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# *Solanum jabrense* Agra & M. Nee (Solanaceae) exhibits spasmolytic activity on guinea-pig ileum

**Fabiana de Andrade Cavalcante[1]*, Joelmir Lucena Veiga da Silva[2], Antonilêni Freire Duarte Medeiros[3], Fladmir de Sousa Claudino[4], Maria de Fátima Agra[3,5], Tania Maria Sarmento da Silva[6], Mário Geraldo de Carvalho[7], Raimundo Braz-Filho[8] and Bagnólia Araújo da Silva[3,5]**

[1]Departamento de Fisiologia e Patologia, CCS, UFPB, Brazil.
[2]Departamento de Saúde, Farmácia-Bioquímica, UNINOVE, Brazil.
[3]Pós-Graduação em Produtos Naturais e Sintéticos Bioativos, CCS, UFPB, Brazil.
[4]Departamento de Biologia Molecular, CCEN, UFPB, Brazil.
[5]Departamento de Ciências Farmacêuticas, CCS, UFPB, Brazil.
[6]Departamento de Ciências Moleculares, UFRPE, Brazil.
[7]Departamento de Química, ICE, UFRRJ, Brazil.
[8]Setor de Química de Produtos Naturais, LCQUI, CCT, UENF, Brazil.

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**Some *Solanum* species are commonly known as "jurubeba" and have a variety of biological activities such as hypotensive, antidiarrheal, and spasmolytic actions. *Solanum jabrense* is a perennial shrub or tree from Solanaceae family. The hexane extract from aerial parts of *S. jabrense* (SJ-Hex) was assayed for possible spasmolytic effect in guinea-pig ileum. SJ-Hex inhibited the contractions induced by carbachol ($IC_{50}$=36.0±6.2 µg/ml) or histamine ($IC_{50}$=37.3±6.1 µg/ml) in a concentration dependent and equipotent manner. SJ-Hex also antagonized cumulative concentration-response curves elicited by histamine. The characterization of the blockade was evaluated by the observation that SJ-Hex shifted to the right cumulative concentration-response curves to histamine in a reversible and non-competitive manner. KCl-, carbachol- and histamine-induced tonic contractions were also inhibited ($IC_{50}$=51.9±11.4; 32.7±11.5, and 31.1±5.3 µg/ml) in a concentration-dependent and reversible manner, suggesting that SJ-Hex could be acting on voltage-operated $Ca^{2+}$ channels. This hypothesis was confirmed by the observation that SJ-Hex inhibited in a concentration-dependent manner $CaCl_2$-induced contractions in depolarizing medium. These results suggest that SJ-Hex produces spasmolytic action in guinea-pig ileum probably by inhibiting the $Ca^{2+}$ influx through voltage-operated $Ca^{2+}$ channels.**

**Key words:** *Solanum jabrense*, Solanaceae, spasmolytic activity, $Ca^{2+}$ channels.

## INTRODUCTION

The *Solanum* genus (Solanaceae) is considered to be one of the largest and most complex among the Angiosperms. It comprised of about 1400 species (Bohs, 2005) and 5000 described epithets. It is distributed mainly throughout tropical and subtropical regions of the world (Nee, 1999). In the Northeast of Brazil, many *Solanum* species are widely used in folk medicine and are commonly known as "jurubeba". This word is originated from the Tupi-guarani, 'yu´beba' which refers to the presence of prickles on some species (Agra and Bhattacharyya, 1999). Several species as *Solanum paniculatum* (Ribeiro et al., 1986), *Solanum melongena*

*Corresponding author. E-mail: fa_cavalcante@hotmail.com. Tel: +55 83 3216-7246. Fax: +55 83 3216-7502.

(Shum and Chiu, 1991), and *Solanum stipulaceum* (Ribeiro, et al., 2002) were reported to induce hypotension in rats, and *Solanum asterophorum* (Silva et al., 2012) presented antidiarrheal activity. Moreover, other species presented spasmolytic effects, such as *S. melongena* (Shum and Chiu, 1991), *Solanum paludosum* (Monteiro et al., 2012), *Solanum agrarium* and *S. stipulaceum* (Santos et al., 2003), *S. asterophorum* (Oliveira et al., 2006a), *Solanum megalonyx* (Oliveira et al., 2006b).

*Solanum jabrense* (SJ-Hex) is a perennial shrub or a small tree from the Solanaceae family, and it is apparently rare, and is only known from few collections in the Pico do Jabre of the state of Paraíba, Brazil (Agra and Nee, 1997). It has an exclusively neotropical distribution and is only found in the marsh or forest islands in the Northeast of Brazil. According to Silva et al. (2002, 2004) and Esteves-Souza et al. (2002), chemotaxonomic important substances as 1,2,3,4-tetrahydro-2-methyl-carboline, solavetivone, *N-trans*-caffeoyl-tyramine, kaempferol 7-methyl ether, quercetin 4',7-dimethyl ether, quercetin 3,3',4',7-tetramethyl ether, and solasodine were isolated from *S. jabrense.* Of these, 1,2,3,4-tetrahydro-2-methyl-carboline and quercetin 4',7-dimethyl ether were first reported in the genus *Solanum*.

Focusing on the spasmolytic properties of *Solanum* spp. from the Northeast of Brazil, we have assayed the possible effects of the hexane extract from *S. jabrense* (SJ-Hex) in guinea-pig ileum.

## MATERIALS AND METHODS

### Plant

The aerial parts of the plant were collected in the summer of 1998 in the "Pico do Jabre", municipality of Maturéia in the state of Paraíba. A voucher specimen (5257) was deposited at the Herbarium of Professor Lauro Pires Xavier (JPB), Universidade Federal da Paraíba. The powdered, dried aerial parts (1.4 kg) were extracted by maceration with EtOH. The EtOH extract was then concentrated in vacuum at 40°C, yielding a dark gum. The EtOH extract was suspended in MeOH:$H_2O$ (4:2) and successively fractionated with hexane and $CHCl_3$. The solvents were evaporated in vacuum furnishing the corresponding residues (Silva et al., 2002).

### General

The composition (mM) of the physiological salt solutions used for the isolated tissues experiments is as follows:

Modified Krebs solution: NaCl (117.0), KCl (4.7), $MgSO_4.7H_2O$ (1.3), $NaH_2PO_4.H_2O$ (1.2), $CaCl_2.2H_2O$ (2.5), glucose (11.0), and $NaHCO_3$ (25.0) were used at 37°C. High-$K^+$ isosmotic solution nominally without $Ca^{2+}$ (depolarizing solution): NaCl (51.7), KCl (70.0), $MgSO_4.7H_2O$ (1.3), $NaH_2PO_4.H_2O$ (1.2), glucose (11.0), and $NaHCO_3$ (25.0) were used at 37°C.

All solutions were bubbled with a 95% $O_2$ and 5% $CO_2$ gas mixture before use. Tissues were suspended in 6 ml organ baths. Tissue responses were set under a resting load of 1.0 g. Force generation was monitored using an isometric transducer coupled to a physiograph (Ugo Basile, Italy) and using isotonic levers kymographs kymographs and smoked drums. The experimental procedure was Universidade Federal da Paraíba.

### Drugs

SJ-Hex was dissolved in Cremophor® and diluted in distilled water. In functional experiments, $NaHCO_3$, KCl, $MgSO_4.7H_2O$ (REAGEN), $CaCl_2.2H_2O$, NaCl, carbachol chloride, $NaH_2PO_4.H_2O$, glucose (Merck), Cremophor®, and histamine dihydrochloride (Sigma-Aldrich) were dissolved and diluted in distilled water.

### Data analysis

Results are expressed as means ± standard error of mean. Statistical analyses were performed by analysis of variance (ANOVA), followed by Bonferroni's test. $EC_{50}$ (concentration of extract that produces 50% of its maximal possible effect) and $IC_{50}$ (concentration of extract that reduces to 50% a maximal response for an agonist) (Jenkinson et al., 1995) values were determined from individual concentration-response curves by non-linear regression. All data were analyzed with the software GraphPad Prism version 3.02.

### Effect of SJ-Hex on carbachol- and histamine-induced phasic contractions on guinea-pig ileum

Guinea-pigs were killed by cervical dislocation. The distal ileum was excised rapidly, and washed thoroughly in modified Krebs solution at room temperature. Segments of ileum (2 to 3 cm) were suspended in a 6 ml organ bath, which contained modified Krebs solution maintained at 37°C, and allowed to equilibrate for 30 min. Two simple concentration-response curves were obtained for both carbachol and histamine. SJ-Hex was then added and after an incubation period of 15 min (time required to produce maximum effect in earlier studies); a third concentration-response curve was induced in the presence of SJ-Hex. The tissue was washed when the agonist responses had returned to resting level. The procedure was repeated in the absence and presence of various concentrations of SJ-Hex. Inhibition was measured comparing the response before and after addition of extract in the organ bath.

### Characteristic of the blockade on histamine-induced contractions

Strips were prepared as previously described. Two cumulative concentration-response curves of approximately equal magnitude were obtained with histamine. After washing, the ileum was incubated with SJ-Hex for 15 min and a third concentration-response curve for histamine was induced in the presence of SJ-Hex. The tissue was washed and when the response to histamine was recovered after repeated washings, the procedure was repeated using different concentrations of the SJ-Hex.

### Effect of SJ-Hex on KCl-, carbachol- or histamine-induced tonic contractions

After stabilization of the preparations, an isometric contraction was elicited with carbachol (1 µM), histamine (1 µM) or KCl (40 mM). Contractile agents remained in contact with the preparation until a plateau was reached, after that, the tissue was washed out. The plateau characterizes the tonic component of the contraction. After about 30 min. the process was repeated and on the plateau of contraction, SJ-Hex was added cumulatively. Subsequent

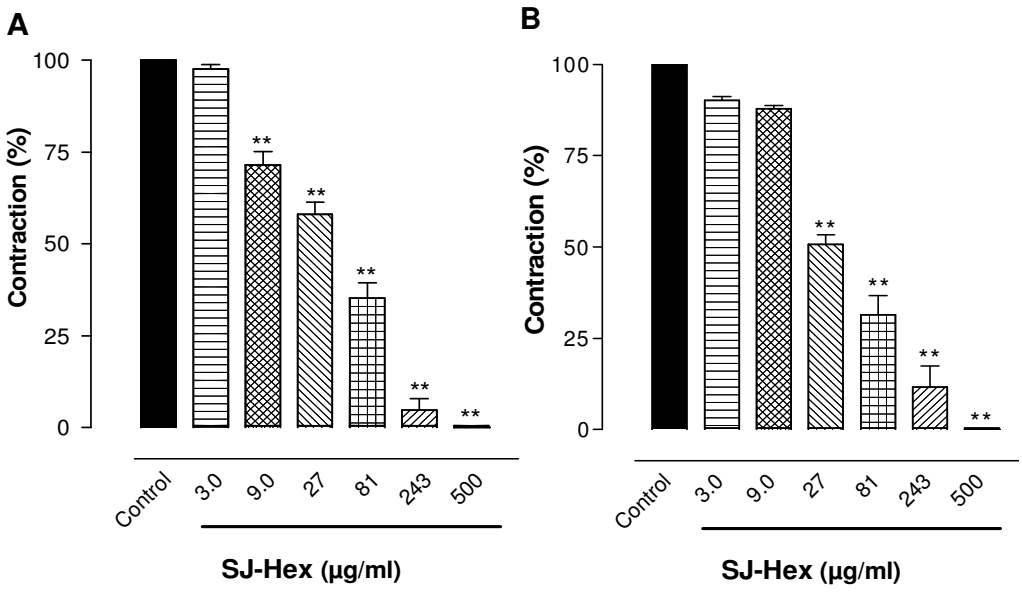


**Figure 1.** Effect of SJ-Hex on phasic contractions induced by $10^{-6}$ M carcachol (A) or histamine (B) in the guinea-pig ileum. Values are mean ± SEM for 5 experiments.

concentrations were added only after the response to the previous concentration became stable. Relaxation was expressed as reversal percentage of the initial contraction elicited by contractile agents.

#### Effect of SJ-Hex on $Ca^{2+}$-induced contractions in depolarizing medium nominally without $Ca^{2+}$

The strips were prepared as described before. To assess the effects of SJ-Hex on the influx of $Ca^{2+}$ through voltage-operated $Ca^{2+}$ channels ($Ca_V$), the strips were bathed for 30 min in modified Krebs solution and then exposed for 45 to 60 min to high-$K^+$ $Ca^{2+}$-free depolarizing solution. In general, two cumulative concentration-response curves to $CaCl_2$ were obtained at 60 min intervals in each preparation (Van Rossum, 1963). After obtaining the first curve, washing and after complete relaxation, different concentrations of SJ-Hex were added to the bath and left in contact with the tissue for 15 min. Then, a second cumulative concentration-response curve to $CaCl_2$ was obtained in the presence of the extract. The maximal contraction obtained with the first concentration-response curve to $CaCl_2$ was taken as 100%, and all contractions were calculated as a function of this value. Each preparation was exposed to only one concentration of SJ-Hex.

## RESULTS

### Effect of SJ-Hex on carbachol- and histamine-induced phasic contractions in guinea-pig ileum

SJ-Hex (0.3 to 500 µg/ml) inhibited phasic contractions induced by both carbachol and histamine (Figure 1). The corresponding values of $IC_{50}$ obtained graphically were 36.0±6.2 and 37.3±6.1 µg/ml for carbachol and histamine, respectively (n=5).

### Characteristic of the blockade on histamine-induced contractions

SJ-Hex (9.0, 27.0, 81.0, and 243.0 µg/ml) antagonized cumulative concentration-response curves with histamine (Figure 2). Concentration-response curves in the presence of SJ-Hex were shifted to the right and suppressed the maximal effect ($E_{max}$). $E_{max}$ values were 86.5±7.9, 67.2±1.7, 39.0±7.7, and 1.5±1.5% for concentrations of 9, 27, 81, and 243 µg/ml, respectively (n = 4), which is characteristic of the non-competitive blockade.

### Effect of SJ-Hex on KCl-, carbachol- or histamine-induced tonic contractions

Cumulative additions of SJ-Hex during the development of the tonic component of contractions resulted in a concentration-dependent relaxation in the guinea pig ileum pre-contracted with both carbachol and histamine1 µM or KCl 40 mM (Figure 3). The $IC_{50}$ values obtained graphically were 32.7±11.5, 31.1±5.3 and 51.9±11.4 µg/ml (n=5).

### Effect of SJ-Hex on $Ca^{2+}$ induced contractions in depolarizing medium nominally without $Ca^{2+}$

SJ-Hex (9.0, 27.0, 81.0, and 243.0 µg/ml) antagonized cumulative concentration-response curves to $CaCl_2$ (Figure 4). Concentration-response curves in presence of SJ-Hex were shifted to the right and suppressed the $E_{max}$ values were 81.5±2.9, 61.6±3.7, 39.9±3.9, and

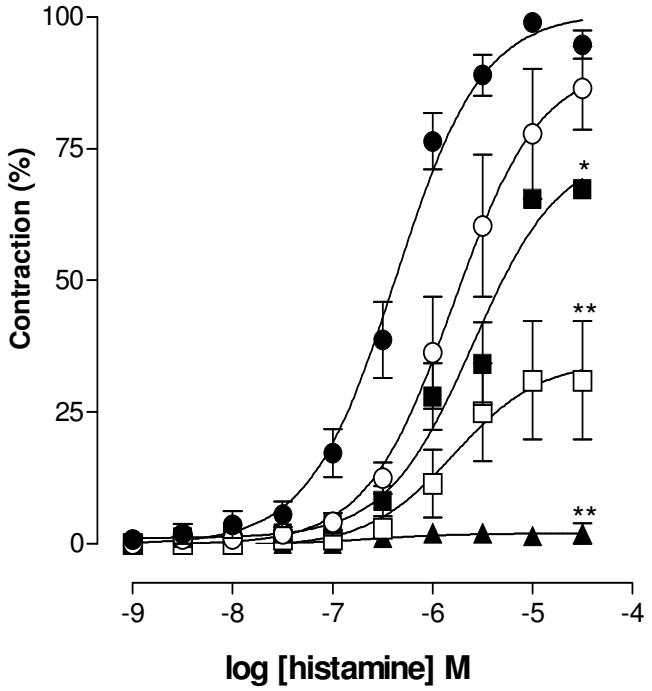


**Figure 2.** Effect of SJ-Hex on histamine-induced cumulative concentration-response curves in guinea-pig ileum. In the absence, control (●), or in the presence of SJ-Hex extract 9 (○); 27 (■), 81 (□) and 243 (▲) µg/ml.
Values are mean ± SEM for 4 experiments.

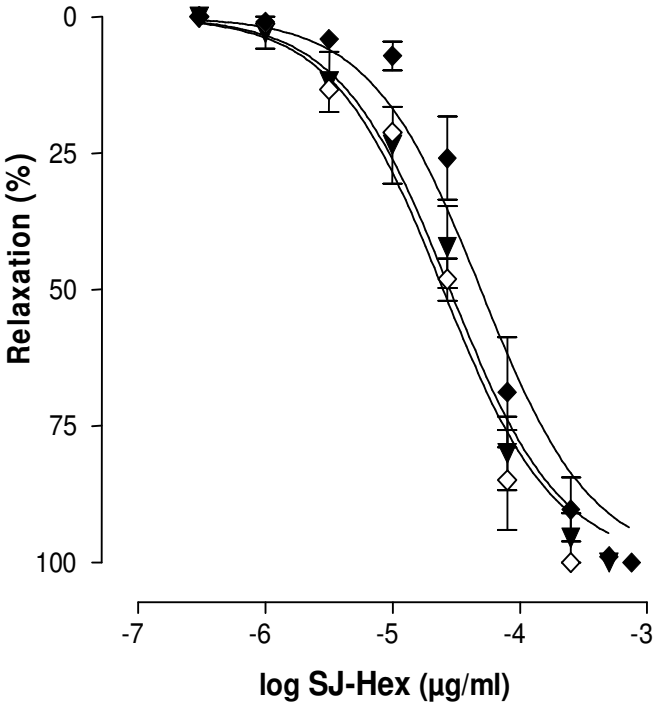


**Figure 3.** Effect of SJ-Hex on 40 mM KCl (◆)-, $10^{-6}$ M carbachol (◇)- or $10^{-6}$ M histamine (▼)-induced tonic contractions in guinea-pig ileum.
Values are mean ± SEM for 5 experiments.

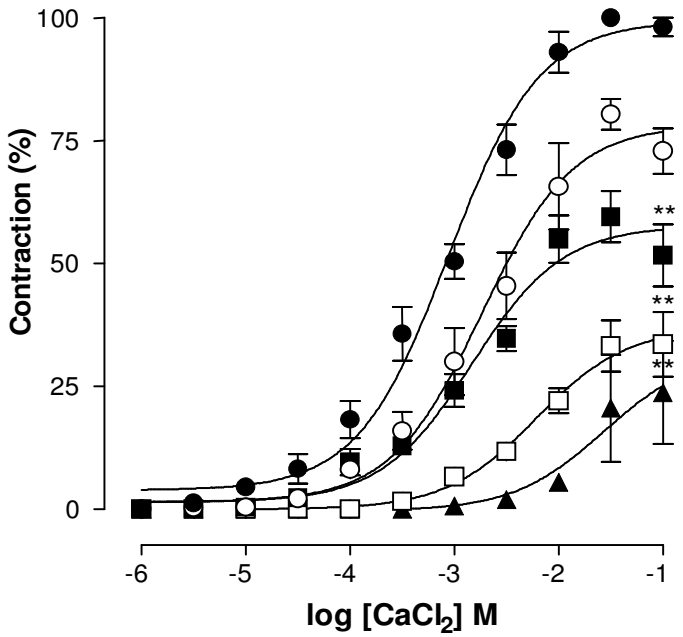


**Figure 4.** Concentration–response curves for $CaCl_2$ ($10^{-6}$ to $10^{-1}$ M) in depolarized guinea-pig ileum (n = 5 in each case, mean ± SEM) incubated in $Ca^{2+}$-free medium. In the absence, control (●), or in the presence of SJ-Hex 9 (○); 27 (■), 81 (□) and 243 (▲) µg/ml. Values are mean ± SEM for 5 experiments.

31.3±12.8% for concentrations 9, 27, 81, and 243 µg/ml, respectively (n=5).

## DISCUSSION

In this study, we investigated the effects of SJ-Hex extract on visceral smooth muscle, and the most important finding in this work is the demonstration for the first time, SJ-Hex exerts a non-selective spasmolytic action in guinea-pig ileum, and that this effect is due in part, to the inhibition of $Ca^{2+}$ influx through voltage-dependent $Ca^{2+}$ channels ($Ca_v$).

We decided to study the spasmolytic action mechanism of this extract, based on the fact that other species of *Solanum* presented spasmolytic activity in guinea-pig ileum, for example, *S. paludosum* (Monteiro et al., 2012), *S. asterophorum* and *S. megalonyx* (Oliveira et al., 2006a, b). The absence of significant differences between the $IC_{50}$ values of the SJ-Hex on carbachol and histamine- induced contractions in guinea-pig ileum is suggestive that SJ-Hex may be acting by a similar mechanism of action to the agonists and not in a receptor level, since each agonist tested has its own receptor system. To verify this hypothesis, we evaluated the characteristic blockade induced by SJ-Hex on cumulative concentration-response curves to histamine, and observed a non-parallel shift to the right and suppression of the maximal effect, suggesting a non-competitive blockade

and the spasmolytic effect induced by SJ-Hex does not require histamine receptor occupancy. Thus, it is probable that SJ-Hex might be acting at another step of the cascade events that leads to smooth muscle contractions.

In order to verify whether SJ-Hex acts on $Ca^{2+}$ influx across the membrane, we evaluated its effect on the tonic component of the contractile response induced by both carbachol and histamine (pharmacomechanical and eletromechanical couplings) and KCl (electromechanical coupling) in the guinea-pig ileum. As shown in Figure 3, SJ-Hex relaxed in an equipotent and concentration-dependent manner of the ileum pre-contracted with carbachol ($IC_{50}$=32.7 ± 11.5 µg/ml), histamine ($IC_{50}$=31.1±5.3 µg/ml) and KCl ($IC_{50}$=51.9±11.4 µg/ml). Independently of the contraction being evoked by either pharmacomechanical or electromechanical coupling, the maintenance of the tonic component involves activation of voltage-operated $Ca^{2+}$ channels (Rembold, 1996). Therefore, we can postulate that SJ-Hex may be blocking these channels to produce non-selective spasmolytic effects.

In order to confirm the aforementioned hypothesis that SJ-Hex blocks $Ca_v$, we evaluated its effects on cumulative curves to $CaCl_2$ in depolarizing medium nominally without $Ca^{2+}$ and we observed a non-competitive blockade on $CaCl_2$ induced curves, since SJ-Hex produced a non-parallel and concentration-dependent rightward displacement of the concentration-response to $CaCl_2$, significantly reducing the maximal response, corroborating the suggestion that SJ-Hex interferes with a $Ca^{2+}$ influx through $Ca_v$.

In conclusion, we have shown that SJ-Hex extract produces nonselective spasmolytic effects in guinea-pig ileum due, in part, to the inhibition of $Ca^{2+}$ influx through $Ca_v$. Further studies need to be carried out in order to detect the spasmolytic effect of solavetivone; however, the major substances obtained from hexane extract of *Solanum jabrense* are required to reinforce this hypothesis.

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