CALCIUM ANTAGONIST EFFECTS OF A CHROMATOGRAPHIC FRACTION FROM *BIDENS PILOSA* L. (ASTERACEAE) LEAF AQUEOUS EXTRACT ON ISOLATED RAT HEART

Kouakou Léandre Kouakou¹, Mathieu Nahounou Bléyéré*, Augustin Kouao Amonkan², André Brou Konan², Jean Claude Kouakou Abo³, Paul Angoué Yapo¹, Etienne Ehouan Ehilé²

¹Laboratory of Physiology, Pharmacology and African Pharmacopoeia of UFR-SN, University of Nangui Abrogoua, Côte d’Ivoire.
²Laboratory of Pharmacology and Nutrition of UFR-Biosciences, University of Felix Houphouet Boigny, Cocody, Côte d’Ivoire.
³Laboratory of Animal Physiology of UFR-Biosciences, University of Felix Houphouet Boigny, Cocody, Côte d’Ivoire.

ABSTRACT

*Bidens pilosa* L. (Asteraceae) is a plant commonly used in traditional medicine to treat several ailments among which hypertension. The aim of this study was to investigate anti-calcic effects of a chromatographic fraction from the leaf aqueous extract of *Bidens pilosa* (BpF₂) on isolated rat heart. The contractions of isolated rat heart were recorded in control conditions and in the presence of BpF₂ in different physiological solutions with a modified Langendorff (1895) apparatus. BpF₂ induced negative inotropic and chronotropic effects on isolated rat heart preparations for concentrations ranging from $10^{-12}$ to $10^{-4}$ mg/ml. BpF₂ significantly influenced the modified media namely calcium depleted, hypercalcic, hyposodic and hypopotassic Mac Ewen solutions. It also significantly attenuated the positive inotropic and chronotropic effects of adrenaline. These results suggested a possible calcium antagonist action of BpF₂ on rat isolated heart.

Key words: Bidens pilosa, Calcium antagonist, Isolated rat heart, Inotropic and chronotropic effects.

INTRODUCTION

Traditional medicine has been practiced for years in developing countries, especially in Africa. According to OMS (2002), this use is widespread and is becoming increasingly important in terms of health and economy, since more than 80% of the population resort to it for health needs. According to the same source, in Asia and Latin America, people still use traditional medicine because of historical circumstances and cultural beliefs. *Bidens pilosa* L. is an herbaceous plant, set up, with regularly cogged and glabrous leaflets. Very widespread pantropical species from Angola to Cameroon, it is also found in Côte d’Ivoire (Bouquet and Debray, 1974; Adjanohoun and Ake Assi, 1979). The plant is used in traditional medicine to treat diverse illnesses such as diarrhoea and inflammation in Côte d’Ivoire (Bouquet and Debray, 1974; Adjanohoun and Ake Assi, 1979) and is prescribed as anti-poison, against flu and haemorrhoids in Congo and Rwanda (Boullard, 2001). Many works carried out on this plant revealed different properties among which anti-microbial, anti-inflammatory (Geissberger and Sequin, 1991), anti-bacterial (Rabe and Van Staden, 1997), anti-malarial...
(Brandao et al., 1997) and anti-gastric ulcer effects (Alvarez et al., 1999; Tan et al., 2000).

The cardiovascular aspect was also subject to many studies (Dimo et al., 1998; Dimo et al., 1999, Nguelefack et al., 2005; Kouakou et al., 2007; Kouakou et al., 2008a and 2008b). Kouakou et al., (2008a and 2008b) showed that a chromatographic fraction from Bidens pilosa L. leaves (BpF2) elicited a decrease of the blood pressure of rabbit via cholinomimetic and β-adrenomimetic agonist substances. The phytochemical study of the fraction revealed flavonoids, polyphenols and catechic tannins.

This study was aimed to evaluate calcium antagonist actions of a chromatographic fraction from the leaves of Bidens pilosa L. (BpF2) on isolated rat heart.

MATERIALS AND METHODS

Animals

Rats (Ratus norvegicus) weighing between 180 and 250 g were used. They were bred in Animal house of Animal Physiology, Pharmacology and Phytotherapy of the University of Nangui Abrogoua (Former University of Abobo-Adjamé, Abidjan, Côte d’Ivoire) according to the principles for the care and use of laboratory animals of the Ethical Committee of the University (Nangui Abrogoua, Abidjan, Côte d’Ivoire).

Plant material

The plant material was described by Kouakou et al. (2008a and 2008b). Authentication of fresh leaves of Bidens pilosa L. (Asteracea) collected from Abidjan, Côte d’Ivoire was implemented by Prof. Aké-assi Laurent, an expert in Botany (Department of Botany, University of Cocody, Abidjan, Côte d’Ivoire). In Côte d’Ivoire, Bidens pilosa L. was first found on November 12, 1966 in Bondoukou and herbarium specimen (voucher n° 9266) was made and deposited at the National Botanic Centre (University of Cocody, Abidjan, Côte d’Ivoire) and then in Kankan (Côte d’Ivoire) on June 20, 1968. Herbarium specimen (voucher n° 10286) was also made and deposited at the National Botanic Centre (University of Cocody, Abidjan, Côte d’Ivoire).

Plant extraction

The extraction and the separation of extracts were as previously described by Kouakou et al. (2008a and 2008b). Hundred grams (100g) of powder from Bidens pilosa L. leaves dried at room temperature were macerated under magnetic shaker during 48h in 2 L of distilled water. The supernatant was filtered on cotton and filter paper Whatman. Two litres of distilled water were added to the base and then mixed during two hours and also filtered. The filtrates were freeze-dried using a lyophilisator TELSTAR (Terrassa, Spain). 0.8 g of the powder obtained were dissolved in 10 ml of distilled water and chromatographed on a fine Sephadex G25 column (3 by 20 cm) packed in distilled water. Elution was carried out with the same solvent. Fractions (5) of 20 ml each were collected then freeze-dried. They were tested on the blood pressure of the rabbit. The fraction 2 (BpF2) was found to be the most active.

Chemicals

Adrenaline was purchased from Sigma Chemical Company (St Louis, MO, USA).

Recording of the isolated rat heart activity

The experimental process used to record and assess the isolated rat inotropic and chronotropic activities was as previously described by Kouakou et al., (2007), Tchikaya et al. (2011) and Atsamo et al., (2013). Rat was anesthetized with intraperitoneal injection of 20% ethyl urethane at 1 g/kg body weight. It was then placed under artificial respiration to avoid anoxia of the heart during operation. To do this, a tracheotomy was performed and a cannula connected to an air pump was placed in the trachea. Then, a thoracotomy was practiced. A hemisection of the aorta was carried out. A cannula fixed to a syringe containing heparinized solution (2500 UI, 0.2 ml/100 g body weight) was inserted in the aorta. The heart was rapidly removed and the heparinized solution injected to dissolve and expel any blood clots that were probably formed in the heart to prevent thrombosis in the coronary circulation. The apex of the heart was fixed by a fine clip and linked to the needle of the modified Langendorff (1895) apparatus for recording. After each treatment, the heart was washed by perfusion fluid for 10 min, time within which the baseline recording was achieved, and the second dose was then given. The recording before the direct perfusion of extract was considered as baseline reading for each dose (control). BpF2 was dissolved in Mac Ewen solution. The isolated heart was perfused with Mac Ewen solution of the following composition (mM): NaCl 130; KCl 2.5; CaCl2 2.4; NaH2PO4 1.18; CO3NaH 11.9; MgCl2 0.24; C6H12O6 2.2 with a pH adjusted to 7.4. The modified media had the same composition as the normal Mac Ewen solution except for the concentration of specific ions which changed: calcium-depleted medium 75% contained 1.8 mM of CaCl2 instead of 2.4; calcium-enriched medium 125% contained 3 mM of CaCl2 instead of 2.4; low potassium medium 75% contained 1.875 mM of KCl instead of 2.5 and hyposodic medium 85% contained 110.5 mM of NaCl instead of 130.

Data analysis

Statistical analysis and graphics were performed with the software GraphPad Instat and GraphPad Prism 4 (San Diego California, USA) respectively. All values are expressed as mean ± standard error on the mean (n ±sem). The differences observed between the values were precised by an analysis of variances (ANOVA) of the
multiple test of comparison of Turkey-Kramer and were considered statistically significant when p < 0.05.

RESULTS

Effect of BpF₂ in normal Mac Ewen solution

The dose-response effect of BpF₂ was achieved when the heart was perfused with a normal Mac Ewen solution. This resulted in a dose-dependent decrease of the cardiac amplitude and frequency compared to control recordings, for concentrations ranging from 10⁻¹² to 10⁻⁴ mg/ml. In this range of concentrations, the decrease of amplitude varied from 11.18 ± 0.93% to 35.30 ± 1.01% and the decrease of the frequency attained 8.98 ± 0.59% to 42.29 ± 2.77%. These effects were reversible after returning to Mac Ewen reference solution. The dose-response curves (Figure 1A and B) permitted to determine the values of EC₅₀ which were 1.98 x 10⁻¹⁰ mg/ml and 1.95 x 10⁻¹⁰ mg/ml respectively for the amplitude and the frequency.

Effect of BpF₂ in high calcium Mac Ewen medium

Perfusion of the heart with a hypercalcic Mac Ewen solution 125% induced positive chronotropic and inotropic effects with an increase of 37.10% ± 1.43 for the amplitude and 22.61 ± 0.84% for the frequency compared to normal recordings. When the heart was perfused with the hypercalcic Mac Ewen medium 125% containing the active fraction BpF₂ at 10⁻⁴ mg/ml, there was a sharp and significant (p < 0.05) decrease in the positive chronotropic and inotropic effects induced by the hypercalcic Mac Ewen solution 125%. The increase of the amplitude and the frequency of the heart contractions attained only 2.19 ± 1.32% and 1.80 ± 1.09% respectively (Figure 2).

Effect of BpF₂ in calcium-depleted Mac Ewen solution

Perfusion of the heart with a hypocalcic Mac Ewen solution 75% caused negative inotropic and chronotropic effects compared to normal recordings. Indeed, drops of amplitude and frequency of heart contractions were respectively 29.15 ± 1.61% and 11.16 ± 1.58%. In the presence of the hypocalcic Mac Ewen solution 75% supplemented with BpF₂ at 10⁻⁴ mg/ml, the negative inotropic and chronotropic effects initially induced by the hypocalcic Mac Ewen solution 75% were significantly (p < 0.05) accentuated. Thus, the decrease of the amplitude and the frequency of heart contractions in presence of the mixture (hypocalcic solution + BpF₂) attained 40.31 ± 0.93% and 34.78 ± 2.09% respectively (Figure 3).

Effect of BpF₂ in a low potassium Mac Ewen solution

Positive inotropic and chronotropic effects were recorded when the isolated rat heart received the administration of low potassium Mac Ewen solution 75%. The amplitude and the frequency of heart contractions augmented to 39.16 ± 1.50% and 20.71 ± 1.84% respectively compared to control recordings. When BpF₂ at 10⁻⁴ mg/ml was added to the hypopotassic Mac Ewen solution 75% and perfused to the isolated heart, the positive inotropic and chronotropic effects induced by the hypopotassic Mac Ewen solution 75% were completely inhibited. Moreover, this solution elicited significant (p < 0.05) negative inotropic and chronotropic effects. Indeed, the decreases registered were 8.30 ± 2.42% and 17.02 ± 2.69% respectively for the amplitude and the heart rate (Figure 4).

Effect of BpF₂ in hyposodic Mac Ewen solution

The hyposodic Mac Ewen solution 85% triggered transient increases of the amplitude of 21.14 ± 1.44% followed by decreases of the amplitude and the frequency of 12.93 ± 1.02 and 19.72 ± 1.72 respectively compared to control recordings. The addition of BpF₂ at 10⁻⁴ mg/ml to the hyposodic Mac Ewen solution 85% completely suppressed the transient positive inotropic effects induced by the medium (Table 1). However, the negative inotropic and chronotropic effects caused by the hyposodic Mac Ewen solution 85% were very little and not significantly affected by BpF₂ in the medium. So, the amplitude and the frequency diminished to 13.26 ± 0.87% and 18.29 ± 0.93% respectively.

Effect of BpF₂ on positive actions induced by adrenaline

As shown in figure 5, adrenaline solution at 10⁻¹⁰ mg/ml caused positive inotropic and chronotropic effects on isolated rat heart. The amplitude and frequency increased to 31.59 ± 1.84% and 50.92 ± 2.69% respectively compared to the control recordings. When the heart was perfused with a mixture of adrenaline at 10⁻¹⁰ mg/ml and BpF₂ at 10⁻⁴ mg/ml, the positive chronotropic effects induced by adrenaline completely vanished and small inotropic effects only persisted. In addition, the mixture was found to diminish the positive chronotropic effect under the control recordings. Thus, the amplitude induced by the mixture only increased to 3.84 ± 1.81% and the frequency decreased to 13.39 ± 1.22%. These changes were significant (p < 0.05).

Table 1. Effect of BpF₂ on the transient positive inotropic effect induced by the sodium-depleted Mac Ewen solution

<table>
<thead>
<tr>
<th>Sodium-depleted medium 85%</th>
<th>Sodium-depleted medium 85% + BpF₂ 10⁻⁴ mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transient increase of cardiac amplitude</td>
<td>21.14 ± 1.44%</td>
</tr>
</tbody>
</table>

The sodium-depleted medium caused transient inotropic effect. This effect was completely and significantly (*p < 0.05, n = 4) reduced in presence of BpF₂.
Fig 1. Dose-response effect of BpF₂ on isolated rat heart perfused with Mac Ewen solution

A

Increasing concentrations of BpF₂ induced a significant (*p < 0.05, n = 4) and dose-dependent decrease of the amplitude (A) and the frequency (B) of the cardiac contractions. The EC₅₀ determined graphically were 1.98 x 10⁻¹₀ mg/ml and 1.95 x 10⁻⁸ mg/ml respectively for the amplitude and the frequency.

Fig 2. Increase of cardiac amplitude and frequency in high calcium Mac Ewen solution in absence and presence of BpF₂

The calcium-enriched solution caused an increase of cardiac amplitude and frequency. The addition of BpF₂ to this solution significantly (*p < 0.05, n = 4) reduced the positive inotropic and chronotropic effects induced by the high calcium medium.

Fig 3. Decrease of cardiac amplitude and frequency in calcium-depleted Mac Ewen solution in absence and presence of BpF₂

The calcium-depleted solution elicited a drop of cardiac amplitude and frequency. The supplementation of BpF₂ to this solution significantly (*p < 0.05, n = 4) accentuated the negative inotropic and chronotropic effects induced by the calcium-depleted medium.
Fig 4. Variation of cardiac amplitude and frequency in low potassium Mac Ewen solution in absence and presence of BpF$_2$

The low potassium medium caused an augmentation of cardiac amplitude and frequency. In presence of BpF$_2$, the positive inotropic and chronotropic effects induced by the potassium-depleted medium were suppressed and negative inotropic and chronotropic effects appeared. These changes were significant (*p < 0.05, n = 4).

Fig 5. Variation of cardiac amplitude and frequency induced by adrenaline in absence and presence of BpF$_2$

Adrenaline induced positive inotropic and chronotropic effects. In presence of BpF$_2$, these effects were significantly (*p < 0.05, n = 4) inhibited for the amplitude and the frequency even decreased (negative chronotropic effect).

**DISCUSSION**

BpF$_2$ caused a dose-dependent decrease of the amplitude and the frequency of the isolated rat heart contractions. These effects were similar to those obtained with plant extracts such as the aqueous-ethanolic extract of Achillea Millefolium (Niazzmand and Saberi, 2010), the aqueous stem bark extract of Anarcadium occidentale (Tchikaya et al., 2011), the methanol extract from the stem bark of Erythrina Senegalensis (Atsamo et al., 2013) and an alkaloid isolated from Crinum macowanii (Mugabo et al., 2012) in isolated rat heart. To investigate a possible action of BpF$_2$ as a calcium antagonist in the cardioinhibitory effect of the extract, a series of experimentations was undertaken. Like BpF$_2$, the hypocalcic medium induced a decrease of the amplitude and the frequency of heart contractions. Hypocalcic solution accentuated the action of BpF$_2$ while the positive chronotropic and inotropic effects of the hypercalcic medium were reduced by BpF$_2$. These results suggested the involvement of calcium in the mechanism of action of BpF$_2$. Strengthening effects of BpF$_2$ in the hypocalcic medium suggested that substances in this fraction could act by reducing calcium influx, since according to some authors, the contraction of the cardiac cell depends
largely on the entry of extracellular calcium into the cell and intracellular calcium mobilization (Kampman et al., 1980; Dresdner and Kline, 1985; Winslow et al., 2000; Kondo et al., 2006). The sharp reduction of positive chronotropic and inotropic effects caused by hypercalcic medium suggested a direct action of BpF₂ on calcium channels. This direct action could be due to anticalcic substances that would block extracellular calcium entry in the cardiac cell.

The effect of this fraction on the decrease in calcium influx was checked in presence of adrenaline. Indeed, adrenaline stimulates cardiac activity by increasing the amplitude and frequency of contractions (Furnival et al., 1971; Dukes and Vaughan Williams, 1984). This increase is due to the activation of a stimulating G protein (Rodbell, 1980; Gilman, 1987), which triggers the activation of adenylate cyclase. Then, adenylyl cyclase stimulates cAMP production, resulting in mobilization of extracellular and then intracellular calcium (Ju and Allen, 1999; DelPrincipe et al., 2000). By reducing the positive chronotropic and inotropic effects induced by adrenaline, BpF₂ could act on the mechanism of calcium mobilization.

The low sodium Mac Ewen solution elicited a transient increase of the amplitude of cardiac contractions as shown by Allen et al. (1983) and Ostádalová et al. (1995). BpF₂ suppressed transient increase caused by hyposodic Mac Ewen solution. This result once again showed the inhibitory effects of this extract on calcium current and suggested that this inhibition may also involve an action of BpF₂ on the Na⁺/Ca²⁺ exchanger by a reduction or inhibition of its activity, especially on calcium ion movements in membrane. Indeed, according to Verkhratskií et al., (1989) and Kolar et al., (1990), the well-known transient increase caused by low sodium medium is due to the action of the Na⁺/Ca²⁺ exchanger on sarclemma. According to Bers (1987), under normal conditions, the exchange system on the Na⁺/Ca²⁺ exchanger causes expulsion of calcium during the cardiac cycle. When the inward gradient of Na⁺ is reduced, the Ca²⁺ influx via the Na⁺/Ca²⁺ exchanger is augmented and the Ca²⁺ efflux is reduced during the cardiac cycle. Sheu and Fozzad (1982) and Kim et al., (1987) showed that the reduction of extracellular Na⁺ concentration induced an increase in the release of intracellular calcium. Rathi et al., (2004) showed that, in addition to the Na⁺/Ca²⁺ exchanger and release of intracellular calcium from the sarcoplasmic reticulum, the Na⁺/K⁺ ATPase pump could also be involved in the release of intracellular calcium in low sodium solution.

BpF₂ reduced the positive inotropic and chronotropic effects induced by hypopotassic Mac Ewen solution. These effects could be due to a possible action of this fraction on the Na⁺/K⁺ ATPase pump and the Na⁺/Ca²⁺ exchanger, resulting in an inhibition of calcium influx. It is known that the hypopotassic medium causes positive inotropic effects in heart (Christe, 1983; Barry et al., 1985; Boyett et al., 1986; White and Terrar, 1991). The inhibition of the Na⁺/K⁺ ATPase pump by hypopotassic medium could be the cause of the positive inotropic effect (Sheu and Fozzard, 1982; Barry et al., 1985; White and Terrar, 1991). Indeed, these authors argued that the inhibition of this pump led to an increase in intracellular sodium concentration, which in turn increased the cytosolic calcium. Barry et al. (1985) even talked of the involvement of the Na⁺/Ca²⁺ exchanger in the intracellular increase in calcium.

CONCLUSION

In conclusion, by inhibiting the effects of the modified Mac Ewen media and adrenaline, BpF₂ could act as a calcium antagonist on isolated rat heart. This action could explain the cardioinhibition induced by this fraction and thus the use of this plant in traditional medicine to treat hypertension.

ACKNOWLEDGEMENTS

The authors are thankful to all other members of Laboratory of Physiology, Pharmacology and Phytotherapy (Nangui Abrogoua University of Abidjan/Côte d’Ivoire) for their encouragement during these investigations.

REFERENCES


DelPrincipe F, Egger M, Niggli E. L-type Ca2+ current as the predominant pathway of Ca2+ entry during INa activation in β-stimulated cardiac myocytes. J Physiol. 2000; 527: 455-466.


Verkhratskii AN, Pronchuk NF, Tepikin AV. Effect of reduced extracellular level of sodium ions on the intracellular level of calcium ions in the cytoplasm of cultured rat cardiomyocytes. *Fiziol Zh.* 1989; 35: 45-49.
