SPASMOLYTIC EFFECTS OF AQUEOUS EXTRACT OF MIXTURE FROM *AFRAMOMUMUM MELEGUETA* (K SCHUM) – *CITRUS AURANTIFOLIA* (CHRISTM AND PANZER) ON ISOLATED TRACHEA FROM RAT

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Abstract

The spasmolytic properties of the aqueous extract of Aframomum *melegueta* (K Schum) and *Citrus aurantifolia* (Christm and Panzer) (AMCA) mixture were tested on isolated rat trachea. Inhibition of the contraction was observed the in presence of the AMCA (EC_{50} = 1.80 ± 0.48 mg/mL) after a pre contraction of the trachea by acetylcholine (10^{-5} M). With propranolol (10^{-6} M), the spasmolytic activity of the mixture was inhibited and the concentration-response curve shifted to the right. The EC_{50} value was then 2.60 ± 0.41 mg/mL. AMCA also inhibited contraction induced by KCl (4.10^{-2} M) with EC_{50} value = 1.86 ± 0.65 mg/mL. These results clearly show the relaxing effect of the aqueous extract on the isolated rat trachea. This effect involved some β-adrenergic receptor inhibition.

Key words: *Aframomum melegueta*; *Citrus aurantifolia*; extract; bronchodilatory; spasmolytic, Rat trachea.

Introduction

Aframomum melegueta (K Schum) is widespread in the forest areas of Gabon, Congo, Islands of the Gulf of Guinea, Cameroun and in Western Africa and it was cultivated in the shaded mediums (Kamtchouing et al.2002). In Africa, *Aframomum melegueta* is very popular in many cookings as condiment and is also used for ritual purposes. It is strongly used in traditional medicine and African pharmacopeia, in the treatment of several diseases such us intestinal infections (Kempkes, 1995) and heartburn; *Aframomun melegueta* also effective in indigestions due to the fact that it stimulates the gastric secretions. In the same way, it is an aphrodisiac (Umukoro and Ashorobi , 2003). In Benin, it is mixed with other plants for the treatment of several pathologies (Adjanohoun, 1986).

Citrus aurantifolia (Christm and Panzer) is cultivated everywhere in the world. The fruit is collected before maturity for food and medicinal use; It was used in particular against obesity, asthma, hypertension, and measles. In this work, we studied the spasmolytic effect of the total aqueous extract from the mixture of *Aframomum melegueta* (K Schum) and *Citrus aurantifolia* (Christm and Panzer) (AMCA) on the isolated rat trachea. This study aimed to identify the pharmacological properties justifying the traditional use of this mixture in the treatment of asthma.

Material and Methods

Plant materials

Aframomum melegueta was collected in Adjarra, locality in the Department of Ouémé in Benin in June 2009. The harvest of the sheets and fruits of *Citrus aurantifolia* was collected in August 2009 at Ouando located in the same department. The identification was made by the National Herbarium of the University of Abomey-Calavi of Benin. Each specimen was deposited at the University (Voucher N° AA6374/HNA for *Aframomum melegueta* (Roscoe) K. Schum. and AA6375/HNB for

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Citrus aurantifolia (Christm. et Panzer) Swingle. The total aqueous extract was prepared by mixing 125g of *Aframomum melegueta* seeds with the filtrate obtained from *Citrus aurantifolia* 125g of leaves and 290g of fruits in 1 liter of distilled water. After 5 hours of stirring, the mixture was filtered, centrifuged and lyophilized (Huet, 1991; Houghton and Amala, 1998).

Animals

Male Wistar rats (weight 288.02 ± 13.41 g) were used. The animals were fasted 18 hours before the test. They were then anaesthetized with urethane (1.5 g/kg of body weight intravenously). The respiratory tract was then and transferred in a sterile container containing a physiological solution from Krebs (Loenders et al., 1992). Wistar rats used in this study were handled according to the Institutional animal safety guidelines (University of Abomey Calavi, Benin and the Animal Company of Human Biology Unit, Health Science Faculty, University of Abomey Calavi, Benin). Animal experiments were approved by the National Health Ethics Committee of Benin (www.ethique-sante.org) under the following registration N°: 002_084/MS/DC/SGM/DFRS/-CNPERS/SA.

Solutions and drugs

The solution of Krebs used was made up of: NaCl, 6.9g; $NaHCO_3$, 2.02g; KCl (LABOSI P 1295 1 kg Batch 083151) 0.35g; KH_2PO_4, 0.16g; $MgSO_4$, 0.30g; $CaCl_2$, 0.37g; D-glucose, 0.6 g in 1L of distilled water. Drugs used were diluted in distilled water. It was about: acetylcholine (SIGMA A-2661 100 g Batch 35H07845) 10^{-5}M, propranolol 10^{-6} M, KCl 4.10^{-2} M; the cumulative dose of atropine were also used. The total aqueous extract of *Aframomum melegueta – Citrus aurantifolia* mixture was used with a cumulative amount after the period of balancing.

Preparation and mounting

The thoracic trachea was transected between the segments of cartilage (4 to 5 rings). These rings were then assembled in the isolated organ bath container of 20 ml from modified krebs solution at 37° C and bubbled with a mixture of oxygen (95%), carbon dioxide (5%)(Schramm, 2000). The ring of the trachea was fixed between one triangular support fixed inside the bath and the other one was connected to the transducer. The preparation was subjected to a tension ranging between 300 mg and 500 mg, during 1 h to obtain regular and spontaneous contractions (Van and Hulsmann , 1999). It was then balanced gradually to a tension of 1.5 g during 30 min. During these 90 min of incubation, a wash was carried out every 15 min. The transducer used for the recording was isometric of F50 type EMKA. It was connected to an analogical amplifier (EMKA). The graphic recording was obtained by the software of acquisition U-vessel (WAGNER University of Strasbourg, France).

Methods

AMCA was used with a cumulative concentration after the 90 min of equilibration period. The Drugs tested were introduced directly into the isolated organ bath using a micro syringe. The volume of administration did not exceed 5% of the volume of the bath (Kouloumenta et al., 2006). The first stimulation was carried out by an injection of acetylcholine (10^{-5}M) in the isolated organ tank. For a relaxation test, a second stimulation was done. When the contraction reached a maximal effect, the AMCA was added in a cumulative concentration manner. In order to check an implication of the β-adrenergic receptor in the relaxing activity of the extract, propranolol (10^{-6} M) was used. The anti-spasmolytic effect of atropine was examined as a reference. This drug was added in cumulative pattern into the bath 15 min after the administration of acetylcholine. The Effect of AMCA was expressed as a percentage (%) of relaxation. The concentration-response curves were plotted and the effective concentrations of 50% were noted.

Statistical analysis

Concentration-response curves and EC_{50} were determined by GraphPad Prism version 2.01 Demo.. The student's t - test was used for the comparisons between each group. The data were expressed in the form of mean ± SEM. The probability of (p) error was regarded as significant with $p < 0.05$.

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Results and discussion

The maximal contractile response to acetylcholine (10^{-5}M) amounted to 4.00 ± 0.30 g (Table1). AMCA (0.03-14 mg/mL) inhibited in a concentration-dependent way, the contraction developed by acetylcholine (Figure 1). The maximal effect (94 .98 ± 2.85%) was observed with 14 mg/mL concentration. EC_{50} value was 1.80 ± 0.48 (table1). Atropine (10^{-8}-10^{-7}M) abolished acetylcholine spasmogenic effect.

It is well known that acetylcholine causes a contraction through muscarinic receptor in tracheal smooth muscles; however this effect was inhibited by atropine (Aruniakshana et al., 1959). The Relaxation on the tracheal smooth muscle can also occur by β-adrenergic receptor stimulation (Torphy et al., 1983; Kume and Kotilkoff, 1991). We examined the relative contribution of β-adrenergic receptor in AMCA-induced relaxation.

The addition of propranolol (10^{-6} M) in the organ bath, slightly influenced AMCA decreased tension developed by acetycholine (10^{-6} M) (compare solid square and triangle in figure 1); EC_{50} value was then 2.60 ± 0.41 mg/mL (Table 1). Indeed β-adrenergic receptors inhibition by propranolol (10^{-6} M) shifted the concentration-response curve to AMCA to the right as regard to the control.

Table 1: Values of maximal contraction, EC_{50} and Emax

	Effectif (n)	Maximal contraction (g)	EC_{50} (mg/mL)	Emax (%)
Acetylcholine	5	4. 00 ± 0.3	1.80 ± 0.48	94 .98 ± 2.85
Propranolol	7	4. 00 ± 0.3	2.60 ± 0.41	88.55 ± 2.74
KCL	6	4.2 ± 0,26	1.86 ± 0.65	98.24 ±1,76
Atropine	5	1.22 ± 0.02	-	-

The Physiological antagonism between β-adrenergic muscarinic receptors was well established on a rat bronchial tissue. These results are comparable with those obtained by Torphy and Cielslinkski (1990) and Kume and Kotilkoff (1991). The stimulation of β_2–adrenergic receptor increases the cyclic Adenosine monophosphate (cAMP) concentration which in turn decreases the intracellular level of calcium Ca^{2+} concentration and then induces smooth muscle relaxation. The Membrane potential regulates smooth muscle contractility by modification of calcium flow through voltage dependent calcium channels (Nelson et al., 1990). We assessed AMCA influence on calcium mobilization through KCL-induced smooth muscle contraction

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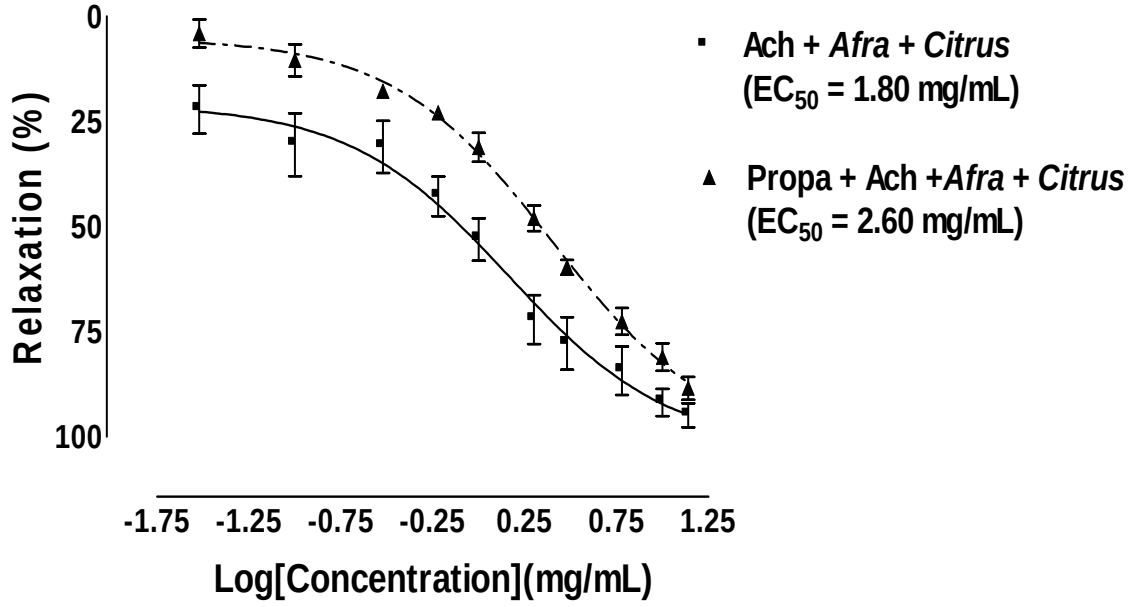

Figure 1: Effect of the AMCA on the contraction induced by Acetylcholine 10^{-5} M (Point) and in the presence of propranolol 10^{-6} M (triangle). Each point represents the mean and the standard deviation. (N: 5-7)

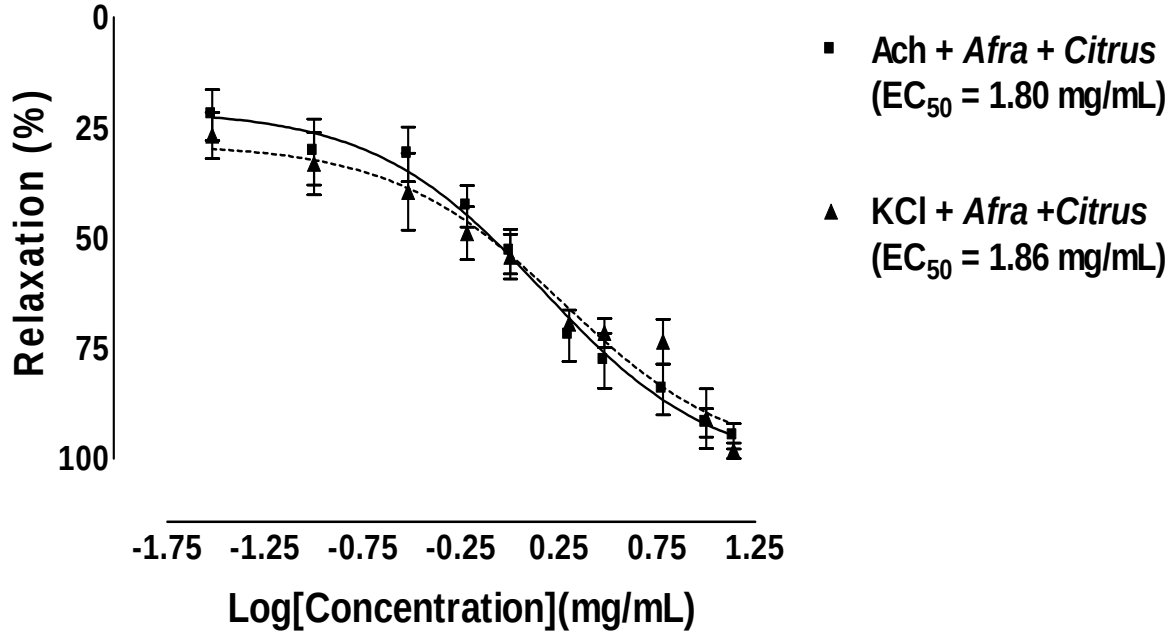

Figure 2: Effect of AMCA on the contraction induced by Acetylcholine 10^{-5} M (square) and KCl 4.10^{-2} M (triangle). Each point represents the mean and the standard deviation. (N: 5-7)

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The maximal contractile response to KCl (4.10^{-2} M) amounted to 4.20 ± 0.26g (Table1). AMCA inhibits KCL-induced-contraction in concentration-dependent manner (Figure 2). EC_{50} value was 1.86 ± 0.65 mg/mL. KCl induced depolarization of smooth muscle cells and this depolarization led to an opening of calcium voltages dependent channels. This mechanism enhances cellular calcium concentration and induces contraction (Nelson et al., 1990). So the relaxation effect of AMCA on these two contractile agents (Acetylcholine and KCL) was similar (see EC_{50} on acetylcholine and KCL-induced contraction) (Table 1). These results indicate that AMCA effect was not only cholinolytic, but also inhibited calcium mobilization. Our results corroborate those of Ouédraogo (2009), which showed that the relaxing effect of *Calotropis procera* Ait.on the trachea isolated from a rabbit involved interaction with calcium.

Conclusion

Our results revealed relaxing properties of AMCA on tracheal smooth muscle isolated from a rat. The extract did not involve muscarinic receptor but likely inhibited cellular calcium. It also involved in part, β-adrenergic receptors.

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