15.0 µg/kg). Action of EGE was inhibited by atropine at doses from 0.3 to 15 µg per kg of body weight challenged with 10 mg/kg of body weight of EGE as seen on typical recording of Figure 6.

Toad heart inhibition by EGE

EGE was tested on the toad, *Amietophrynus regularis*, isolated and perfused heart at constant temperature, 37°C. The device used and the sensor recorded both heart mechanical activity and global electric activity, electrocardiogram. Injection of 0.1 ml of Amphibian's Ringer (perfusion liquid) showed that the recording setup was well functional and was not disturbed by injections (Figure 7). It is first tested Ach action on heart (Figure 8) and then inhibition of Ach activity by Atr (Figure 9). Injection of EGE (10 mg) has an inhibitory effect on both mechanical and electrical heart activities (Figure 10). This inhibition began within two seconds following the injection. However when we realized the Atr/EGE interactions, we did not get inhibition but inhibitory effect of the EGE was delayed from 15 to 20 s after injection (Figure 11). These two results have been systematically obtained for 10 toads' hearts.

DISCUSSION

As shown in the introduction, there was little work on *E. grahamii*, probably because of its reputation for toxicity (Schmelzer, 2007). But it is important to evaluate the pharmacological properties of *Excoecaria* genus plants. Indeed, some of these species (*E. agallocha* and *E. acerifolia*) investigated in China, India and Australia contained phorbol esters (tetracyclic diterpene) and other terpenoids with anti-HIV properties (Konoshima et al., 2001; Zou et al., 2006; Yang-Li et al., 2010; Huang et al., 2013a, 2013b). Similarly, use as biopesticides were also encountered for Asian and Indian *Excoecaria* genus species (Goel et al., 2007).

The acute toxicity that we have found for leaves extracts (LD_{50} = 141.49 mg/kg) placed this plant among

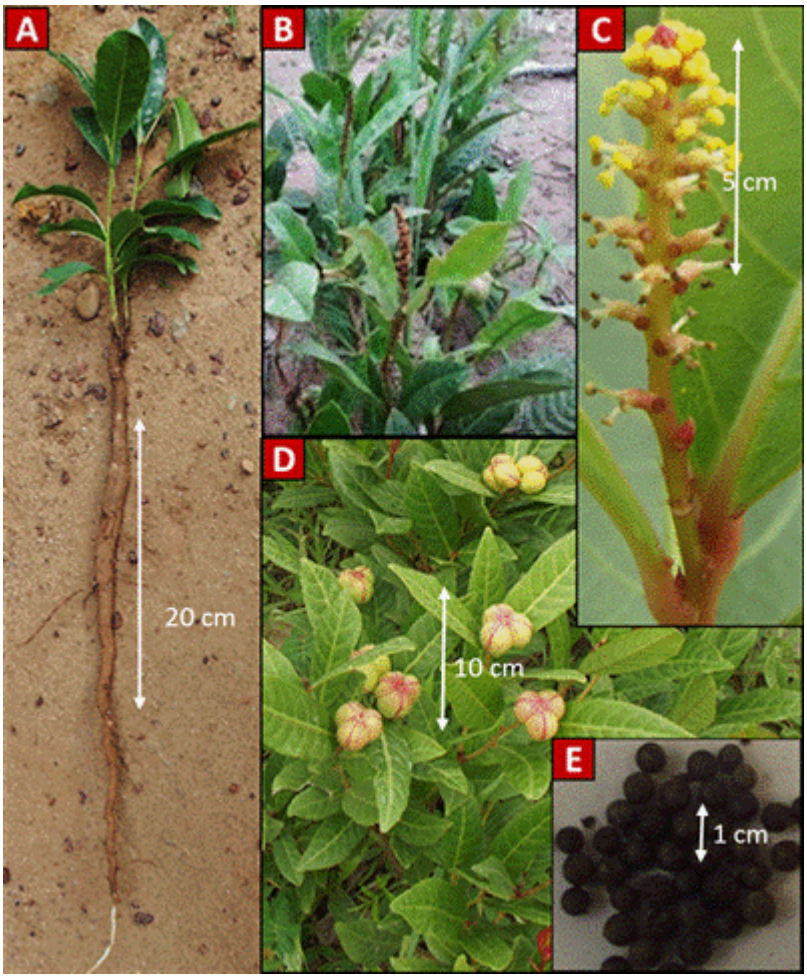

Figure 1. *Excoecaria grahamii.* A = whole plant, B and C = inflorescence and flowers, D = fruits, E = seeds.

the moderately toxic according to International Programme on Chemical Safety (IPCS) (2002) scale adapted from Hodge and Sterner (1943). But security index is too weak for potential uses (< 5). However, Traoré et al. (2014) found lower acute toxicity LD_{50} = 389 mg/kg in 24 h and a good security index, 35.9.

In 1976, Ouédraogo highlighted, on the isolated duodenum from rabbit and guinea pig isolated ileum, hypotonic activity followed by hypertonic one (the latter can be inhibited by atropine). This result led to the hypothesis of the existence in the plant extracts, of two or more different active principles. The hypotonic principle would be temperature sensitive since the extract heat-treated (100°C for 10 days, 120°C for 3 days or 140°C for 90 min) removed this activity. Belemtougri et al. (1995) found hypertensive and cardiotonic activities for the alcoholic extract of the plant at doses ranging from 0.6 to 1.8 mg. In our works, we highlighted a hypotensive activity, comparable to acetylcholine muscarinic activity but at doses ranging from 10 to 30 mg/kg body weight.

The presence of two contradictory effects and therefore two active principles was confirmed. The first would be hypotonic on intestinal smooth muscle, and cardiotonic and hypertensive. It would also be thermal sensible with more affinity for organic solvents. The second would be hypertonic on the intestinal smooth muscle, muscarinic type hypotensive (inhibited by atropine). It has more affinity for water and would be heat-resistant.

Antihypertensive drugs may act on the heart or blood vessels. The present study showed that EGE produced a negative inotropic effect on the heart which can be attributed to the stimulation of nitric oxide synthase (NOS) or caused by different mediators such as β-receptor antagonist, cholinergic receptors agonists and

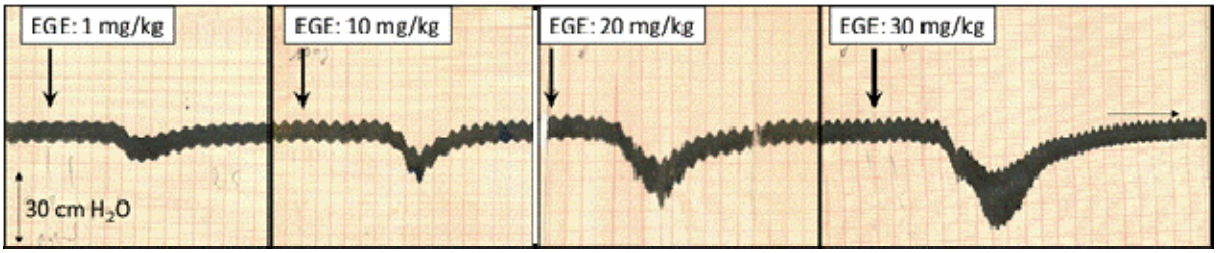

Figure 2. Typical recording of the action of EGE on rabbit blood pressure.

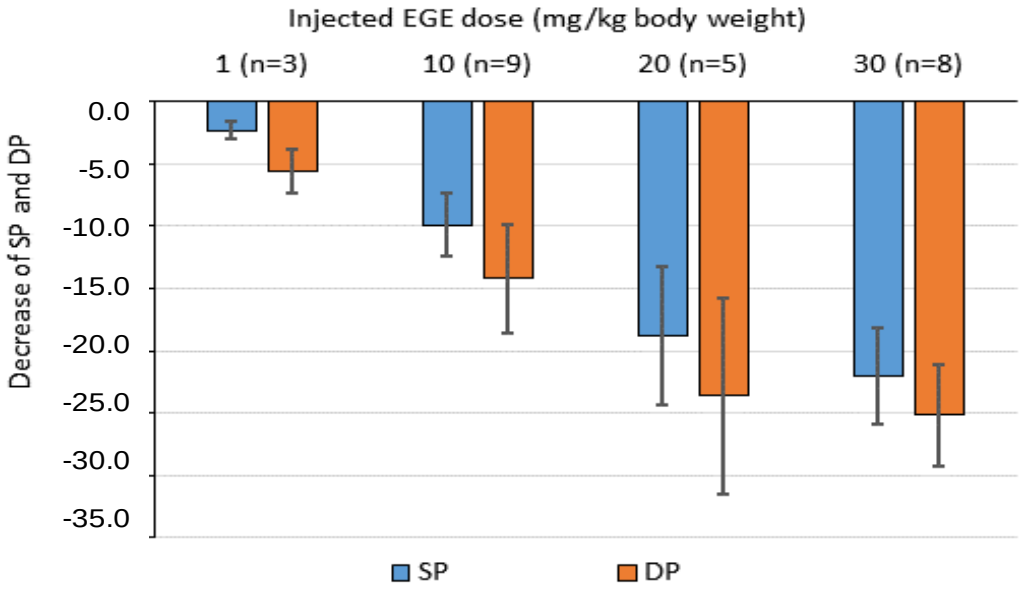

Figure 3. Decrease of systolic pressure (SP) and diastolic pressure (DP) after *E. grahamii* extract injection.

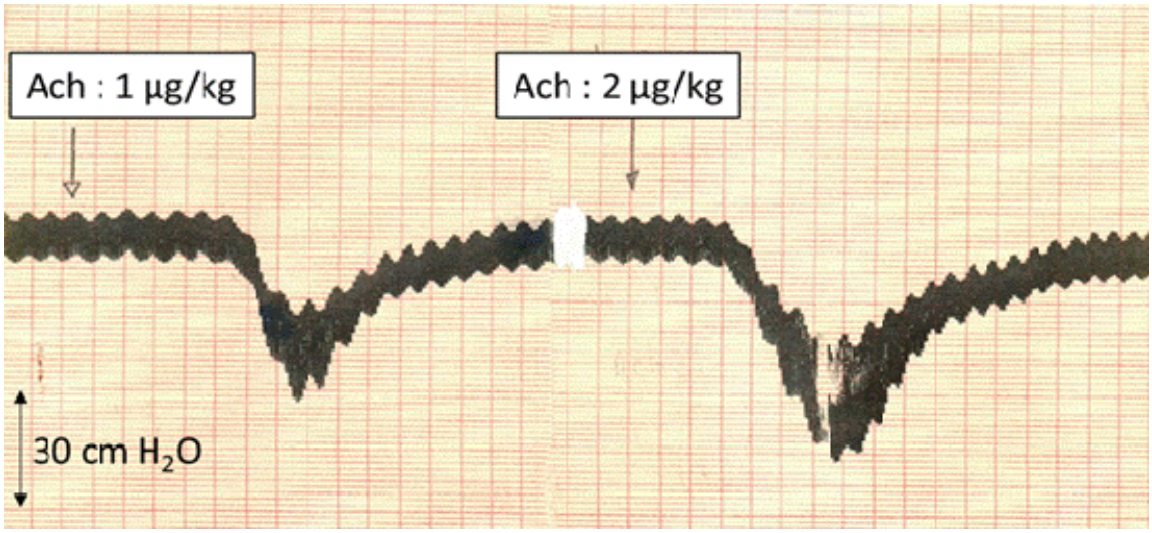

Figure 4. Typical recording of the action of Ach on rabbit blood pressure.

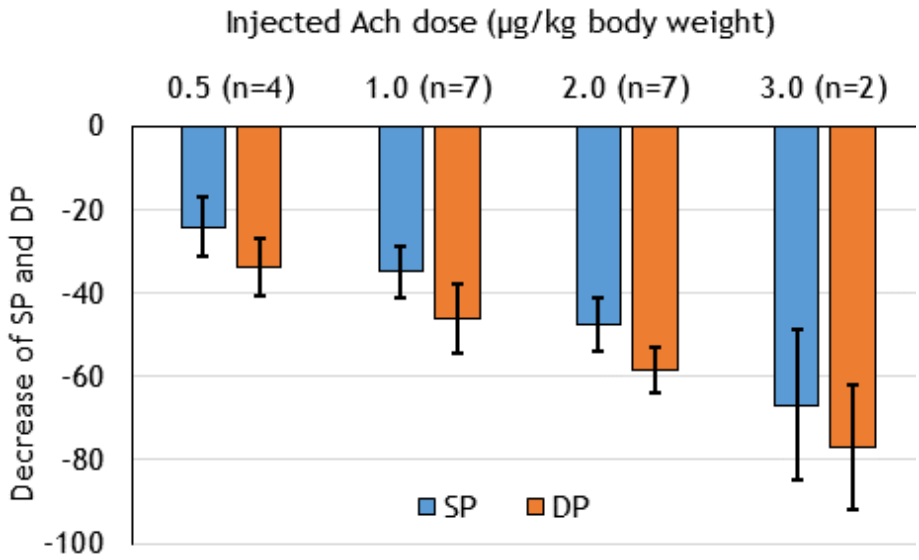

Figure 5. Decrease of systolic pressure (SP) and diastolic pressure (DP) after Ach injection.

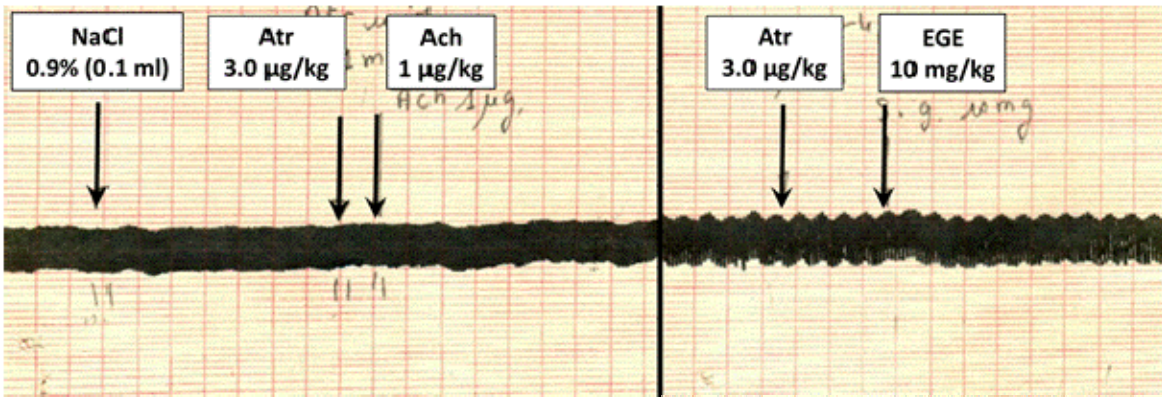

Figure 6. Typical recording of inhibition of Ach and EGE action on rabbit blood pressure.

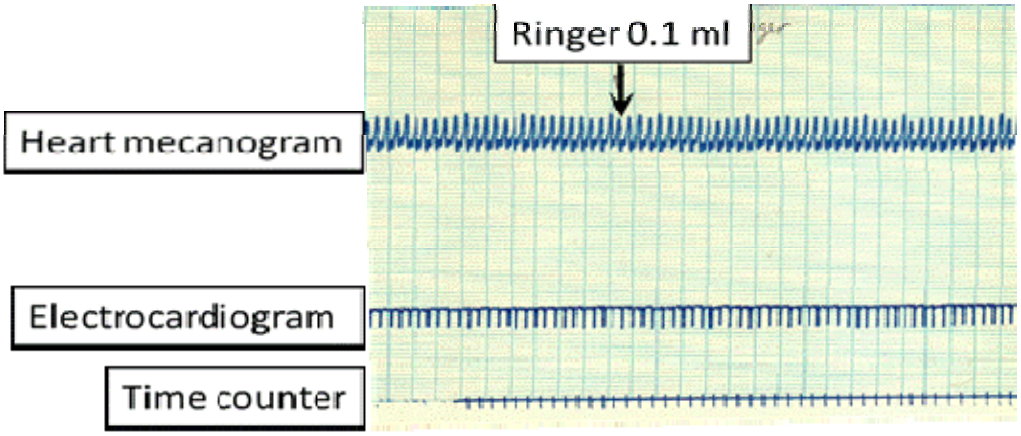

Figure 7. Typical recording of mecanogram et electrocardiogram of toad isolated perfused heart.

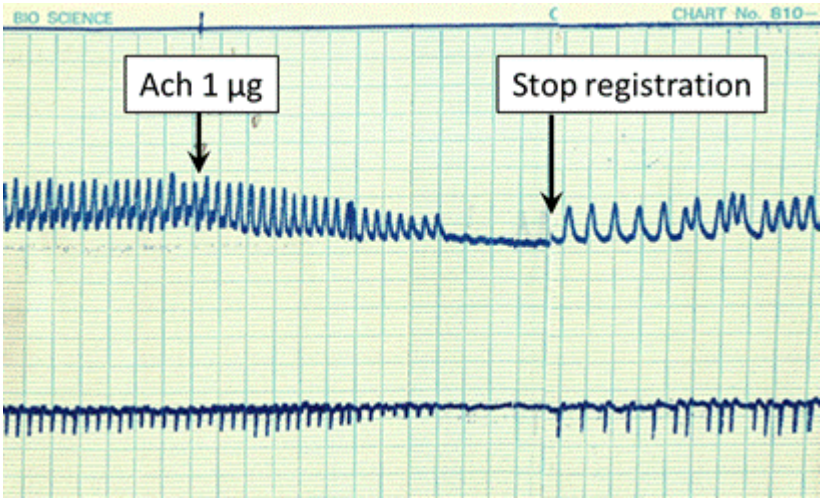

Figure 8. Ach effect on toad isolated perfused heart activity.

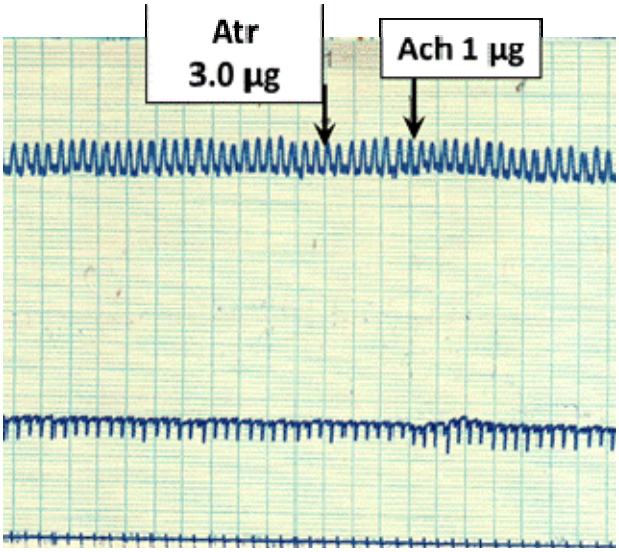

Figure 9. Inhibition of Ach activity by Atr.

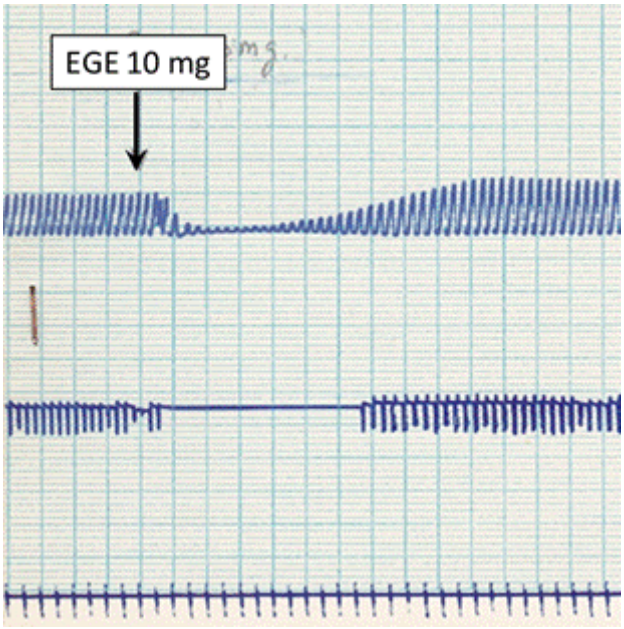

Figure 10. Inhibition of cardiac activities by EGE (10 mg/kg body weight).

endothelium derived relaxing factor (Shabi et al., 2012). The effect of EGE was reduced in presence of atropine, an antagonist of muscarinic receptor of Ach. According to Kurian et al. (2010) and Kurian and Paddikkala, (2012), the methanol extract of the root of *Desmodium gangeticum* induced the same response on the isolated frog heart.

Ach has been known to lower the blood pressure and to induce bradycardia through its muscarinic receptor. Then our extract which has effect like Ach can induce its effect by blocking calcium entry through calcium type T channel in myocardium and lowered heart beat which results by blood pressure decrease in our experimental study in rabbit blood pressure. Toad isolated perfused heart results obtained suggested that EGE effects on blood pressure is underpinned by cardiac inhibition. Subsequent investigations on mammals should allow better assess of the respective shares of the heart and vascular system in the blood pressure decrease effects of aqueous extract. In the same order, further steps of our works will be toxicity investigations with whole plant and the roots

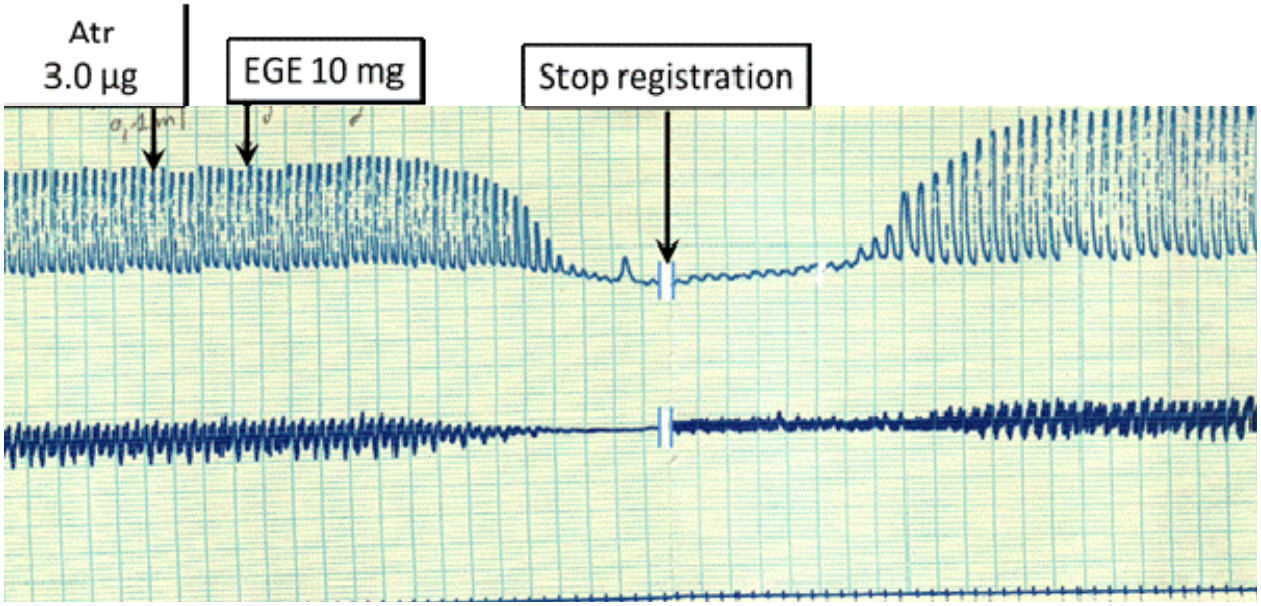

Figure 11. Delayage of EGE effect on cardiac activities by Atr.

extracts for a better understanding of the real toxicity of the plant, and then, we will be able to understand the presence of two contradictory activities principles in plants extracts and their main pharmacological properties.

Conflict of interest

Authors declare that there are no conflicts of interest.

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