

Cardiodepressor effects of an aqueous trunk bark extract of *Parkia Biglobosa* (Jacq. Benth (Mimosaceae) on the spontaneous contractile activity of the isolated heart of rat

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Abstract : *Parkia biglobosa* (Mimosaceae) is a plant commonly used in African traditional medicine to treat several diseases among which hypertension and heart disorders. The present work, carried out with the total aqueous extract of the trunk bark of *Parkia biglobosa* (AEPB) on the mechanical activity of isolated rat heart proposes to deepen studies on mechanisms of action underlying its effects in Cardiac level. Spontaneous Contractile Activity of the isolated rat heart has been recorded. The aqueous extract of *Parkia biglobosa* (AEPB), at concentrations between 10^{-14} and 10^{-4} mg/ml induces dose dependent cardiodepression. However, these negative cardiotropic, inotropic and chronotropic effects are not inhibited by atropine, a well-known muscarinic antagonist. The adrenaline-AEPB interaction, suppresses the positive inotropic and chronotropic effects induced by adrenaline alone. This action of AEPB was the same as that induced by atenolol, a well known β_1 -adrenergic receptors blocker. It was therefore concluded that AEPB action was not mediated by cholinergic muscarinic receptors, but may imply an adrenergic β -blocker.

Keywords: Isolated rat heart, *Parkia biglobosa*, Atropine, Adrenaline, Atenolol, Cardiodepression

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I. Introduction

Parkia biglobosa (Mimosaceae) is a Sudanese-Zambezian species found throughout West Africa, from Senegal to Togo [1]. These authors indicate that the bark of *Parkia biglobosa* is used in the treatment of wounds, febrile bouts of measles and chicken pox.

Adjanohoun et al. [2] have also reported several healing qualities of *Parkia biglobosa*. It is generally used in the treatment of amoebiasis, hookworm, ascariidiosis, asthma, infertility, diarrhea and gastro duodenal ulcers. It is also advocated in the treatment of dental pain and heart disorders.

Already Kerharo and Bouquet [3] have indicated that the bark of this plant is often used in Burkina Faso by healers in the treatment of wounds and ulcers.

Kerharo and Adam [4] also reported that the bark of *Parkia biglobosa* is used in Senegalese therapeutics to treat infertility, bronchitis, tracheitis, pneumonia, leprosy and venereal diseases.

The work of Kouadio et al. [5], made from hexanic, methanolic and aqueous extracts of bark, also demonstrated analgesic and anti-inflammatory properties of *Parkia biglobosa*.

Finally, Assane et al. [6], Bonnah et al. [7] and Kassi et al. [8], reported respectively that the seeds and the trunk bark of this plant exhibit antihypertensive activity.

The present work, carried out with the total aqueous extract of the bark of *Parkia biglobosa* (AEPB) on the mechanical activity of isolated heart of rat proposes to deepen the studies on the mechanisms of action underlying its effects in Cardiac level.

II. Material and methods

Biological material

Preparation of the aqueous extract of *Parkia biglobosa* (AEPB)

Parkia biglobosa (Mimosaceae) is a tree of 10 to 13 meters in height. This plant grows in tropical Africa, especially in the savannahs of northern Côte d'Ivoire [9].

The sample was collected in March 2003 behind the Amphitheater C of UFR Biosciences of Felix Houphouet-Boigny (Former University of Cocody), Abidjan, Côte d'Ivoire. Authentication was made by Professor Ake Assi Laurent, thanks to the herbarium of the National Center of Floristics (CNF), which is the herbarium of Côte d'Ivoire, on samples number 10933 of 22 -12-1969, 13329 of 8-02-1976 and 13336 of 9-02-1976

The bark is cut into small pieces, dried in ambient air and then crushed in a mechanical ball mill for at least an hour. A sufficiently fine powder of brown color is obtained.

Fifty grams (50 g) of ground material are mixed in 1 liter of distilled water under slow magnetic stirring for 24 hours. The solution obtained is filtered on hydrophilic cotton and Wattman filter paper according to the method described by Kouakou *et al* [10]. The filtrate collected in a flask is then evaporated under vacuum at 90°C using a rotary evaporator of the rotavapor type and dried in an oven at 70°C [10]. A perfectly water-soluble fine brown powder, the crude aqueous extract of the bark of *Parkia biglobosa* (AEPB), was obtained and kept in the fridge. A stock solution from which the experimental solutions will be made with the Mac Ewen (ME) is prepared.

Animal material

The experiments were carried out with albino, male and female white rats, *Rattus norvegicus*, of Wistar strain, genus *Musa*. These animals come from the animal house of Animal Physiology Laboratory of the Training and Research Unit (UFR) of Biosciences of the University of Cocody (University Félix Houphouet-Boigny Abidjan-Cocody). They had access to food and water *ad libitum*. They benefited from the light and the darkness (12 hours/12 hours). The specimens used weigh between 150 and 300 g. All procedures are in accordance with the guide for the health and use of laboratory animals published by the National Institute of Public Health.

Experimental and technical recording device

The experimental device used for recording the mechanical activity of isolated Rat heart by infusion is the same as that performed by Kouakou *et al* [10].

The rat is anesthetized by intraperitoneal injection of 20% ethylcarbamate at a rate of 1 g / kg of body weight. It is then put under artificial respiration. A median thoracotomy is performed and the pericardium is cleared. The aortic arch is intubated with a cannula and then ligatured together with the collateral arteries.

The heart is then isolated and the free end of the intubation cannula is attached to the outlet of the multi-way valve of the perfusion of a Langendorff-type perfusion device.

The physiological solutions to be tested are maintained at a constant temperature of 37 °c and oxygenated using an aquarium bubbler. The isolated heart is perfused with the normal Mac Ewen-type (ME) physiological solution or test solution.

Pharmacodynamic Substances

The reference substances used in this study are: atropine (ATR) and atenolol (AT) from laboratory Sigma (St Louis, MO, USA), adrenaline (ADr), from the laboratory Renaudin (France).

These pharmacodynamic substances, as well as the aqueous extracts of *Parkia biglobosa*, are previously dissolved in a solution of Mac Ewen with glucose before the perfusion. It is composed in mM of: NaCl, 130.05; KCl, 5.63; CaCl₂, 2.16; NaH₂PO₄, 0.91; NaHCO₃, 11.90; MgCl₂, 0.53; glucose; 11, 11; PH = 7.4.

Statistical analysis

Data analysis was done using the software GraphPad Instat (San Diego CA USA). Statistical analysis of the results was carried out using the variance analysis (ANOVA) of the Tukey-Kramer multiple comparison tests. $P < 0.05$ is considered significant. All values are presented as mean \pm SEM.

The curves were plotted with mean values assigned to standard error on mean ($M \pm$ ESM) using GraphPad Prism version 4 (Microsoft, San Diego, California, USA).

III.Results

During these series of experiments, each AEPB concentration is tested on a single preparation in order to avoid the cumulative effects of the product.

Also, after each test a systematic "washing" is done in order to allow the return to the contractile activity of reference.

Beforehand, the stylus is calibrated with a mass marked 0.071g. Thus, a recording of one centimeter corresponds to 0.071 g/f (gram/force) for amplitude and 6s for frequency.

Dose-response effect of AEPB on heart contractions

The experiments were carried out at AEPB concentrations between 10^{-14} and 10^{-4} mg / ml. Measure are made following two (2) minutes of infusion.

AEPB, in the range of concentrations between 10^{-14} and 10^{-4} mg/ml, induces a dose-dependent decrease of the amplitude (negative inotropic) and the frequency (negative chronotropic) on the contractions of isolated rat heart. Thus at 10^{-14} , 10^{-8} and 10^{-4} mg/ml, the decreasing of the amplitude is 7%, 31% and 68% respectively. As for the frequency, it decreases to 9%, 17% and 58% respectively for the same concentrations.

It should be noted that the effects of AEPB are totally reversible in the chosen concentration range. Moreover, at the 10^{-8} mg / ml concentration, not only the negative inotropic and chronotropic effects are well marked but the reversibility is more rapid. That justifies the choice of this concentration for subsequent studies.

Figure 1, based on five (5) experiments, shows the mean variations in the amplitude and frequency of contractions of the isolated rat heart as a function of the increasing doses of AEPB. For the amplitude and the frequency, two sigmoid curves having the same appearance are observed. These curves permitted to determine the values of the EC_{50} which are $1.2 \cdot 10^{-8}$ and $1.6 \cdot 10^{-6}$ mg/ml respectively for the amplitude and the frequency of the contractions.

From this dose-response study, AEPB, in the range of concentrations between 10^{-14} and 10^{-4} mg / ml, induces dose-dependent negative inotropic and chronotropic effects on the isolated rat heart.

Interaction atropine - AEPB on the mechanical activity of the isolated heart of rat.

The results of three (3) experiments are consigned in the **table I**.

AEPB alone, at 10^{-8} mg/ml induces a decrease in the amplitude and frequency of heart contractions (negative inotropic and chronotropic effects) of $26.33\% \pm 2.91$ and $22.33\% \pm 3.13$ respectively.

In the presence of atropine 10^{-5} mg/ml, the negative inotropic and chronotropic effects induced by AEPB persist and are $25.33\% \pm 2.33$ and $32.33\% \pm 2.85$ respectively.

Thus, atropine does not inhibited the negative inotropic and chronotropic effects of EAPB.

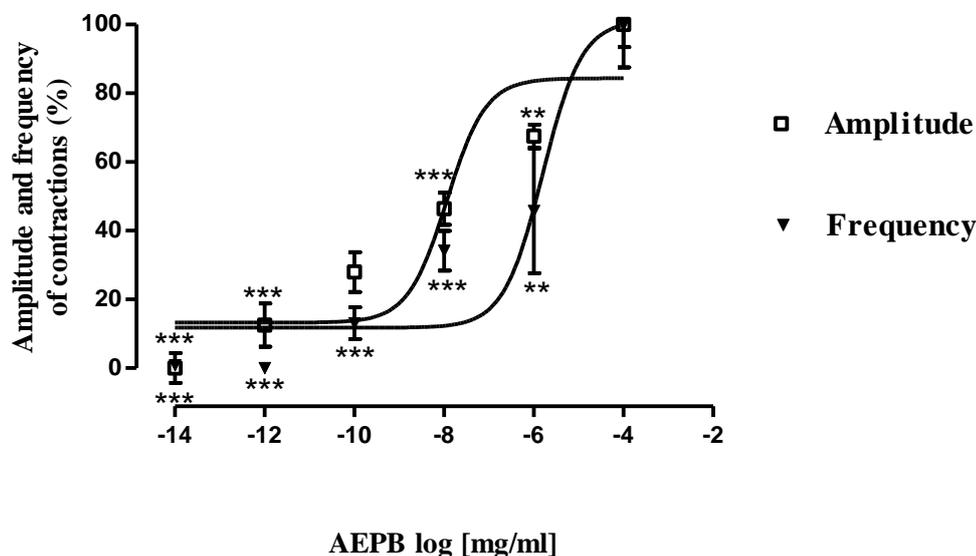


Figure 1: Curves of percentage of diminution of amplitude and frequency of heart contractions as a function of the concentration of AEPB

(Mean± ESM; **p<0.01; ***p<0.001; n=5)

Table I: Interaction atropine - AEPB on the isolated heart of rat.

Substances	AEPB (10^{-8} mg/ml) alone	AEPB(10^{-8} mg/ml) + ATR (10^{-5} mg/ml)
Percentage of decreasing		
Amplitude	26.33 ± 2.91	25.33 ± 2.33
Frequency	22.33 ± 3.53	32.33 ± 2.85

Influence of AEPB on the contraction induced by adrenaline

In this study, adrenaline is first perfused alone. Figure 2 shows at A, a control record, followed by heart perfusion with adrenaline at 10^{-7} mg / ml alone. Adrenaline induces an increase in the amplitude and frequency of heart contractions of 27% and 18%, respectively.

The perfusion of the heart with AEPB at 10^{-8} mg / ml and adrenaline at 10^{-7} mg / ml in the same mixture (Figure 2B) causes a decrease in amplitude and frequency of heart contractions of 13% and 29% respectively.

We note that AEPB cancels the adrenaline-induced positive inotropic and chronotropic effects

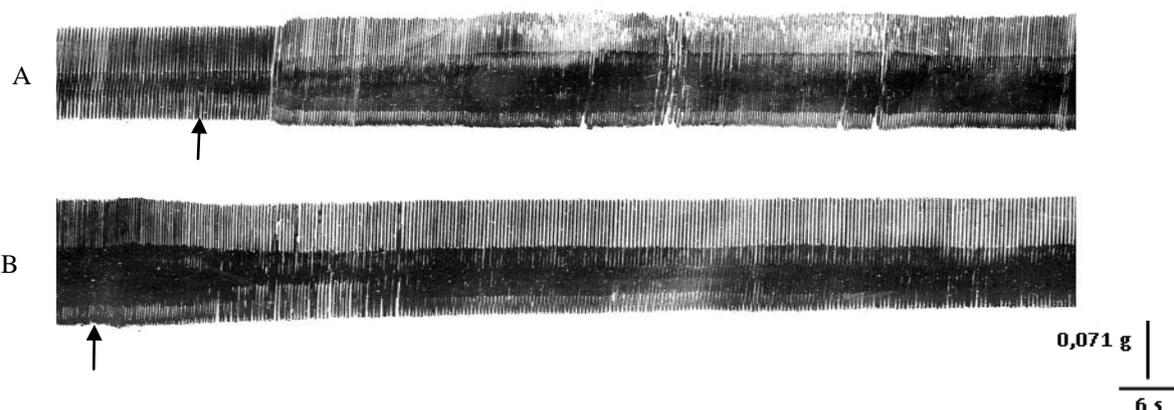


Figure 2: Influence of AEPB on adrenaline-induced contractile activity of isolated rat heart

A: Normal recording before the arrow followed by adrenaline only

B: Normal recording before the arrow followed by adrenaline 10^{-7} mg / ml and AEPB 10^{-6} mg/ml (mixture)

Influence of atenolol on the contraction induced by adrenaline

The injection of adrenaline induces an increase in amplitude and frequency as demonstrated in the previous experiment.

In this series of experiments, atenolol is added to the adrenaline solution and the mixture is injected into the heart.

The perfusion of the heart with the atenolol 10^{-6} mg / ml and adrenaline 10^{-7} mg / ml (Figure 3) cancels the effects induced by adrenaline alone. There was even a slight decrease in the amplitude and frequency of heart contractions of 13% and 11%, respectively.

IV. Discussion

Our results show an important activity of the aqueous extract of *Parkia biglobosa* (AEPB) on the functioning of the isolated heart of rat. Indeed, AEPB induces negative inotropic and chronotropic dose-dependent effects.

This cardiodepression is due to the presence of alkaloids in this extract according to the work of Kassi et al. [11]. In fact, alkaloids induce bradycardia [12, 13].

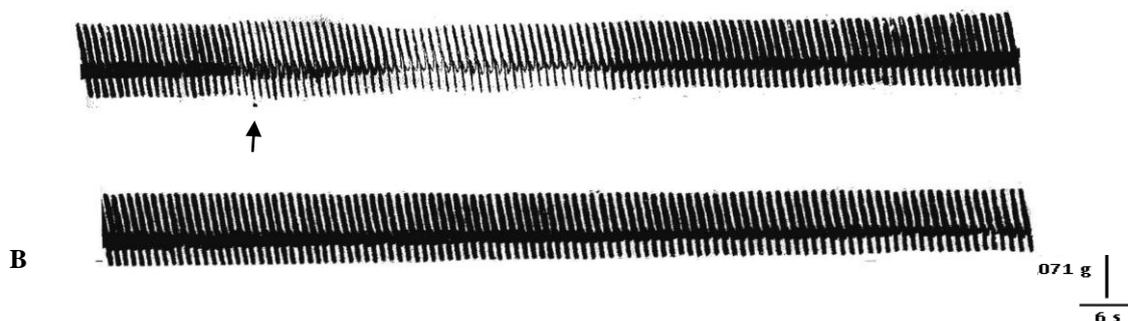


Figure 3: Influence of atenolol on adrenaline-induced contractile activity of isolated rat heart

A: Normal recording before the arrow followed by adrenaline 10^{-7} and atenolol at 10^{-6} mg / ml mixture

B: continuation of A

Moreover, these cardiodepressor effects of (AEPB) are comparable to those of acetylcholine (ACh) on the isolated heart already reported by several authors [14, 15, 16, and 17].

Acetylcholine (ACh) released on vagal stimulation reduces the heart rate by increasing K^+ conductance of pacemaker cells in the sinoatrial (S-A) node. Fluctuation analysis of ACh-activated currents in pacemaker tissue showed this to be due to opening of a separate class of K^+ channels gated by muscarinic receptors [18, 19].

This suggests that AEPB would behave as a cholinergic substance. However, the negative inotropic and chronotropic cardiodepressant effects induced by this substance are not inhibited by atropine. Yet, the presence of muscarinic cholinergic receptors in the mammals heart is known [20, 21, 22].

Consequently, the cardioinhibitory action of AEPB would not be mediated by muscarinic cholinergic receptors. Cardiodepressor effects independent of muscarinic cholinergic receptors were reported by Gauthier et al. [23, 24] and Heidecker et al. [25].

AEPB would therefore act via other receptor sites through which it would cause a decrease in calcium influx resulting in a reduction in the contractile force of the myocardium [26, 27].

On the other hand, it was observed that AEPB cancels the adrenaline-induced inotropic and chronotropic effects as well as atenolol.

According to Herberg et al. [28], atenolol is a reference antagonist β_1 of cardioactive substances.

Indeed, it is known that two types of β -adrenergic receptors (β_1 and β_2) exist in the heart [29, 30].

However, cardiac β_1 adrenergic receptors are the most common. There would be approximately 80% adrenergic β_1 receptors and 20% adrenergic β_2 receptors [31].

Moreover, the cardiostimulating action of adrenaline is due to a stimulation of cardiac β_1 receptors resulting in an increase in calcium influx [32].

In addition, the cardiostimulatory action of adrenaline is due to a stimulation of the cardiac β_1 receptors which results in an increase in the activity of the adenylylase and the concentration of cAMP in the cell resulting in an increase of calcium influx, therefore the concentration of cytosolic calcium [31, 33 and 34]. The results obtained with adrenaline in the presence of atenolol favor the blocking of β_1 -adrenergic receptors.

It can therefore be suggested that the inhibition of the cardiostimulating action of adrenaline in the presence of EAPB would be due to a blockade of the cardiac β_1 receptors by this substance. This β -blocking activity was revealed by Kassi et al. [8] investigating the comparative effect of AEPB and propranolol (a β -adrenergic receptor blocker) on adrenaline-induced hypertension. EAPB is therefore an adrenergic type β_1 antagonist

V. Conclusion

Our results show that the aqueous extract of *Parkia biglobosa* (EAPB) contains cardiodepressant active principles. However, this muscarinic non-cholinergic cardiodepression would be mediated by a blockade of β_1 -adrenergic cardiac receptors.

This action could explain thus the use of this plant in traditional African medicine to treat cardiac disorders and hypertension.

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