ANTISPASMODIC ACTIVITY OF *TEUCRIUM STOCKSIANUM* BOISS.

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ABSTRACT
The aim of the study was to explore the traditional antispasmodic use of *Teucrium stocksianum* on scientific grounds, and preliminary screen the specie for phytochemical constituents. Crude methanolic extract of aerial parts of *Teucrium stocksianum* (Ts.Cr) was studied for possible relaxant effects on spontaneous rabbits' jejunum preparations at concentrations of 0.01, 0.03, 0.1, 0.3, 3.0, 5.0 and 10.0 mg/ml. Effects of Ts.Cr was also studied on 80 mM KCl-induced contractions to find out possible mechanism as antispasmodic. Calcium chloride curves were constructed in the presence and absence of Ts.Cr at different concentrations in decalcified rabbits' jejunum preparations and were compared with curves of verapamil. Plant was also screened for different classes of phytochemicals. The plant gave positive tests for the presence of different classes of phytochemicals like carbohydrates, proteins and amino acids, saponins, tannins, flavonoids and sterols. It gave negative tests for alkaloids, anthraquinone glycosides and cardiac glycosides. Ts.Cr caused 100 % relaxation of the spontaneous rabbit's jejunum preparations at dose of 5.0 mg/ml (EC_{50} = 1.98 mg/ml ± 0.07, n=6). Contractions induced by 80 mM potassium chloride (KCl) were also relaxed 100 % by Ts.Cr at dose of 5.0 mg/ml. When tested for possible calcium channel blockade in calcium free K^+-rich medium, Ts.Cr at concentration of 0.3 mg/ml produced a right shift in the calcium chloride curves vs. control (log EC_{50} =-1.78 ± 0.03 [Ca^{++}] M vs. control log EC_{50} = -2.57 ± 0.07 [Ca^{++}] M). In similar fashion, a right shift was observed for the calcium chloride curves in the tissues treated with 0.1 µM verapamil. When tested on histamine and barium chloride induced contractions, the extract produced no significant effect. The results confirm the folkloric use of *Teucrium stocksianum* as antispasmodic possibly through the calcium channel blocking mechanism.

Keywords: *Teucrium stocksianum*; folkloric use; antispasmodic; calcium channel blocking activity; verapamil.

INTRODUCTION

Teucrium stocksianum Boiss belongs to family Lamiaceae that have extensive traditional uses as medicinal plant (Naghibi *et al*., 2005). Reported activities for the Teucrium genus are anti-inflammatory, antifungal, antibacterial, antispasmodic and antioxidant (Bashir *et al*., 2007). Interests in the other species of Teucrium genus developed as its hypotensive effects were reported (Calatayud *et al*., 1998). Moreover, the importance of the biological activities of the Teucrium species is evident as larvicidal, antispasmodic, antinocieceptive, hypoglycemic, hepatoprotective (Ali *et al*., 2008; Bashir *et al*., 2007). Specie like *Teucrium royleanum* has been used as antispasmodic, astringent, antipyretic and anti allergic (Bashir *et al*., 2007). *Teucrium stocksianum* is an aromatic shrub with reported gastro protective effect (Islam *et al*., 2002; Jaimand *et al*., 2006). In addition to the gastro protective effect, decoction of *Teucrium stocksianum* has been used for the treatment of diabetes and burning feet syndrome (Barkatullah *et al*., 2009). Aerial parts of *Teucrium stocksianum* has been used in treatment of pyrexia (Naghibi *et al*., 2005), sore throat, and as an expectorant (Wazir *et al*., 2007). Extract of *Teucrium stocksianum* has been used as stomachic and in the management of diarrhea. On the basis of reported traditional uses of *Teucrium* genus and *Teucrium stocksianum* specie as antispasmodic, we carried out current experimental work to explore the plant on scientific grounds.

MATERIALS AND METHODS

Collection and identification of plant materials

The fresh aerial parts of Ts.Cr was collected in the month of July-August 2009 from the nearby hills of the campus-I of University of Malakand, Chakdara, Dir lower, Khyber Pakhtunkhwa, Pakistan. Professor Dr. Jehandar Shah identified the plant. A specimen numbered as T-01-2009 has been submitted to the herbarium Malakand University.

Preparation of the extract

The aerial parts of the shade dried materials (600g) of *T. stocksianum* were soaked in methanol and subjected to occasional shaking. The materials were filtered off after 10-15 days and the process was done thrice (Bashir *et al*., 2007). The filtrates were combined and concentrated under reduced pressure at 40 ^oC using rotary evaporator. A blackish extract (13.0 g) was obtained and stored in freezer.

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Drugs and animals

Acetylcholine was purchased from BDH Chemicals, Poole, England. Rests of the chemicals were purchased from E. Merck Germany. All the solutions were prepared on the same day of experiments. Either sex rabbit's weighing 1.5-2.0 kg were used in the experiments. The animals had a free access to water. They were kept in fasting conditions 24 hours prior to start of the experiments. The animals were treated as per the "Animals Bylaws 2008 of the University of Malakand (Scientific Procedures Issue- 1)" complying with international standards for dealing the experimental animals duly approved by the legal bodies of the University of Malakand.

Data recording

Force Transducer (MLT 0210/A) connected with Power lab ADInstruments, Australia was used to record the tissue(s) responses. Setting parameters were 5Hz X 10 gain (input 1) @ 40/S, Low pass, Range 20mv.

Phytochemical screening

Preliminary phytochemical screening of the Ts.Cr for presence of saponins, alkaloids, tannins, anthraquinone glycosides, flavonoids, cardiac glycosides (Aduragbenro *et al*, 2009), carbohydrates, proteins and amino acids, and sterols (Walis, 1985; Harborne, 1973; Kokate *et al*., 1994) was carried out.

Rabbit's jejunum preparations

Effects of Ts.Cr on spontaneous jejunum preparations were carried out at concentration of 0.01, 0.03, 0.1, 0.3, 3.0, 5.0 and 10.0 mg/ml (Gilani *et al*., 2005; Niaz *et al*., 2009). Abdomens of the cervical dislocated animals were opened and jejunum's portions of about 1.5-2.0 cm were removed. Each preparation was cleared off the mesentery so that it could freely give spontaneous contractions. Once the preparation was stabilized, the Ts.Cr was tried at concentration of 0.01, 0.03, 0.1, 0.3, 3.0, 5.0 and 10.0 mg/ml at a minute interval in cumulative manner and the effects were recorded (n=6).

Effects on KCl-induced contractions in rabbit's jejunum preparation

To explain the possible mode of actions, we carried out experiments on rabbit's jejunum preparations. The tissues were maintained in form of sustained contractions by giving 80 mM KCl (Final bath concentration). The sustained contractions were then treated by the Ts.Cr in similar fashion at concentration of 0.01, 0.03, 0.1, 0.3, 3.0, 5.0 and 10.0 mg/ml as per reported work (Gilani *et al*., 2005; Niaz *et al*., 2009). Again the responses were recorded (n=6).

Calcium channel blocking activity

The tissues were stabilized for at least 30 minutes in Tyrode's solution. The tissues were exposed to calcium free Tyrode's solution (K^+-Normal Tyrode's solution) followed by K^+-Rich Tyrode's solution to decalcify the tissues (Gilani *et al*., 2005). The decalcified tissues were then calcified with known concentration of calcium and normal control curves (n=3) were constructed in the tested tissues. The tissues were then treated with known concentration of Ts.Cr (0.3 and 1.0mg/ml) and an incubation period of about one hour was given. Calcium curves were constructed (n=6). In similar fashion, curves for verapamil, a standard calcium channel blocker, were also constructed and compared.

Effects on Barium chloride and histamine induced contractions

The Ts.Cr was also studied for possible relaxant effects on barium chloride and histamine induced contraction to study that whether the relaxant effect is either through the release of calcium from internal stores or through the receptor operated channels (Gutierrez and Solis, 2009).

Statistics and interpretation

Chart 5, purchased from AD Instruments was used to interpret the graph tracings. Student "t" test was used at 95 % confidence interval (CI). 'P' values less or equal to 0.05 was considered as statistically significant.

RESULTS

Preliminary Phytochemical screening

The preliminary phytochemical screening gave positive tests for the presence of different classes of phytochemicals like carbohydrates, proteins and amino acids, saponins, tannins, flavonoids and sterols. It gave negative tests for alkaloids, anthraquinone glycosides and cardiac glycosides.

Effects on spontaneous and KCl–induced contractions on rabbit's jejunum preparations

Self-explanatory effects of Ts.Cr are shown in fig. 1. Ts.Cr produced a relaxant effect on spontaneous rabbit's jejunum preparations with EC_{50} value of 1.98mg/ml ± 0.07 (1.7-2.2, n=6). There was 100 % relaxation at concentration of 5.0 mg/ml. High concentration (80 mM) of KCl-induced contractions were also relaxed by Ts.Cr with EC_{50} value of 2.0 mg/ml ± 0.081 (1.7-2.3, n=6). The nearly same EC_{50} values for effects on both spontaneous and KCl-induced contractions predicted that relaxant effect may be through single mode of action possible through calcium channel blockade (statistically non significant, calculated t value=0.0996 at $P \leq 0.05$, two tailed distribution).

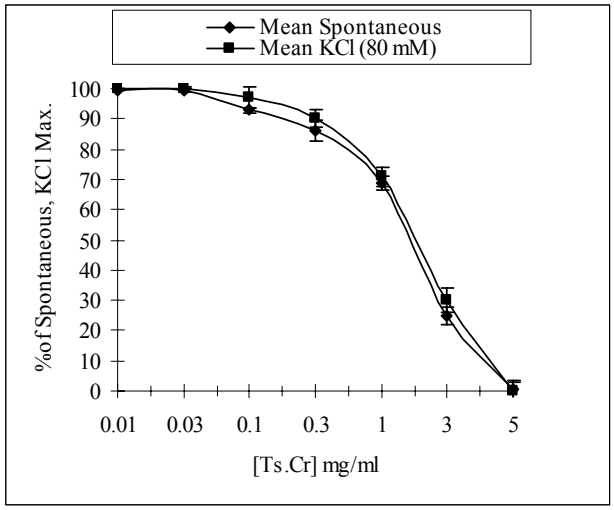


Fig. 1: Effects of Ts.Cr on spontaneous activity and contractions induced by KCl (Mean ± SEM, *n*=6).

Calcium channel blocking activity

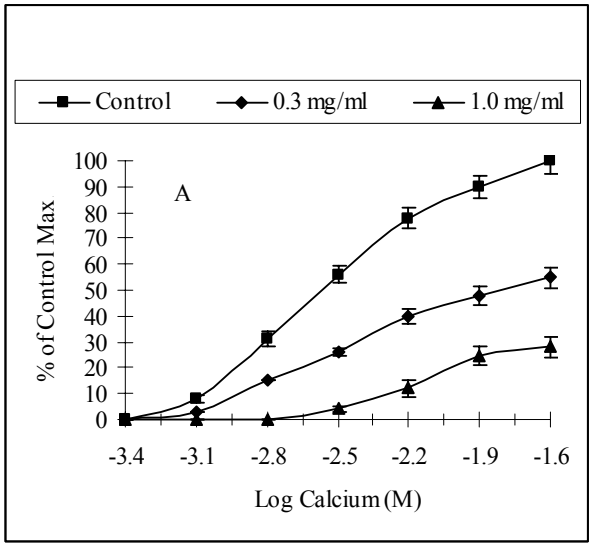
As positive relaxant effects by the test drug or extract do not always give positive blockade of calcium channels (Kobayashi *et al*., 1989), therefore, we carried out work to study the effects of Ts.Cr on calcium channels. Ts.Cr at concentration of 0.3 mg/ml produced rightward shift in the calcium chloride curves (fig. 2a) with log EC_{50} value of -1.78 ± 0.03 [Ca^{++}] M vs. control with log EC_{50} value of -2.57 ± 0.07 for calcium chloride curves. In similar fashion, the verapamil at a concentration of 0.01 µM (fig. 2b) produced rightward shift with EC_{50} value of -1.66 ± 0.03 log [Ca^{++}] M (-1.6 to -1.9, n=6) vs. control with EC_{50} value of -2.67 ± 0.07 log [Ca^{++}] M (-2.2 to -2.9, n=6).

Effects on barium chloride and histamine induced contractions

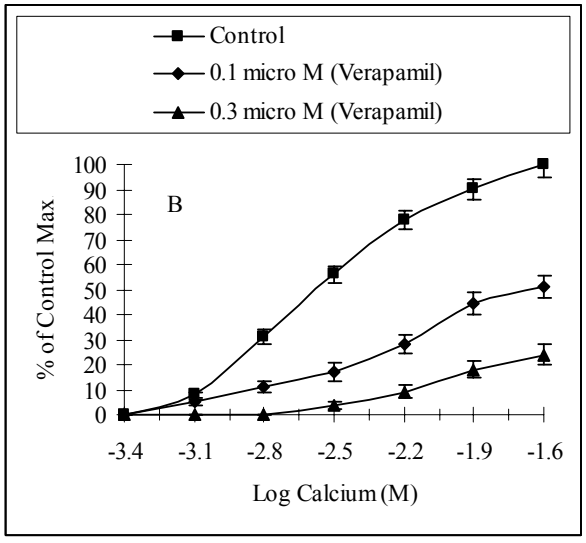
The extract produced no statistical significant effect on barium chloride and histamine induced contractions (data not shown).

DISCUSSION

The current study was carried out to validate some of the traditional uses of *T. Stocksianum* as anti-spasmodic. The extract relaxed the spontaneous rabbit's jejunum preparations showing an antispasmodic action. The extract also relaxed the KCl-induced contractions with nearly same EC_{50} values suggesting that possible mode of actions may be through calcium channel blockade (Ali *et al*., 2009; Gilani *et al*., 2005). Contractile effects of the intestine are due to the cytosolic free calcium levels. Moreover, intracellular and extracellular calcium stores exchange with each other. Periodic depolarization and repolarization of the tissues are due to influx of calcium into sarcoplasmic reticulum through voltage dependent calcium channel. These events are responsible for spontaneous intestinal responses (Bolton, 1979; Karaki and Wiess, 1983; Godfraind *et al*., 1986; Carl *et al*., 1996). It is not necessary that relaxing effects on contractions induced by KCl do not always suggest for calcium channel blocking activity mechanism (Kobayashi *et al*., 1989), therefore, we developed calcium chloride curves in presence of Ts.Cr in calcium free depolarizing medium. The results confirmed that the mode of action of *T. stocksianum* is through the calcium channels as the right shift effects were resembling the effects of verapamil (Cortes *et al*., 2006). Moreover, Ts.Cr did not produce relaxant effects on histamine induced



(a) In presence of Ts.Cr



(b) In presence of verapamil. (Mean ± SEM *n*=6).

Fig. 2: Effects of Ts Cr on calcium chloride curves.

contractions, suggesting that the relaxant effect is not through receptor operated channels that occur with H1 receptor agonists (data not shown). Also there was no significant effect on the barium chloride (an agent that releases bound calcium) induced contractions. Hence, it is concluded that *T. stocksianum* produced antispasmodic actions possibly through calcium channel blocking mechanism of voltage dependent calcium channels and not through the histaminergic receptors or through the release of bound calcium.

It is thus postulated that the relaxant effect of *T. Stocksianum* on the rabbit's jejunum preparation may be attributed to the phytochemical constituents such as saponins, flavonoids, tannins and triterpenes present in the plant as similar type of studies in medicinal plants with same phytochemicals have been reported to have antispasmodic activity (Cortes *et al*., 2006).

CONCLUSION

The antispasmodic study supports the rationale for use of *T. Stocksianum* in treatment of gastrointestinal disorders. Moreover, the relaxant effects on KCl-induced contractions, and no significant effects on barium chloride and histamine induced contractions confirm that the relaxant effect is dominantly through voltage dependent calcium channels and not through the receptor operated channels or through the release of internal calcium stores.

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