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ANTICALCIC EFFECT 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

Objective: *Parkia biglobosa* (Mimosaceae) is a plant used in African traditional medicine to treat hypertension and heart disorders. Cardiac activity is underlain by particularly calcium ionic flow through the myocardial membrane. We undertook this study of the effects of an aqueous trunk bark extract of *Parkia biglobosa* (EAPB) on the isolated heart of rat in modified physiological solutions in order to investigate its influence on the transmembrane transport of the calcium ion which is the main activator of the heart muscle or muscle contraction. **Methods:** The spontaneous contractile activity of the isolated rat heart is recorded in normal physiological solution and modified physiological solutions with a Langendorff- type device. **Results:** An aqueous extract of Parkia biglobosa (EAPB), at concentrations between 10^{-14} and 10^{-4} mg / ml induces cardiodepressant effects (negative inotropic and chronotropic effects) dose dependent. EAPB inhibits the positive inotropic effect induced by hypercalcic, hyposodic or hypopotassic physiological media that raises the calcium level; which allows to affirm that this substance is a calcium influx inhibitor or a slow calcium channel or voltage-dependent blocker (L-type channels). This characteristic is confirmed by the accentuation of the inhibitory action of EAPB in hypocalcic physiological medium, which reduces the calcium influx, and consequently the concentration of cytosolic calcium. **Conclusion**: This study shows that EAPB exerts on the isolated heart an anti-calcic effect responsible for its cardiodepressor effects.

KEYS-WORDS: *Parkia biglobosa*, isolated rat heart, calcium antagonist effect, cardiodepression.

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, ascaridiosis, 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.

We undertook this study of the effects of an aqueous trunk bark extract of *Parkia biglobosa* (EAPB) on the isolated heart of rat in modified physiological solutions in order to investigate its influence on the transmembrane transport of the calcium ion which is the main activator of the heart muscle or muscle contraction.

MATERIAL AND METHODS

Biological material

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

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* (EAPB), 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.

Physiological solutions

In this mammalian study, Mac Ewen was used as a physiological solution. For our manipulations we used so-called normal physiological solutions and modified physiological solutions.

The mM composition of normal Mac Ewen is as follows: NaCl, 130.05; KCl, 5.63; CaCl2, 2.16; NaH2PO4, 0.91, NaHCO3, 11.90; MgCl 2, 0.53; glucose; 11.11; The pH of this solution is adjusted to 7.4. The aqueous extract of Parkia biglobosa is previously dissolved in a solution of Mac Ewen with glucose before the perfusion.

The modified physiological solution has the same ionic composition as the normal Mac Ewen; only the concentration of the concerned or specific ion is modified.

Thus 75% hypocalcic, 85% hyposodic and 75% hypopotassic solutions are obtained by reducing the respective amounts of calcium, sodium and potassium of the normal Mac Ewen solution by 75%, 85% and 75%. The 125% hypercalcic solution is obtained by increasing the amount of calcium in the normal Mac Ewen solution by 125%.

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 ± SEM.

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

RESULTS

During these series of experiments, each EAPB 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 EAPB on heart contractions

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

EAPB, 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 isolated rat heart contractions. 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 EAPB 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 EAPB. 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 10^{-8} and 1.6 10^{-6} mg/ml respectively for the amplitude and the frequency of the contractions.

From this dose-response study, EAPB, 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.

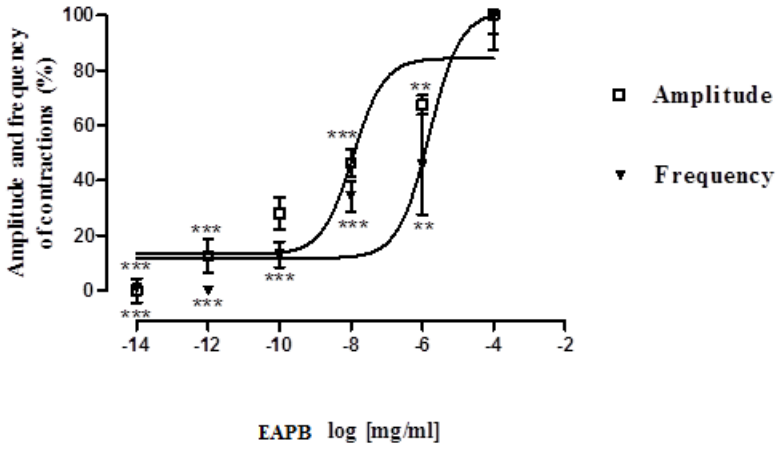

Figure 1: Curves of percentage of diminution of amplitude and frequency of heart contractions as a function of the concentration of EAPB.
(Mean± ESM; **p<0.01; ***p<0.001; n=5)

Effects of EAPB on the rhythmic and spontaneous contractions of the isolated rat heart in hypocalcic medium

Figure. 2, based on three (3) experiments, shows the average variations of the amplitude and the frequency of the contractions of the isolated rat heart in hypocalcic medium 75% alone and in hypocalcic medium 75% containing EAPB (10^{-8} mg / ml).

Perfusion of heart with a hypocalcic solution 75% causes negative inotropic and chronotropic effects. In fact, the decreases of the amplitude and the frequency of cardiac contractions are respectively 31 ± 2.08% and 10.33 ± 1.45%.

In a hypocalcic medium 75% containing EAPB (10^{-8} mg / ml), the negative inotropic and chronotropic effects initially induced by the hypocalcic medium are significantly accentuated ($p < 0.05$). Thus, the decreases of the amplitude and the frequency of cardiac contractions are respectively 63.33 ± 8.82% and 15.33 ± 1.45%. EAPB thus, accentuates the negative inotropic effect induced by the hypocalcic solution.

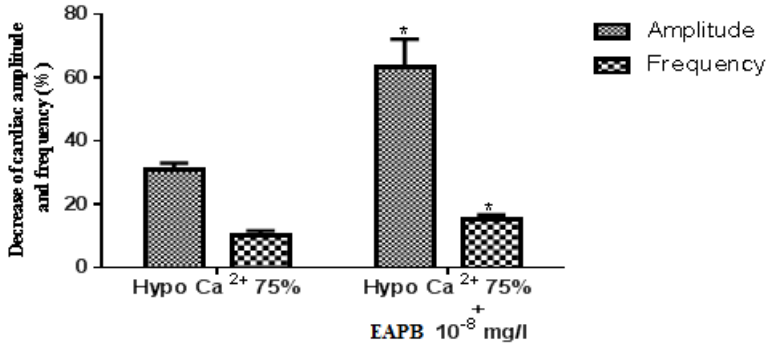

Figure 2: Decrease of cardiac amplitude and frequency in low calcium medium in absence and presence of EAPB.
*p<0.05 ; n=3

Effects of EAPB on the rhythmic and spontaneous contractions of the isolated rat heart in hypercalcic medium

Figure. 3, based on three (3) experiments, shows the average variations of the amplitude and the frequency of the contractions of the isolated rat heart in hypercalcic medium 125% alone and in hypocalcic medium 125% containing EAPB (10^{-8} mg / ml).

Perfusion of heart with a hypercalcic solution 125% causes positive inotropic and chronotropic effects with an increase of the amplitude and the frequency of contractions respectively 51.66 ± 5.90% and 37.33 ± 7.84%.

When the heart is perfused with a hypercalcic solution 125% containing EAPB (10-^{8} mg / ml), the positive inotropic and chronotropic effects induced by the hypercalcic medium are extremely reduced. Thus, the increases of the amplitude and the frequency of cardiac contractions are only 2.00 ± 0.29% and 13.00 ± 0.57%.

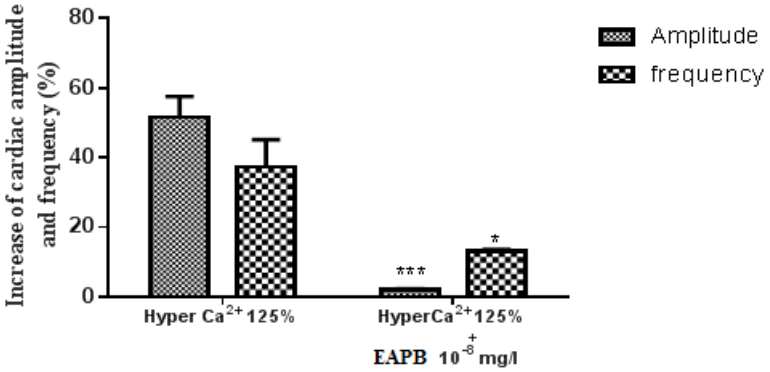

Figure 3: Increase of cardiac amplitude and frequency in high calcium medium in absence and presence of EAPB.
***p<0.05; ***p<0.001; n=3**

Effects of EAPB on the rhythmic and spontaneous contractions of the isolated rat heart in hyposodic medium

In figure 4A, the perfusion of the heart with a hyposodic physiological solution (85% NaCl) results in a transient increase of the amplitude of 15 ± 0.58% followed by a decrease of the amplitude and the frequency of cardiac contractions of 10 ± 2.71% and 17 ± 1.58% compared to their initial values.

When the isolated rat heart is perfused with a hyposodic physiological solution (85% NaCl) containing EAPB at 10^{-8} mg / ml (figure 4B), the transient increase of the amplitude (transient positive inotropic effect) induced by the hyposodium medium (85% NaCl) is completely suppressed. On the other hand, a concomitant decrease of the amplitude and frequency of cardiac contractions of 18 ± 1.33% and 23 ± 0.76% is observed respectively. EAPB cancels the transient positive inotropic effect induced by the hyposodic solution.

3.5 - Effets de EAPB sur les contractions rythmiques et spontanées du cœur isolé de Rat en milieu hypopotassique

Effects of EAPB on the rhythmic and spontaneous contractions of the isolated rat heart in hypopotassic medium

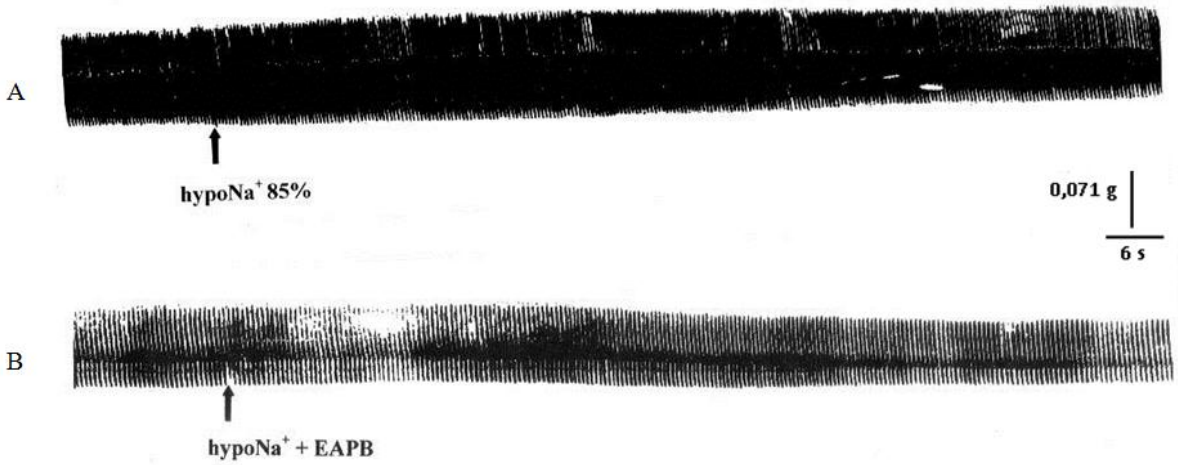

Figure 4: Effects of medium hyposodic alone and of EAPB in medium hyposodic on spontaneous contractile activity of isolated heart of rat.

A: normal recording before the arrow followed by the effect of medium hyposodic 85%

B: normal recording before the arrow followed by the effect of EAPB in medium hyposodic 85%

Perfusion of heart with a hypopotassic solution 75% (Figure 5A) causes a transient increase of the amplitude of cardiac contractions of 94 ± 1.15%.

In the presence of EAPB at 10^{-8} mg / ml (figure5B), the positive inotropic effect induced by the hypopotassic medium is completely suppressed. Statistically significant negative inotropic and chronotropic effects are noted ($p < 0.001$). Thus, the amplitude and the frequency of the cardiac contractions decrease to 25 ± 0.58% and 15.66 ± 0.88% respectively.

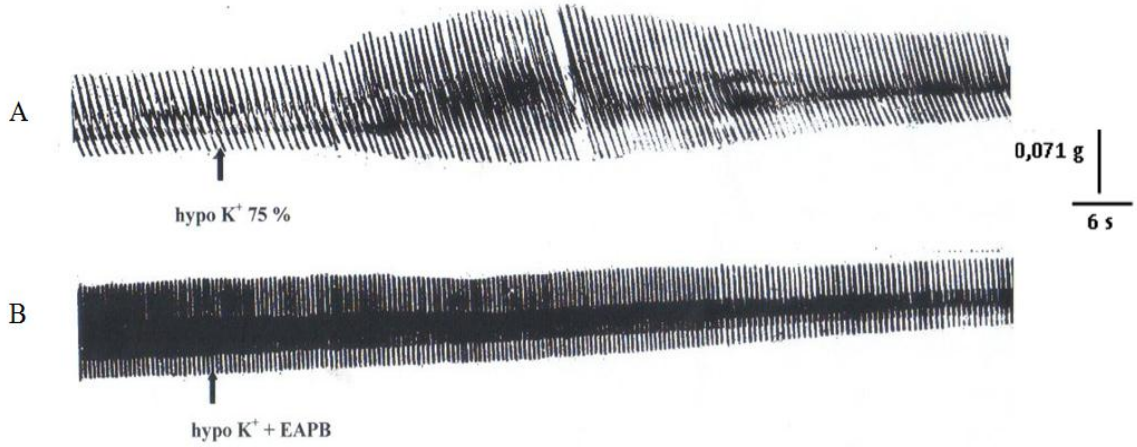

Figure 5: Effects of medium hypopotassic alone and of EAPB in medium hypopotassic on spontaneous contractile activity of isolated heart of rat.

A: normal recording before the arrow followed by the effect of medium hypopotassic 75%

B: normal recording before the arrow followed by the effect of EAPB in medium hypopotassic 75%

DISCUSSION

Our results show that aqueous extract of *Parkia biglobosa* (EAPB) causes dose-dependent negative inotropic and chronotropic effects on rat isolated heart.

These effects are similar to those obtained on the isolated rat heart with plant extracts such as the crude aqueous extracts of *Bidens pilosa* and *Heliotropium indicum*[10,11], the methanolic extract stem bark of *Erythrema senegalensis*[12] and the F2 fraction of the aqueous extract of *Bidens pilosa*.[13]

The cardiodepressor effects of EAPB would be due to the presence of alkaloids in this extract according to the work of kassi et *al.*[14] In fact, alkaloids induce bradycardia.[15,16]

In addition, Kassi and *al.*[8], studying the EAPB - Nifédipne (a calcium channel blocker of slow calcium channel or voltage-gated calcium channels, L type

channel) interaction on rabbit blood pressure, found EAPB enhancement or potentiation of hypotensive effect of Nifedipne or the character of calcium channel blocker of EAPB.

To know if the cardioinhibitor effect of EAPB is due for a possible purpose anticalcic, a series of experiments in modified physiological solutions was carried out.

Our results revealed a suppression of the transient positive inotropic effect observed in hyposodic medium by EAPB.

However, this transient increase in contractile force of the myocardium is underlain by a transient increase in calcium influx.[17-20]

Thus, the suppression of the transient positive inotropic effect observed in a hyposodic medium by EAPB confirms the fact that this substance behaves like a calcium channel blocker.

Moreover, in a hypopotassic medium, there is an increase of the amplitude of cardiac contractions resulting from a sustained calcium influx[21,22], probably through the Na^+/Ca^+ exchange channel[20,23,24] to compensate for the decline in activity of the Na^+/K^+ pump.[25,26]

EAPB also cancels the positive inotropic effect up to the reduction of the reference amplitude. The frequency of contractions also decreases.

EAPB seems to act as a calcium influx inhibitor either only in the calcium influx by the voltage-gated calcium channel, or on the Na^+/Ca^+ exchanger, or certainly on both types of channels.

Thus, EAPB not only accentuates cardio-depression induced hypocalcic medium (which reduces calcium influx), but also reduced the myocardium contractile force induced by the hypercalcic medium which promotes increased calcium influx.[27-30]

This study shows that the action of EAPB involves the blocking of voltage-gated calcium channels, thus leading to an inhibition of calcium influx.[31-43]

Ultimately the reduction of the amplitude of the cardiac contractions and the inhibition of the positive inotropic effect observed in the hyposodic, hypopotassic and hypercalcic media by EAPB confirm well that this substance is an anti-calcium drug.

CONCLUSION

This study shows that EAPB exerts on the isolated heart an anti-calcic effect responsible for its cardiodepressor effects; which justifies the use of this plant in African traditional medicine to treat hypertension and heart disorders.

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