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Spasmolytic Effects of Aqueous Extract of *Sterculia setigera* Delile on Isolated Rat Trachea

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Abstract

The spasmolytic properties of aqueous extract of Sterculia setigera was tested on Rat isolated trachea. Inhibition of the contraction was observed in presence of the aqueous extract (EC50 = 0.91mg/mL ± 0.02) after a pre contraction of the trachea by acetylcholine (10-5M). In the presence of propranolol (10-6M), the spasmolytic activity of the extract was inhibited. The EC50 value was 0.46mg/mL ± 0,11. The aqueous extract of Sterculia setigera also inhibited contraction induced by KCl (4.10-2 M) with EC50 value = 1.9mg/mL ± 0.48). These results clearly show a relaxing effect of aqueous extract of Sterculia setigera on the trachea isolated from rat. This effect involves at least in part β-adrenergic receptor inhibition.

Key words: *Sterculia setigera*; bronchodilatory; spasmolytic; Rat trachea.

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1. Introduction

Sterculia setigera was plant of Sterculiaceae family. These plant pushed especially at north of Benin. *Sterculia setigera* was used as ornamental tree or tree of shade, because of its dense foliage. Its light and hard wood is required for the construction of the roofs. A study carried out in Senegal showed that the density of the cultures (groundnut, millet and sorghum) increases with the distance to trunk of *Sterculia setigera* [1]. It was used in decoction to preserve against the diseases pregnant woman. It is also a remedy to relieve the pulmonary pains at the asthmatic people. The fruits are used in the anti inflammatory treatment. Its roots are used like diuretic. The bark is used against for vomiting and the snake bite. The leaves were treated the constipation, the diarrhoea, paludism of child and are used as fodder in dry season for the cattle [2]. In north of Benin, the populations used it to treat asthma. In this work, we studied the spasmolytic effect of aqueous extract from *Sterculia setigera* on the rat isolated trachea. This study aims to identify the pharmacological properties justifying the traditional use of this extract in the treatment of asthma.

2. Materiel and Methods

2.1 Plant material and extraction

Sterculia setigera leaves were collected in Okpara, locality in department of Borgou in Benin at July 2009. The identification was made by the National Herbarium of the University of Abomey-Calavi of Benin and the specimen was deposited at the University (Voucher N° AA6376/HNB). The aqueous extract was obtained by the decoction of 125g of *Sterculia setigera* leaves in 1 liter of distilled water. The filtrate was centrifuged and lyophilized. The phytochemical analysis of aqueous extracts, based on methods of precipitation and differential staining of [3].

2.2 Animals

Male Wistar rats (weight 288.02 ± 13.41g) were used. The animals were fasted 18 hours before the test. They were then anaesthetized with urethan (1.5 g/kg of body weight by intravenous way). The respiratory tract was then taken and transferred in a sterile container containing a physiological solution from Krebs [4]. 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.

2.3 Solutions and drugs

The Krebs solution 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 (0.6g) in 1L of distilled water. Drugs used were diluted in distilled water. It was about: acetylcholine (SIGMA A-2661 100g Batch 35H07845) 10^{-5}M, propranolol 10^{-6}M, KCl 4.10^{-2}M; the cumulative dose of atropine were also used. The aqueous extract

of *Sterculia setigera* was used with cumulative amount after the period of balancing.

2.4 Preparation and mounting

The thoracic trachea was cut out transversely between the segments of cartilage (4 to 5 rings). These rings are then assembled in the isolated organ bath container 20ml from modified krebs solution at 37°C and bubbled with a mixture oxygenates (95%), carbon dioxide (5%). The ring of trachea is fixed between one triangular supports fixed inside the bath and the other one connected to the transducer.

The preparation was subjected to a tension ranging between 300mg and 500mg, during 1h to obtain regular and spontaneous contractions [5]. It was then balanced gradually to the tension of 1,5g during 30min. During these 90 min of incubation, a washing is carried out every 15 min.

The transducer used for the recording is isometric of F50 mark EMKA. It is connected to an analogical amplifier (EMKA). The graphic recording is made by the software of acquisition U-vessel (WAGNER University of Strasbourg, France).

3. Methods

Sterculia setigera was used with cumulative concentration after the period of balancing. Drugs tested are introduced directly into the isolated organ bath using micro syringe. The volume of administration does not exceed 5% of the volume of the bath [6]. The first stimulation is carried out by injection of acetylcholine (10^{-5}M) in the isolated organ tank. For relaxation test, a second stimulation is realized [7]. When the contraction is reaches maximal effect, *Sterculia setigera* extract is added in 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 the atropine is examined as a reference. This drug is added in cumulative (10^{-8}M - 10^{-5}M) into the bath 15 min after the administration of acetylcholine.

The effect of *Sterculia setigera* was expressed as a percentage (%) of relaxation. The concentration-response curves are plotted and the effective concentrations 50% are noted.

4. Statistical analysis

The maximum effect (E_{max}) and the pD_2 (Log of the concentration producing 50% of the maximum) response given graphically and were calculated by non-linear regression using the software Graph Pad PRISM 2.01 Demo. The student - t - test was used for the comparisons between each group. The data are expressed in the form of mean ± SEM. The probability of (p) error is regarded as significant with $p < 0.05$.

5. Results

The maximal contractile response to acetylcholine (10^{-5}M) amounted to 4.00 ± 0.30g (table1).

Table 1: Values of maximal contraction, EC50 and Emax

	Effectif (n)	Maximal contraction (g)	Emax (%)	EC50 (mg/mL)
Acetylcholine	5	4. 00 ± 0.3	94.98 ± 2.85	0.91 ± 0.02
Propranolol	7	4. 00 ± 0.3	88.55 ± 2.74	0.46 ± 0.11
KCL	6	4.2 ± 0.26	98.24 ±1.76	1.39 ± 0.48

Sterculia setigera extract inhibited in a concentration-dependent way, the contraction developed by acetylcholine. The maximal effect (94.98 ± 2.85) was observed with 14mg/mL concentration. EC_{50} value was 0.91mg/mL ± 0.02 (Figure 1).

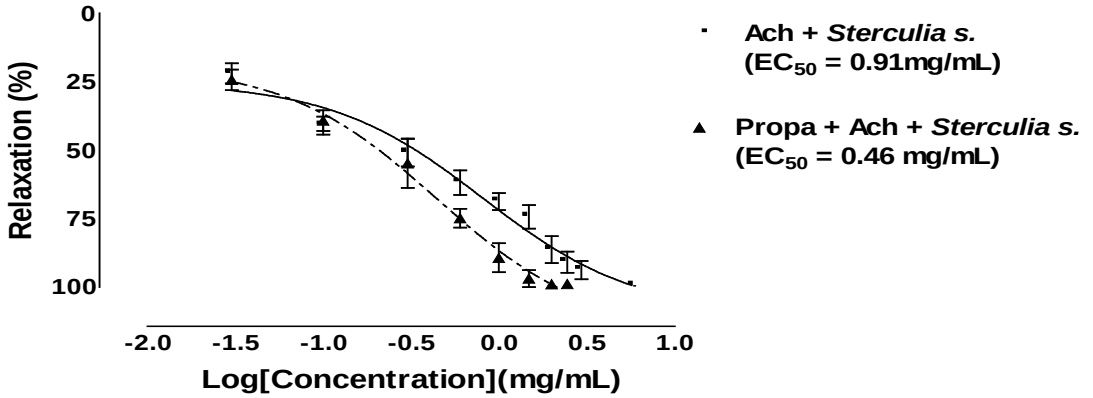

Figure 1: Effect of *Sterculia setigera* on the contraction induced by Acetylcholine 10^{-5}M (Point) and in the presence of propranolol 10^{-6}M (triangle).Each point represents the mean and the standard deviation. (N: 5-7)

The administration of the propranolol (10^{-6}M) have not of remarkable influence on the spasmolytic effect of the extract. Nevertheless we have an increase in the releasing effect of the aqueous extract of *Sterculia setigera* and the value of the EC50 is 0.46mg/mL ± 0.11 (Fig. 1 and table I).

KCl (4.10^{-2} M) produced a maximum contraction 4.2 ± 0.26g on the trachea. The aqueous extract of *Sterculia setigera* caused a relaxation of the trachea. EC50 was 1.39mg/mL ± 0.48. (Figure 2 and table 1).

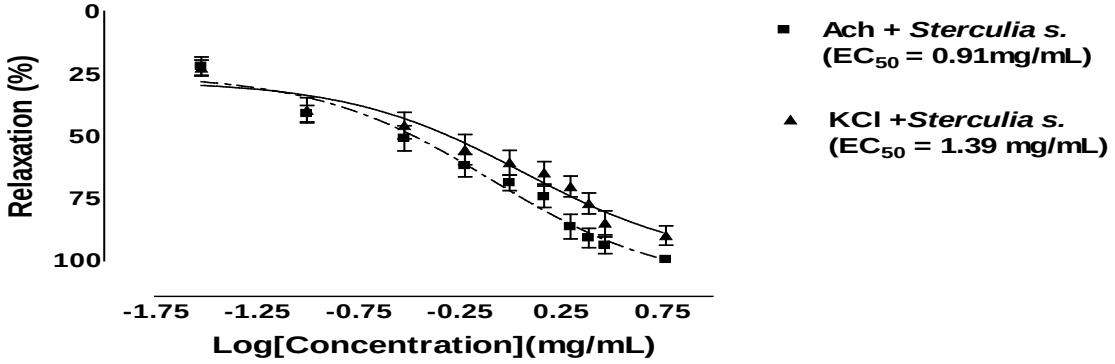

Figure 2: Effect of *Sterculia setigera* on the contraction induced by Acetylcholine 10^{-5}M (square) and KCl 4.10^{-2}M (triangle). Each point represents the mean and the standard deviation. (N: 5-7)

On the scraps of trachea contracted with acetylcholine, the cumulative addition of atropine (10^{-8} to 10^{-7}), caused a reduction of the contracting effect. (figure 3).

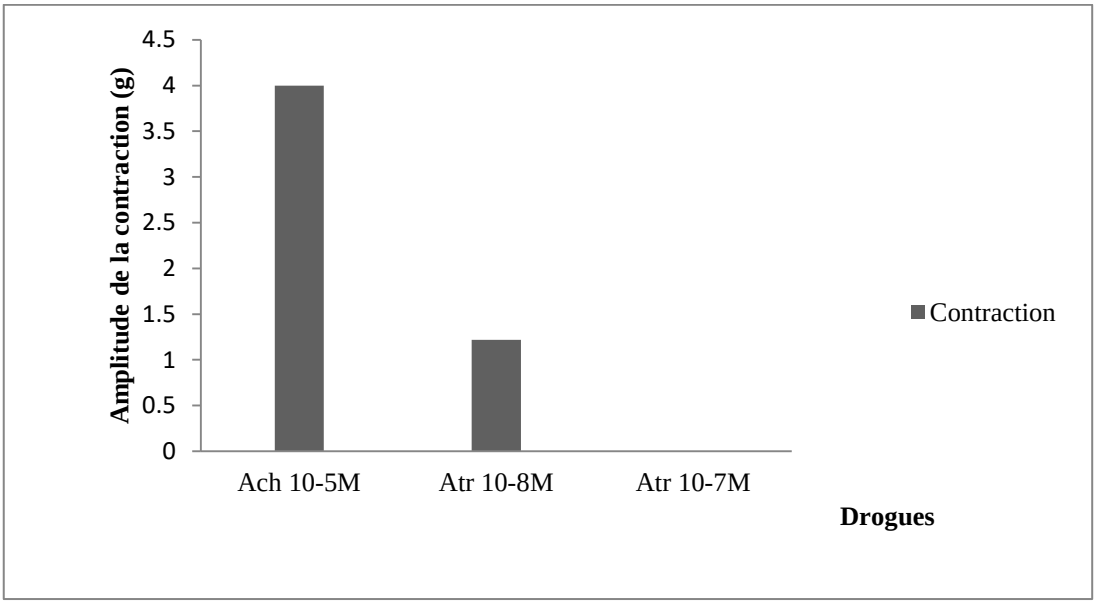

Figure 3: Effect of the atropine on contraction induced by acetylcholine

6. Discussion

It is widely know that acetylcholine causes a contraction through muscarinic receptor of in tracheal smooth muscles. This effect was inhibiting by atropine [8]. Relaxation on tracheal smooth muscle can also occur by β-adrenergic receptor stimulation [9, 10]. We examined the relative contribution of β-adrenergic receptor in

Sterculia setigera induced relaxation.

The addition of propranolol (10^{-6}M) in the organ bath, slightly influences *Sterculia setigera* decreases tension developed by acetycholine (10^{-5}M). Indeed β-adrenergic receptors inhibition by propranolol (10^{-6}M) shifted concentration-response curve to *Sterculia setigera* to the right as regard to the control.

Physiological antagonism between β-adrenergic muscarinic receptors was well established on rat bronchial tissue. These results are comparable with other studies [9, 11]. The stimulation of β_2–adrenergic receptor increases cyclic Adenosin Monophosphat (cAMP) concentration which in turn decreases intracellular Ca^{2+} concentration and then induces smooth muscle relaxation. Membrane potential regulates smooth muscle contractility by modification of calcium flow through voltage dependent calcium channels [12]. *Sterculia setigera* influence on calcium mobilization through KCL-induced smooth muscle contraction. *Sterculia setigera* inhibit KCL-induced- contraction in concentration-dependent manner.

KCl induces depolarization of smooth muscle cells and this depolarization lead to an opening of calcium voltages dependent channels. This mechanism enhances cellular calcium concentration and induces contraction [12].

These results indicate that *Sterculia setigera* effect, was not only cholinolytic, but also inhibited calcium mobilization.

Our results corroborate those of the literature [13,14] which showed that the relaxing effect of *Calotropis procera* Ait.on the trachea isolated from rabbit involved interaction with calcium.

7. Conclusion

Our results revealed relaxing properties of *Sterculia setigera* on tracheal smooth muscle isolated from Rat. The extract does not involved muscarinic receptor but likely inhibited cellular calcium. It also involved, in part, β-adrenergic receptors.

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