

## Myorelaxant Effect of an Alcoholic Extract of *Sphenocentrum jollyanum* Roots in Rabbit Aortic Strip and Corpus Cavernosum

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### ABSTRACT

**Objective:** In the present study, we demonstrate the myorelaxant actions of an ethanolic root extract of *Sphenocentrum jollyanum* (SJE) in isolated smooth muscle preparations—corpus cavernosum and rabbit aortic strip. **Materials and Methods:** Isolated tissue experiments using tissue/organ bath were conducted to evaluate the myorelaxant effect of *Sphenocentrum jollyanum*. SJE dose-dependently suppressed spasmogenic actions of Phenylephrine (PE) and  $K^+$  in rabbit aortic strip ( $IC_{50}$ s were  $0.75 \pm 0.11$  and  $2.58 \pm 0.20$  mg  $mL^{-1}$ , respectively). Also, SJE ( $0.1$ – $10$  mg  $mL^{-1}$ ) relaxed corpus cavernosal smooth muscle pre-contracted with PE and  $K^+$  with  $IC_{50}$ s of  $3.99 \pm 0.45$  and  $7.23 \pm 1.02$   $\mu g$   $mL^{-1}$  for PE and  $K^+$ -induced contractions, respectively. Various antagonists were employed to determine the possible mechanism of SJE. **Results:** The relaxation caused by SJE was not affected by cholinergic antagonist (atropine),  $\beta$ -adrenoceptor antagonist (propranolol), nitric oxide synthase inhibitor ( $N_{\omega}$ -nitro-L-arginine methyl ester), soluble guanylate cyclase inhibitors, (methylene blue: or 1H-[1,2,4]-oxadiazole-[4,3-a]-quinoxalin-1-one), cyclooxygenase inhibitor (indomethacin): and  $K^+$  channels antagonists (glibenclamide and tetraethylammonium). However SJE caused non-competitive inhibition of  $Ca^{2+}$ -induced contraction of rabbit aortic strip. **Conclusion:** These results suggest that SJE has myorelaxants effects and may be acting further downstream of the signal transduction pathway, possibly at the level of  $Ca^{2+}$  mobilization.

**Key words:** Erectile dysfunction, aphrodisiac, smooth muscle

Pharmacologia 4 (5): 371-382, 2013

### INTRODUCTION

Arteriolar and cavernous smooth muscles play key roles in the process of penile erection. Smooth muscle cells of the arterioles and sinuses of the erectile tissue and vasculature of the penis mediate the prominent actions of dilators and constrictors involved in erection<sup>1,2</sup>. Relaxation of these smooth muscles allow for increased permeation through the arterioles leading to the filling of cavernosal sinuses with blood. Also, the veno-occlusive mechanism is activated to reduce the rate of blood outflow. The resultant effect is the high intracavernous pressure associated with the erect penis<sup>3,4,5,6</sup>. Several neural events are synchronized to release endogenous mediators such as NO, at the level of the penile smooth muscle to induce relaxation. Any action that disrupts these series of events can lead to Erectile Dysfunction (ED)<sup>7,8</sup>. ED is considered one of the most important public health problems worldwide, since it affects a high percentage of men<sup>8,10</sup>. Although several orthodox medicines are available, herbal remedies continue to

provide a popular alternative for men seeking to improve their sexual life<sup>11,12</sup>. The root of *Sphenocentrum jollyanum* is chewed as a central nervous system stimulant and to enhance libido and sexual function<sup>9,13,14,15,16</sup>. We have previously provided evidence to support the use of *S. jollyanum* in the treatment of ED in traditional medicine<sup>17</sup> and that central effects may account for its acute action whilst the long term effect may be due to its effect on testosterone<sup>17,18</sup>. However, evidence for peripheral actions of the plant is not yet available. Currently, type-5 phosphodiesterase inhibitors such as sildenafil, tadalafil and vardenafil are widely-used therapy for management of erectile dysfunction. Sildenafil causes cavernosal vasorelaxation via a NO-mediated mechanism<sup>8,19,20</sup>. Although NO is considered an important stimulator of cavernosal smooth muscle relaxation<sup>21,22</sup>, the inhibition of vasoconstrictors such as norepinephrine and endothelin-1 could as well be a potential regulator of penile erection<sup>23,24,25</sup>. This is true of drugs used for the treatment of ED which have peripheral site of action such as phosphodiesterase inhibitors (papaverine),  $\beta$ -adrenoceptor antagonists (phentolamine and thymoxamine), prostaglandin E<sub>1</sub> (alprostadil), vasoactive intestinal polypeptide, calcitonin gene-related peptide,

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organic nitrates (nitroglycerin) and K<sup>+</sup> channel openers (pinacidil and cromakalim)<sup>2</sup>. The most significant property common to all these pharmacological agents is their ability to either inhibit contractions or induce relaxations of penile smooth muscles, which may ultimately result in penile erections. The present study therefore seeks to investigate the vasorelaxation and corporal relaxation properties of an ethanolic extract of *S. jollyanum* in order to evaluate its possible peripheral site of action in smooth muscles of rabbit aortic strip and corpus cavernosal smooth muscle.

## MATERIALS AND METHODS

**Animals:** Male New Zealand rabbits (3.0-4.0 kg) purchased from a nearby animal farm, fed on normal commercial pellet diet (GAFCO, Tema) and given water *ad libitum* were used in these studies. The animals were housed at the animal facility of the Department of Pharmacology, KNUST, Kumasi, Ghana, and maintained at a temperature of 24-28°C, relative humidity 60-70%, and 12 h light-dark cycle. All animals used in these studies were treated according to the Guide for the Care and Use of Laboratory Animals<sup>26</sup> and were approved by the College Ethics Committee.

**Drugs and chemicals:** Potassium chloride and calcium chloride were obtained from BDH Chemicals Ltd, Poole, England, tetraethylammonium, phenylephrine, phentolamine, atropine, propranolol, sodium nitroprusside, N<sub>ω</sub>-nitro-L-arginine methyl ester (L-NAME), 1H-[1,2,4]-oxadiazole-[4,3-a]-quinoxalin-1-one (ODQ), sildenafil, indomethacin, nifedipine, glibenclamide from Sigma Aldrich Inc., St Louis, MO, USA., and methylene blue from May and Baker Ltd, Dagenham, U.K.

**Plant material:** Dried roots of *Sphenocentrum jollyanum* Pierre (family Menispermaceae) was purchased from the Central Market, Kumasi and authenticated by Prof. T.C. Fleischer, Department of Pharmacognosy, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana where a voucher specimen (FP/017/06) was deposited.

**Preparation of the extract:** The dried root was pulverized with a hammer-mill to obtain a coarse powder. The powdered material (5 kg) was macerated with 70% (v/v) ethanol by cold percolation for 48 h. The filtrate from the macerate was concentrated using a rotary evaporator and dried in an oven to obtain a yellowish syrupy mass which was air-dried at room temperature (28°C). The dried extract (478 g) was kept in a desiccator and is subsequently referred to as the extract or SJE.

**Experiments on rabbit aortic strip:** Helically cut aortic strips<sup>27</sup> were prepared from the thoracic aorta of male rabbits (3.5±0.5 kg; n= 6). Each Rabbit aortic strip (RbAS) was mounted in a 10-mL organ bath containing Ringer-Locke's solution of the following composition (mM): NaCl, 155.0; KCl, 5.7; NaHCO<sub>3</sub>, 6.0; CaCl<sub>2</sub>, 1.0; and glucose, 5.5, at 37°C and gassed with 95% O<sub>2</sub>/5% CO<sub>2</sub>. The RbAS was placed under an initial passive tension of 2 g and allowed to equilibrate for about 1 h. During this equilibration period the buffer was changed every 10 min. The final resting tension was adjusted to 1 g and force displacement transducer (modelA-6360; Harvard Apparatus Ltd, Kent, England) was used to measure changes in isotonic contraction of the tissues, which were displayed on a Harvard Universal Oscillograph (Model50-8622; Harvard Apparatus, Kent, England).

For the evaluation of relaxation, SJE (0.1-10 mg mL<sup>-1</sup>) was added cumulatively to the KCl (80 mM) or phenylephrine (PE, 0.1 μM)-induced contraction of RbAS. A 10 min time interval was allowed to obtain the maximal effect with each concentration of SJE. The results were presented as concentration-response curves for SJE relaxation of the agonist (KCl or PE)-induced contraction. A 100% relaxation was considered attained when the pre-contracted RbAS returned to the baseline position. To determine the inhibitory effect of SJE on agonist (KCl or PE)-induced contractions, RbAS was pre-incubated for 10 min with SJE (0.1-30 mg mL<sup>-1</sup>) before obtaining the cumulative concentration-response curves of KCl and PE. Control concentration-response curves were obtained in the absence of SJE.

To ascertain the mechanism of action of SJE, the possible contribution of K<sup>+</sup> channels to the relaxation effect of SJE was determined by pre-incubating RbAS with a K<sup>+</sup> channel blocker-glibenclamide (10 and 100 μM) or tetraethylammonium (TEA) (1 and 10 mM) for 20 min before PE-induced contraction. Cumulative concentrations of SJE (0.01-3.0 mg mL<sup>-1</sup>) were then applied during the sustained phase of the PE-induced contraction and the percentage relaxations induced by SJE calculated to construct concentration-response curves in the presence of the K<sup>+</sup> channel blocker. Control concentration-response curves were obtained in the absence of the respective blocker.

To investigate the possible involvement of muscarinic receptors, β-adrenergic receptors, prostacyclin, nitric oxide or guanylyl cyclase in the vasorelaxing effects of SJE, RbAS were preincubated separately with muscarinic antagonist-atropine (1-10 μM), β-adrenoceptor antagonist-propranolol (1-10 μM), cyclooxygenase inhibitor-indomethacin (10-100 μM), nitric oxide synthase inhibitor-L-NAME

(10-100  $\mu\text{M}$ ) and the guanylyl cyclase inhibitors-methylene blue (10-100  $\mu\text{M}$ ) or ODQ (1-10  $\mu\text{M}$ ) for 15-30 min. Cumulative concentrations of SJE (0.01-3.0  $\text{mg mL}^{-1}$ ) were then applied during the sustained phase of the PE-induced contraction. Percentage relaxations were calculated and concentration-response curves constructed. Control concentration-response curves were obtained in the absence of the respective inhibitors. Further experiment was conducted to investigate the possible involvement of  $\text{Ca}^{2+}$  influx through Voltage Dependent Calcium Channels (VDCC). Depolarized RbAS contracted by  $\text{Ca}^{2+}$  were chosen as the model for such study. Experiments were carried out under  $\text{Ca}^{2+}$ -free conditions after equilibration. Subsequent to the addition of  $\text{K}^+$  (100 mM) to depolarize the membrane potential, cumulative concentrations of  $\text{Ca}^{2+}$  (0.1-3 mM) were applied. The stepwise increments in tension represented the vasoconstriction dependent on extracellular  $\text{Ca}^{2+}$  influx induced by  $\text{K}^+$ . The RbAS were then washed and equilibrated for 60 min, followed by repetition of the experiment in the presence of SJE (0.1-1.0  $\text{mg mL}^{-1}$ ) or a reference  $\text{Ca}^{2+}$  channel antagonist, nifedipine (0.1  $\mu\text{M}$ ). Control concentration-response curves were obtained in the absence of inhibitors. Cumulative concentration-response curves to  $\text{Ca}^{2+}$  were constructed after 10 min exposure of the preparation to the treatments.

### Experiments on rabbit corpus cavernosal muscle preparation:

Male New Zealand rabbits ( $3.5 \pm 0.5$  kg;  $n = 6$ ) were sacrificed and the penis was surgically removed en bloc, with care being taken to keep the tunica albuginea intact. The corpus cavernosal tissue was carefully dissected free from the surrounding tunica albuginea. One or two strips of the Rabbit corpus cavernosum (RbCC) smooth muscle (12 mm long and 1-2 mm thick) were dissected from each penis<sup>28</sup>. These were kept in Krebs physiological salt solution of the following composition (mM): NaCl 119, KCl 4.7,  $\text{CaCl}_2$  1.5,  $\text{MgCl}_2$  1.2,  $\text{KH}_2\text{PO}_4$  1.2,  $\text{NaHCO}_3$  24 and glucose 11.0 (pH 7.3 to 7.4), constantly aerated with 95.0%  $\text{O}_2$  and 5.0%  $\text{CO}_2$ . Strips were mounted in a perfusion bath, with one end tied to the inside bottom of the perfusion bath and the other end to a thin wire connected to a force displacement transducer (model A-6360; Harvard Apparatus Ltd, Kent, England) for tension measurements. Resting tension of 1 g was applied and the tension developed was measured with the force displacement transducer (model A-6360; Harvard Apparatus Ltd, Kent, England) coupled to Harvard Universal Oscillograph (Model 50-8622; Harvard Apparatus, Kent, England).

To observe the myorelaxant effect of SJE on RbCC, the tissue was stimulated with PE (0.1  $\mu\text{M}$ ) or high- $\text{K}^+$  solution (80 mM KCl). When the plateau of PE- and  $\text{K}^+$ -induced RbCC tone was reached, cumulative concentrations of SJE (0.001-0.3  $\text{mg mL}^{-1}$ ) were added to the tissue baths successively with a contact time of 5 min for each concentration. The relaxation effects of sodium nitroprusside (SNP, 0.01-10  $\mu\text{g mL}^{-1}$ ), sildenafil (0.01-1  $\mu\text{g mL}^{-1}$ ) and phentolamine (0.1-30.0  $\mu\text{g mL}^{-1}$ ) were also evaluated on the RbCC stimulated with PE.

To investigate the possible involvement of nitric oxide pathway in the relaxing effects of SJE, RbCC were preincubated separately with nitric oxide synthase inhibitor-L-NAME (100-300  $\mu\text{M}$ )-and the guanylyl cyclase inhibitor-ODQ (10-100  $\mu\text{M}$ )-for 30 min. SJE (0.003-0.3  $\text{mg mL}^{-1}$ ) was then applied during the sustained phase of PE-induced contraction. Control concentration-response curves were obtained in the absence of the respective inhibitors. The percentage relaxation induced by SJE in the presence of these inhibitors was calculated to make a concentration-response curve. A 100% relaxation was considered attained when the pre-contracted rings returned to the baseline position. Effect of ODQ (1-10  $\mu\text{M}$ ) on SNP and sildenafil were also studied.

**Statistical analysis:** All graphs were fitted and analyzed with GraphPad Prism for Windows version 4.02 (GraphPad Software, San Diego, CA, USA). For the determination of  $\text{IC}_{50}$  (concentration responsible for 50% of the maximal inhibitory effect), drugs were analyzed by using an iterative computer least squares method using the following nonlinear regression (four-parameter logistic equation):

$$Y = \frac{a + (a - b)}{1 + 10^{(\log \text{ED}_{50} - X) \cdot \text{Hill slope}}}$$

where, X is the logarithm of concentration. Y is the response and starts at a and goes to b with a sigmoid shape.

Statistical analyses were by one-way ANOVA followed by Student-Newman-Keuls or Tukey-Kramer's post test using GraphPad Prism.

## RESULTS

**Experiments on rabbit aortic strip:** PE and  $\text{K}^+$ -induced tension in the RbAS was relaxed by SJE (0.1-10.0  $\text{mg mL}^{-1}$ ) in a concentration-dependent manner as shown in Fig. 1. However, the concentration-response curve for high- $\text{K}^+$  solution (80 mM) was shifted to the right of the PE curve as confirmed by their  $\text{IC}_{50}$  values of  $0.76 \pm 0.11$  and  $2.58 \pm 0.20$   $\text{mg mL}^{-1}$  for the PE and  $\text{K}^+$  curves, respectively (Fig. 1). Further analysis of the

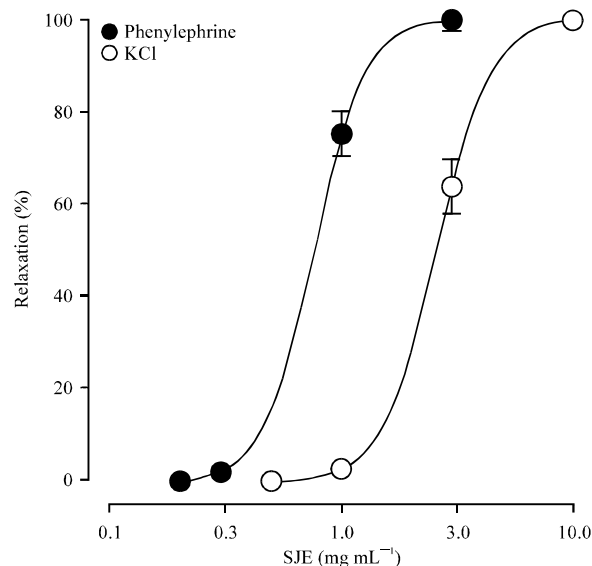


Fig. 1: Relaxation effects of SJE on PE (0.1  $\mu$ M) and KCl (80 mM)-precontracted rabbit aortic strips. Relaxations are expressed as percentage of PE-induced contraction according to concentration in logarithmic scale and given as Means $\pm$ SE mean ( $n = 6$  to  $8$ ). IC<sub>50</sub> (mg mL<sup>-1</sup>) of SJE for: PE-induced contraction,  $0.7546\pm 0.11$ ; KCl-induced contraction,  $2.579\pm 0.20$

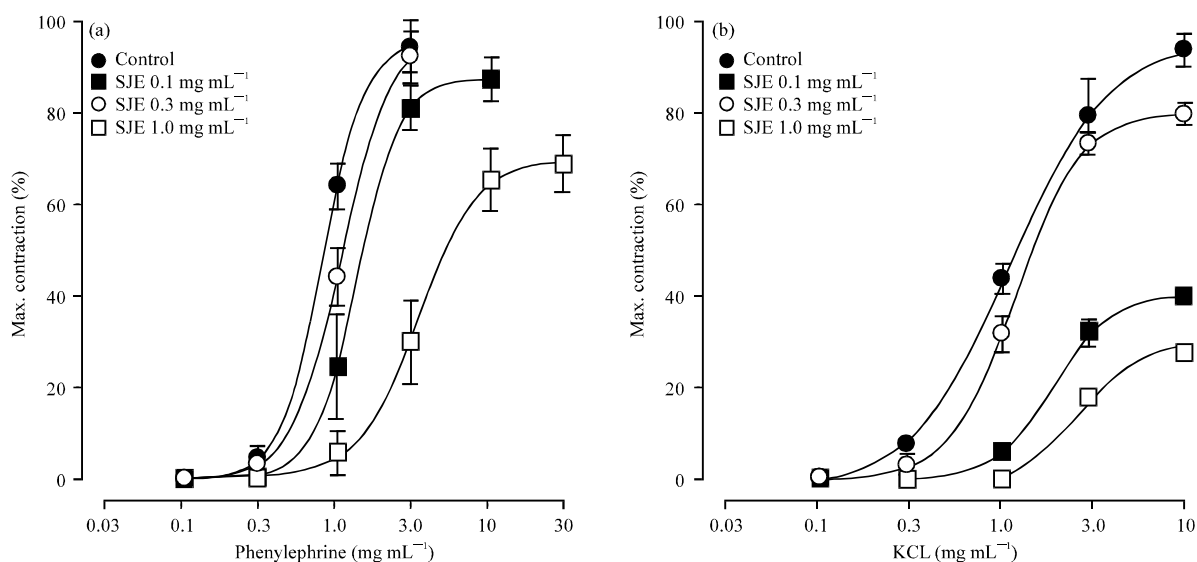


Fig. 2(a-b): Effect of SJE on concentration-response curves of (a) PE and (b) KCl on isolated rabbit aortic strip preparation. Responses are expressed as percentages of maximum control response. Each point represents the Mean $\pm$ SEM ( $n = 3$ )

inhibitory effect of SJE on the contractile response to PE and high K<sup>+</sup> solution (80 mM) showed that pre-treatment with SJE (0.1-1.0 mg mL<sup>-1</sup>) caused a rightward shift of concentration-response curves of both PE and K<sup>+</sup> with depression of the maximum responses when compared to the control (Fig. 2).

In the experiments designed to investigate the mechanisms responsible for the relaxation induced by SJE, the K<sup>+</sup> channel blockers-glibenclamide (10-100  $\mu$ M) and TEA (1-10 mM)-did not inhibit SJE-induced vasorelaxation in RbAS (Fig. 3 a, b): suggesting that K<sup>+</sup> channels might not be involved SJE-induced

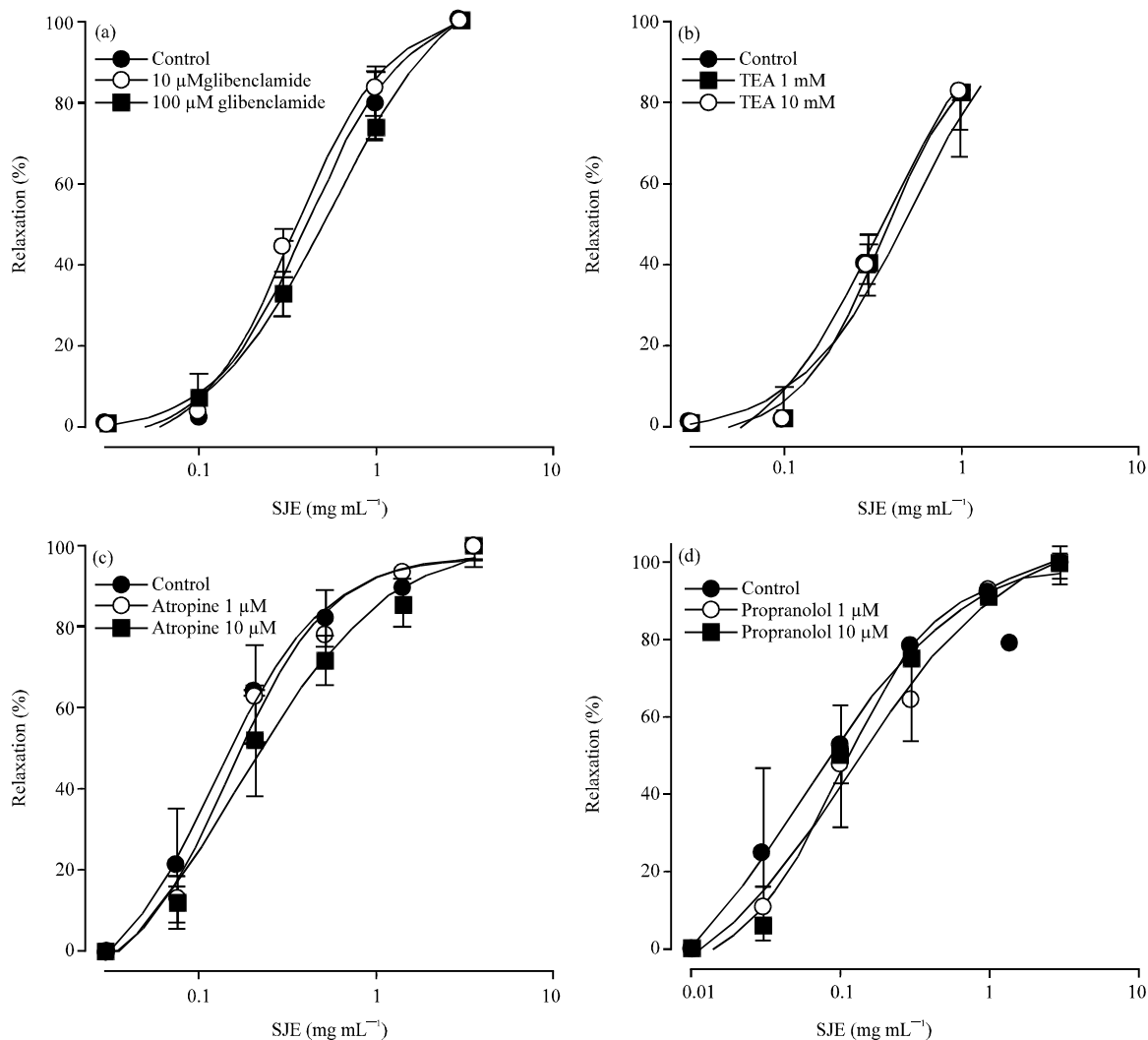


Fig. 3(a-d): Relaxation effects of SJE on PE-induced contraction of rabbit aortic strips in the presence of (a) Glibenclamide (10-100  $\mu\text{M}$ ), (b) TEA (1-10 mM), (c) Atropine (1-10  $\mu\text{M}$ ) and (d) Propranolol (1-10  $\mu\text{M}$ ). Relaxations are expressed as percentage of PE-induced contraction according to concentration in logarithmic scale and given as Means  $\pm$  SE mean ( $n = 4$  to 6)

vasorelaxation. Also SJE-induced vasorelaxation was not affected by preincubation of the aortic rings with atropine (1 and 10  $\mu\text{M}$ ) or propranolol (1 and 10  $\mu\text{M}$ ) (Fig. 3c, d). This indicates SJE did not interact with neither muscarinic nor  $\beta$ -adrenoreceptors to induce vascular relaxation. Furthermore, indomethacin (10 and 100  $\mu\text{M}$ ), L-NAME (10 and 100  $\mu\text{M}$ ), ODQ (1 and 10  $\mu\text{M}$ ) and methylene blue (10 and 100  $\mu\text{M}$ ) did not significantly affect the relaxing effect of SJE (Fig. 4) as confirmed by the similar  $E_{\text{max}}$  and  $\text{IC}_{50}$  values compared to the their respective controls (Table 1). These observations rule out the involvement of the prostacyclin and the NO-guanylyl cyclase pathway in the

vasorelaxation caused by SJE. On the contrary, pre-treatment of RbAS with SJE (0.1 and 1.0  $\text{mg mL}^{-1}$ ) caused a rightward shift of the concentration-response curves for  $\text{Ca}^{2+}$ -induced contractions with depressed maximum responses as was observed for nifedipine (0.1  $\mu\text{g mL}^{-1}$ ) (Fig. 5). This is suggestive of possible non-competitive antagonism of  $\text{Ca}^{2+}$  effects by SJE.

**Experiments on corpus cavernosal smooth muscle preparation:** PE (0.1  $\mu\text{M}$ ) and high- $\text{K}^{+}$ -solution (80 mM) provoked sustained tonic contraction of the RbCC which was concentration-dependently relaxed by the cumulative addition of SJE (0.001–0.3  $\text{mg mL}^{-1}$ ) with

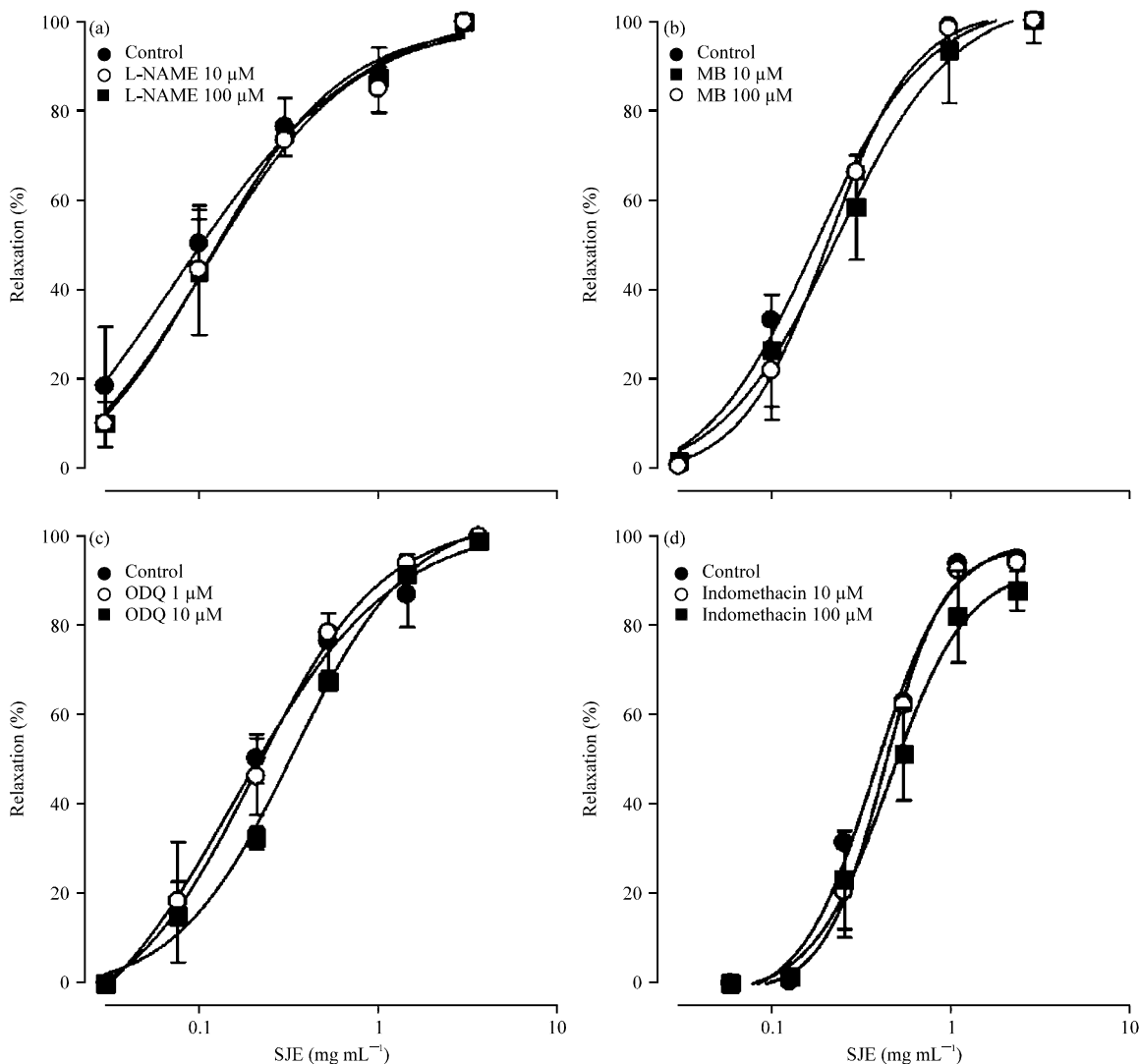


Fig. 4(a-d): Relaxation effects of SJE on PE-induced contraction of rabbit aortic strips in the presence of (a) L-NAME (10-100 μM), (b) Methylene blue (10-100 μM), (c) ODQ (1-10 μM) and (d) Indomethacin (10-100 μM). Relaxations are expressed as percentage of PE-induced contraction according to concentration in logarithmic scale and given as Means ± SE, mean (n = 4 to 6)

IC<sub>50</sub> values of  $3.99 \pm 0.45$  and  $7.23 \pm 0.58$  μg mL<sup>-1</sup> for PE- and K<sup>+</sup>-induced contractions, respectively (Fig. 6a). It was also observed that at the highest concentration of SJE (0.3 mg mL<sup>-1</sup>), both PE and K<sup>+</sup>-induced tone of the RbCC were completely abolished (Fig. 6a). The reference drugs SNP (IC<sub>50</sub>  $0.13 \pm 3.47$  μg mL<sup>-1</sup>), phentolamine (IC<sub>50</sub>  $0.30 \pm 1.90$  μg mL<sup>-1</sup>) and sildenafil (IC<sub>50</sub>  $0.15 \pm 1.23$  μg mL<sup>-1</sup>) also displayed concentration-dependent relaxations of PE-induced tension (Fig. 6b). A comparison of the IC<sub>50</sub> values shows that all the reference drugs were more potent than SJE (Table 2). In

the experiment to elucidate the mechanism of the relaxation produced by SJE, it was observed that ODQ caused a rightward shift in the concentration response curves of both SNP (a nitric oxide donor) and sildenafil (a phosphodiesterase inhibitor) (Fig. 7a, b). Moreover, the relaxing effect of SJE was significantly affected by both L-NAME and ODQ (Fig. 7c, d) as indicated by their IC<sub>50</sub>s when compared to the control (Table 2). It thus appeared that the carvenosal smooth muscle relaxation caused by SJE was partly mediated by the NO-guanylyl cyclase pathway.

Table 1:  $E_{max}$  and  $IC_{50}$  for the relaxation effects of SJE on PE-induced contraction of rabbit aortic strips in the presence of (A) L-NAME (10-100  $\mu$ M), (B) methylene blue (10-100 mM), (C) ODQ (1-10  $\mu$ M), (D) indomethacin (10-100  $\mu$ M) (n = 4 to 6)

	$E_{max}$ (mm)	$IC_{50}$ (mg mL <sup>-1</sup> )
<b>A</b>		
Control	50.81 ± 5.28	0.0721 ± 0.06
L-NAME (10 $\mu$ M),	51.80 ± 5.46	0.1073 ± 0.04
L-NAME (100 $\mu$ M),	54.91 ± 5.87	0.1102 ± 0.03
<b>B</b>		
Control	52.05 ± 1.53	0.1758 ± 0.02
Methylene blue (10 mM)	51.35 ± 2.53	0.2074 ± 0.03
Methylene blue (100 mM)	52.60 ± 6.86	0.2308 ± 0.09
<b>C</b>		
Control	51.65 ± 6.55	0.0721 ± 0.06
ODQ (1 $\mu$ M)	52.75 ± 2.76	0.0967 ± 0.02
ODQ (10 $\mu$ M)	52.55 ± 1.77	0.1740 ± 0.02
<b>D</b>		
Control	52.25 ± 2.00	0.1799 ± 0.07
Indomethacin (10 $\mu$ M)	51.00 ± 2.46	0.2186 ± 0.10
Indomethacin (100 $\mu$ M)	51.45 ± 4.47	0.2319 ± 0.30

L-NAME = N<sup>o</sup>-nitro-L-arginine methyl ester; ODQ = 1H-[1,2,4]-oxadiazole-[4,3-a]-quinoxalin-1-one; PE = phenylephrine; SJE = *Sphenocentrum jollyanum*

Table 2:  $E_{max}$  and  $IC_{50}$  for relaxation effects of SNP, sildenafil and SJE on PE-induced contraction of rabbit corpus cavernosum in the presence of L-NAME and ODQ (n = 3 to 5)

	$E_{max}$ (mm)	$IC_{50}$ ( $\mu$ g mL <sup>-1</sup> )
<b>SNP</b>		
Control	48.51 ± 7.71	0.0003 ± 0.00
ODQ (10 $\mu$ M)	14.13 ± 0.68	0.0021 ± 0.00
<b>Sildenafil</b>		
Control	50.30 ± 9.08	0.0036 ± 0.00
ODQ (10 $\mu$ M)	44.23 ± 6.09	0.9124 ± 0.16
<b>SJE</b>		
Control	52.55 ± 7.98	3.991 ± 4.11
L-NAME (100 $\mu$ M),	50.75 ± 5.57	5.000 ± 3.17
L-NAME (300 $\mu$ M),	44.53 ± 2.17	11.00 ± 2.00
<b>SJE</b>		
Control	47.58 ± 6.51	4.100 ± 2.33
ODQ (1 $\mu$ M)	46.54 ± 4.07	5.500 ± 1.60
ODQ (10 $\mu$ M)	41.67 ± 4.39	10.30 ± 2.97

L-NAME = N<sup>o</sup>-nitro-L-arginine methyl ester; ODQ = 1H-[1,2,4]-oxadiazole-[4,3-a]-quinoxalin-1-one; PE = phenylephrine; SJE = *Sphenocentrum jollyanum*; SNP = sodium nitroprusside.

## DISCUSSION

The use of herbal remedies as an alternative in the management of ED is popular in many parts of the world<sup>11,12</sup>. *Sphenocentrum jollyanum* is one of several plants used for their alleged aphrodisiac properties to enhance libido and sexual function in West Africa<sup>9,13,14,15,16</sup>. The goal of the present study is to ascertain the possible peripheral mechanism of action of SJE. This could then complement our previous study<sup>17</sup> to adequately substantiate claims for its use.

**Experiments on Rabbit aortic strip:** In the vascular tension studies, SJE induce concentration-dependent relaxation of RbAS pre-contracted by PE or K<sup>+</sup>, demonstrating a vasorelaxant activity of the extract. This

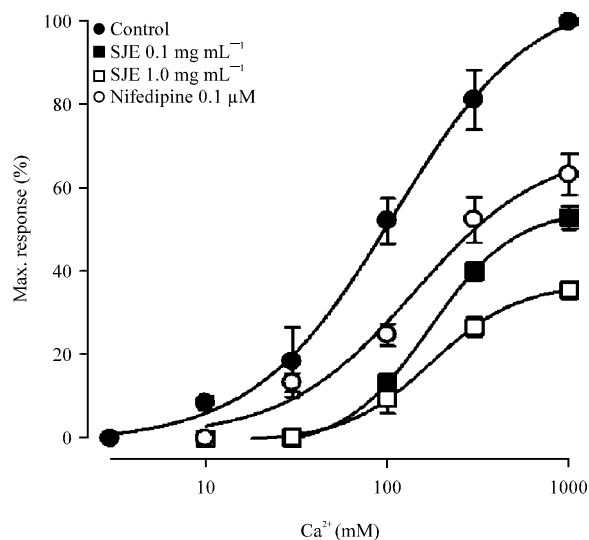


Fig. 5: Effect of nifedipine and SJE on concentration-response curves of CaCl<sub>2</sub>-induced contractions of isolated rabbit aortic strip depolarized with K<sup>+</sup> (100 mM). The concentration-response relationships were obtained by addition of doses of CaCl<sub>2</sub> following 15 minutes pre-treatment with of nifedipine (0.1  $\mu$ M) or SJE (0.1 and 1.0 mg mL<sup>-1</sup>) in Ca<sup>2+</sup> free, isotonic K<sup>+</sup> (100 mM) medium as described in the method. Responses are expressed as percentages of maximum CaCl<sub>2</sub>-induced response. Each point represents the Mean  $\pm$  SEM (n = 3)

also shows that the inhibitory effects of SJE are not only on receptor-mediated contractions such as those produced by PE, but also on those produced by ions such as K<sup>+</sup>. It was also observed that pretreatment of the RbAS with SJE caused a rightward shift of concentration-response curves for PE and K<sup>+</sup> in a dose dependent fashion with depression of the maximum responses. Although, the reason for the depression of the maximum response is unknown, it suggests that the interaction between SJE and the agonists (PE and K<sup>+</sup>) is not truly competitive. These results suggests that SJE either acts further downstream on a receptor that links the action of the agonists to the final response observed, or it interferes with other post-receptor events that contribute to the tissue response, such as blocking the influx of Ca<sup>2+</sup> necessary for sustained smooth muscle contraction<sup>29</sup>.

Additionally, SJE-induced relaxations were not affected by K<sup>+</sup> channel blockers, (glibenclamide and TEA), muscarinic antagonist (atropine) and  $\beta$ -adrenoreceptor antagonist (propranolol). This suggests that SJE did not activate K<sup>+</sup> channel nor interacted with muscarinic or  $\beta$ -adrenoreceptors to induce the relaxation

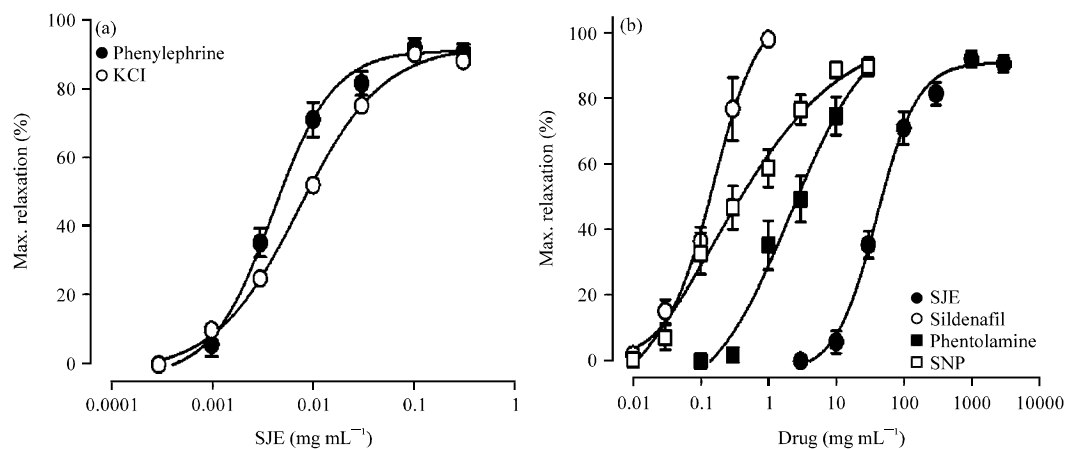


Fig. 6(a-b): (a) SJE caused relaxation of PE (0.1  $\mu$ M) and KCl (80 mM)-induced contraction of rabbit corpus cavernosum strips. IC<sub>50</sub> ( $\mu$ g mL<sup>-1</sup>) of SJE for: PE-induced contraction,  $3.99 \pm 0.45$ ; KCl-induced contraction,  $7.23 \pm 0.58$  (b) Relaxation effect of SJE, SNP, sildenafil and phentolamine on PE-induced contraction of rabbit corpus cavernosum strips. Relaxations are expressed as percentage of PE-induced contraction according to concentration in logarithmic scale and given as Means  $\pm$  SE mean (n = 6 to 8). IC<sub>50</sub> ( $\mu$ g mL<sup>-1</sup>) of SJE,  $3.991 \pm 0.45$ ; SNP,  $0.1591 \pm 0.22$ ; phentolamine,  $2.146 \pm 1.25$ ; sildenafil,  $0.1559 \pm 0.04$

observed in the rabbit aortic strip and the corpus cavernosal smooth muscle preparation. Furthermore, the inability of indomethacin, L-NAME or methylene blue to inhibit the myorelaxant actions of SJE shows that the relaxation caused by SJE was not mediated by prostacyclin nor the NO-guanylyl cyclase pathway. Whereas indomethacin inhibits the production of prostacyclin, methylene blue and ODQ have been reported to inhibit the activation of guanylyl cyclase<sup>30</sup>. Contraction of smooth muscle is dependent upon an increase in the concentration of cytoplasmic free Ca<sup>2+</sup> that activates the contractile elements, the source of which may be extracellular or intracellular<sup>31,32</sup>. Potential sensitive calcium channels are activated by depolarization of the plasma membrane when the extracellular K<sup>+</sup> concentration is increased. Several evidences have revealed that the relaxant effect of a calcium channel blocker becomes more pronounced when smooth muscle is depolarized<sup>33</sup>. Pretreatment with SJE caused a rightward shift as well as suppressed, in a concentration-dependent manner, the aortic contractile response to CaCl<sub>2</sub> in Ca<sup>2+</sup>-free high K<sup>+</sup> (100 mM) medium, suggesting that the effects of the extract may be mediated partly by inhibition of Ca<sup>2+</sup> mobilization.

**Experiments on corpus cavernosal smooth muscle preparation:** SJE caused a concentration-dependent relaxation of both PE-and K<sup>+</sup>-induced tension in the RbCC. The extract exhibited relatively more potent effect on the cavernosal smooth muscle compared to vascular smooth muscle. This is very important because

it indicates that the concentration required for enhancing erection is unlikely to cause any significant hypotensive side effect. Subsequent analysis with nitric pathway inhibitors, L-NAME and ODQ significantly modified the concentration-response curve of SJE in RbCC suggesting that the vasorelaxation caused by SJE was partly mediated by the NO-guanylyl cyclase pathway. The contracted state of the RbCC smooth muscle is considered to be mediated by release of norepinephrine, endothelin-1, neuropeptide Y, prostanoids and angiotensin II<sup>3,28,34,35</sup>. Any inhibition of these agonists at the receptor level or in their downstream signaling pathways may lead to a decrease in myosin regulatory light chain (RLC20) phosphorylation to cause relaxation through decreased cytoplasmic [Ca<sup>2+</sup>] and/or inhibition of Ca<sup>2+</sup> sensitization<sup>36</sup>. Ca<sup>2+</sup> sensitization has been shown to make a significant contribution to agonist-induced contraction and is, at least in part, under the control of the RhoA/Rho-kinase systems<sup>36,37,38</sup>. Thus, the contractile activity of cavernosal smooth muscles is regulated by the concentration of free Ca<sup>2+</sup> in the cytosol, which depends partially on extracellular Ca<sup>2+</sup> influx and mobilization of internal Ca<sup>2+</sup> store<sup>39</sup>. The contraction of cavernosal smooth muscles are inhibited by Ca<sup>2+</sup> channel blockers<sup>39,40,41</sup>. The ability of SJE to cause significant relaxation of K<sup>+</sup>-and PE-induced contraction of the RCC, as observed in the present study, suggests that inhibition of Ca<sup>2+</sup> signalling (as was observed for the RbAS) could be involved in its relaxant effect on cavernosal smooth muscle<sup>41,42</sup>. Our finding that *S. jollyanum* relaxes vascular and cavernosal smooth



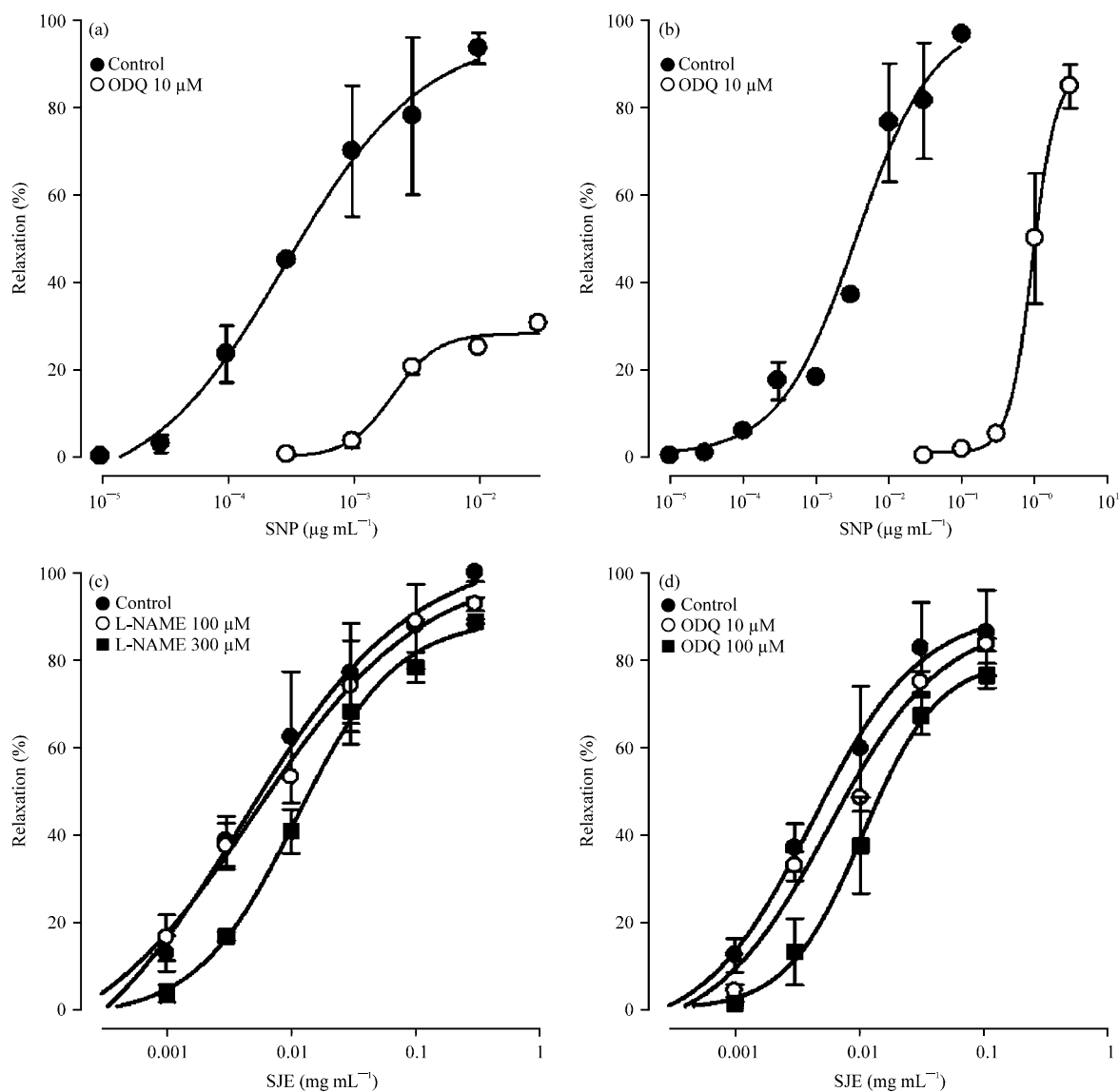


Fig. 7(a-d): Relaxation effect of SNP (a), Sildenafil, (b) SJE, (c and d) on PE-induced contraction of rabbit corpus cavernosum strips in the presence of L-NAME and ODQ. Relaxations are expressed as percentage of PE-induced contraction according to concentration in logarithmic scale and given as Means  $\pm$  SE mean (n = 3 to 5)

muscles thus provides evidence of another possible mechanism of action of the plant which may contribute to validating its folkloric use in the management of ED.

The non-specific inhibitory effect of SJE observed is not surprising considering the fact that plant extracts contain myriads of chemical agents with a multiplicity of pharmacological activity<sup>43</sup>. Phytochemical screening shows that SJE contain terpenoids, flavanoids, saponins, tannins, anthraquinones, glycosides and alkaloids<sup>44</sup>.

Indeed, the presence of an alkaloid that corresponded to berberine, which possess smooth muscle relaxant activities, has been confirmed<sup>45,46</sup>. It has been shown that berberine possesses a relaxant effect on the rabbit corpus cavernosum tissue<sup>47,48</sup>. Moreover, flavonoids have been shown to possess relaxant properties,<sup>49,50</sup> an effect attributed to action on the mobilization of  $\text{Ca}^{2+}$ <sup>51,52,53</sup>. Thus, the peripheral action of SJE involved in the treatment for ED is probably by antagonizing  $\text{Ca}^{2+}$ <sup>54</sup>.

## CONCLUSION

The present study has demonstrated the ability of SJE to relax tension development in RbAS and RbCC induced by PE and high-K<sup>+</sup> solution. The results have provided evidence to support the use of *S. jollyanum* in the treatment of ED in traditional medicine. Other experimental approaches will be necessary to delineate the exact constituent of SJE responsible this action in smooth muscles.

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