BJP

Bangladesh Journal of Pharmacology

Research Article

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A Journal of the Bangladesh Pharmacological Society (BDPS) **Bangladesh J Pharmacol 2014; 9: 203-207**
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index
ISSN: 1991-0088

Functional nature of the spasmolytic effect, phytochemical composition and acute toxicity studies on *Sauromatum guttatum*

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Article Info

Received: 15 April 2014
Accepted: 22 April 2014
Available Online: 26 April 2014

DOI: 10.3329/bjp.v9i2.18602

Cite this article:
Shah N, Shah AJ, Ahmed M, Gilani AH. Functional nature of the spasmolytic effect, phytochemical composition and acute toxicity studies on *Sauromatum guttatum.* Bangladesh J Pharmacol. 2014; 9: 203-07.

Abstract

The aim of the present work was to study the functional nature of the potential spasmolytic effect of the crude extract of *Sauromatum guttaum*. It was found positive for the presence of alkaloid and tannins. In isolated rabbit jejunum preparation, *S. guttaum* caused inhibition of spontaneous and high K^+ (80 mM)-induced contractions, with respective EC_{50} values (95% confidence intervals) of 1.5 mg/mL (0.7-3.0) and 1.2 mg/mL (0.8-1.6), similar to verapamil. Inhibition of high K^+ -induced contractions suggests Ca^{++} antagonistic effect. The Ca^{++} channel blocker activity of *S. guttaum* was confirmed when pre-treatment of the tissues with extract (0.3-3 mg/mL) caused a rightward displacement in the Ca^{++} concentration-response curves. Moreover, in the acute toxicity test, *S. guttaum* was found safe up to the dose of 3 g/kg. The findings of the current study suggest that the *S. guttaum* exhibited spasmolytic activity, possibly mediated through inhibitory effect on Ca^{++} entry and was found safe and this current study provides first evidence to the potential use of this plant as antispasmodic and can play a possible role as antidiarrheal.

Introduction

Sauromatum guttatum Schott., (Syn: *S. vencsum)* belongs to the family Araceae and is locally known as "Sanp Ki Booti" and commonly called as "Voodoo lilly or Snake Plant" (Baquar, 1989). It is a shade loving plant and found in the Northern areas of Pakistan. The plant has not been traditionally explored for its medicinal uses, although the family Araceae has wide reputation in the traditional medicine. However, *Acorus calamus* belongs to family Araceae has traditionally been used in a variety of conditions, such as diarrhea, spasm, colic, flatulence and asthma (Baquar, 1989; Gilani et al., 2006). Similarly, we also have reported *A. calamus* as antispasmodic (Gilani et al., 2006), vasomodulator (Shah et al., 2009) and bronchodilator (Shah et al., 2010).

To our knowledge, there is no report in the literature on pharmacological and or biological activities of *S. guttatum*. The present study was therefore carried out to investigate the potential usefulness of *S. guttatum* in hyperactive gut disorders using *in vitro* pharmacological protocols.

Materials and Methods

Plant materials

Crude extract of the rhizome of *S. guttatum* was obtained from HEJ Research Institute of Chemistry, Interna-

tional Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan. A voucher specimen (Sg-231) was deposited at the herbarium of the same Institute.

Preliminary phytochemical analysis

Crude extract of *S. guttatum* was tested for the presence of different phytochemical constituents, such as saponins, flavonoids, flavanols, flavones, tannins, phenols, coumarins, sterols, terpenes, alkaloids and anthraquinones by using methods described previously (Edeoga et al., 2005).

Drugs and standards

The following reference chemicals were obtained from the sources specified: acetylcholine chloride, potassium chloride, calcium chloride and verapamil (Sigma Chemical Company, USA). All other chemicals used were of the highest purity grade. Stock solutions were made in distilled water and the dilutions were made fresh in normal saline on the day of experiment.

Animals

Local rabbits (1.5-2 kg) of either gender used in the study were given water *ad libitum* and a standard animal diet. The animals were maintained in the animal house facility of the Department of Biological and Biomedical Sciences, the Aga Khan University and Hospital, Karachi, Pakistan. Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (NRC, 1996).

Isolated rabbit jejunum preparations

The isolated tissue experiments were carried out as previously described (Gilani et al., 2005; Shah et al., 2011a; Khan et al., 2012). The animals had free access to water but were fasted for 24 hours before the experiment. The animals were sacrificed by cervical dislocation and segment of the jejunum about 2-3 cm long was removed. The intestinal contents were removed by flushing with Tyrode's solution and placed in Petri dishes containing Tyrode's solution. The tissues were constantly aerated with carbogen gas (5%carbon dioxide in oxygen). The composition of the Tyrode's solution was as follows in mM: KCl 2.7, NaCl 136.9, $MgCl_2$ 1.1, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.6 and $CaCl_2$ 1.8 (pH 7.4). These tissues were then mounted in 10 mL tissue baths containing Tyrode's solution. Temperature was maintained at 37°C and aerated with carbogen gas. An initial tension of 1 g was applied to each tissue and kept undisturbed for an equilibrium period of 30 min with physiological solution changed every five min.

Effects of the extract on isolated rabbit jejunum

At the end of equilibration period, control responses to a sub-maximal concentration (0.3 mM) of acetylcholine were obtained and the tissue presumed stable only after the reproducibility of the said responses. Under these experimental conditions, rabbit jejunum exhibits spontaneous rhythmic contractions, allowing testing the relaxant (spasmolytic) activity directly without the use of an agonist (Gilani et al., 2005; Shah et al., 2011a).

Determination of calcium antagonist activity

To understand the possible mechanism of action, the extract was tested on KCl-induced contractions in rabbits' jejunum preparation (Farre et al., 1991). Extract of *S. guttatum* and standard, such as verapamil were then added cumulatively to obtain concentration-dependent inhibitory responses (van-Rossum, 1963). The relaxation of jejunal preparations, pre-contracted with K^+ (80 mM) was expressed as percent of the control.

For the confirmation of possible Ca^{++} entry blocking activity of the crude extract, the tissues were allowed to stabilize in normal Tyrode's solution, which was then replaced with Ca^{++}-free Tyrode's solution containing EDTA. An incubation of 20-30 min was given in order to remove Ca^{++} from the tissues and surroundings. This solution was further replaced with K^+-rich and Ca^{++}-free Tyrode's solution, having the following composition (mM): KCl 50, NaCl 91.04, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, glucose 5.55 and EDTA 0.1. Following an incubation period of 30 min, control concentration-response curves of Ca^{++} were constructed. When the control CRCs of Ca^{++}, were found superimposable, the tissues were pre-treated with the plant extract for 40-50 min to test the possible calcium entry blocking effect. The concentration-response curves of Ca^{++} were re-constructed in the presence of different concentrations of the crude extract and verapamil.

Acute toxicity test

The method previously described by (Gilani et al., 2005) was adopted. Briefly, 15 mice in three groups five in each were used in the experiment. The test was performed using increasing doses of the crude extract of *S. guttatum* (1-3 g/kg body weight), given orally, in 10 mL/kg volume to different groups serving as test groups. Another group of mice was administered saline (10 mL/kg) served as negative control. The mice were allowed food *ad libitum* and kept under regular observation for 6 hours while the lethality was recorded after 24 hours.

Statistics

All data expressed are mean ± standard error mean (SEM) and the median effective concentration (EC_{50} values) with 95% confidence intervals (CI). The statistical parameter applied to the Student's t-test (paired or unpaired) with $p<0.05$ as significantly different (GraphPAD program, GraphPAD, San Diego, Ca, USA),

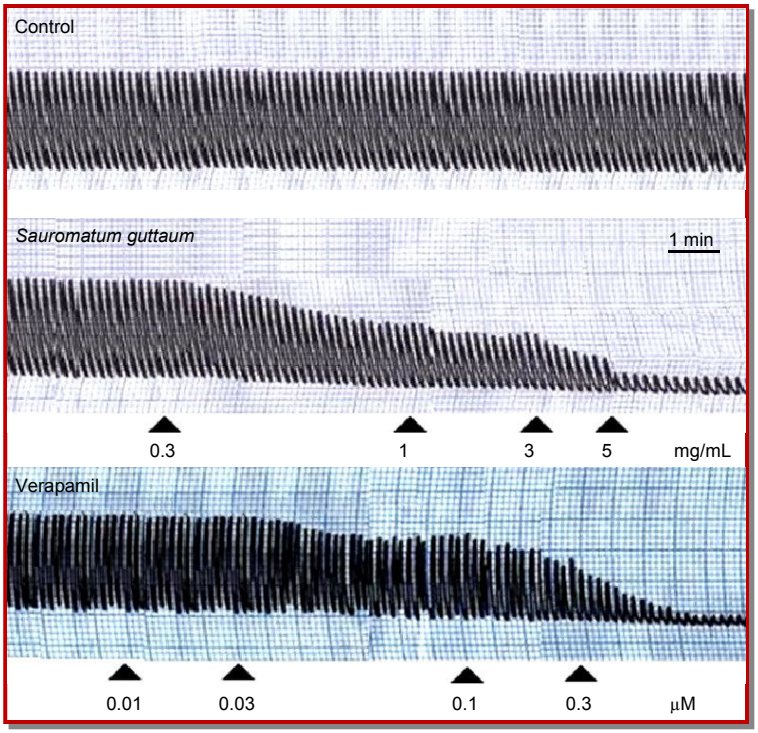

Figure 1: A representative tracing shows the spasmolytic effect of the crude extract of *S. guttaum* and verapamil on spontaneous contractions in isolated rabbit jejunum preparation

concentration response curves were analyzed by non-linear regression (GraphPAD program).

Results and Discussion

In isolated rabbit jejunum preparations, cumulative addition of the crude extract of *S. guttatum* and verapamil suppressed the spontaneous contractions (Figure 1). The median effective concentration (EC_{50} value) of extract was 1.5 mg/mL (0.7-3.0), as shown in Figure 2A, this shows intestinal smooth muscle relaxant (spasmolytic) activity.

The contraction of smooth muscle preparations including rabbit jejunum is dependent upon an increase in the cytoplasmic free [Ca^{++}], which activates the contractile elements (Karaki, 1987). The increase in cellular Ca^{++} occurs either through influx via voltage-dependant Ca^{++} channels or release from intracellular stores in the sarcoplasmic reticulum. Periodic depolarization and repolarization regulates the spontaneous movements of the intestine and at the height of depolarization, the action potential appears as a rapid influx of Ca^{++} via voltage-dependant Ca^{++} channels (Brading, 1981).

Intracellular and extracellular calcium stores also exchange with one other that are responsible for the periodic depolarization and repolarization of jejunal tissues (Abouzid et al., 2008). Hence, it is suggested that the inhibitory effect of *S. guttatum* crude extract on spontaneous movements of rabbit jejunum may be due to interference either with the Ca^{++} release or with the Ca^{++} influx through voltage-dependant Ca^{++} channels.

We previously observed that the spasmolytic constituents present in different medicinal plants mediate their effect through Ca^{++} channel blockade (Gilani et al., 2005; Shah et al., 2010; Khan et al., 2011; Shah et al., 2011b). To see, whether the spasmolytic effect of the crude extract of *S. guttatum* in the current study is mediated through inhibition of calcium entry, a high concentration of K^+ (80 mM) was introduced to produce sustained contraction. Crude extract of *S. guttatum* was then added in a cumulative fashion, where it caused a dose-dependent relaxation of the induced contraction with EC_{-50} value of 1.2 mg/mL (0.8-1.6) suggesting inhibitory effect on calcium entry (Figure 2A). The curves resembled those of verapamil (Figure 1 and 2C).

Figure 2C and D, show respectively, the effect of the extract and verapamil on the Ca^{++} concentration-response curves in isolated rabbit jejunum preparations, constructed in Ca^{++} -free medium. Values shown are mean ± SEM (n = 3-8).

The contraction induced by high K^+ (80 mM) is dependent on the entry of Ca^{++} through voltage-dependant Ca^{++} channels (Bolton, 1979). It is evident that, subs-tances which can inhibit high K^+-induced contractions is therefore, considered to have a possible

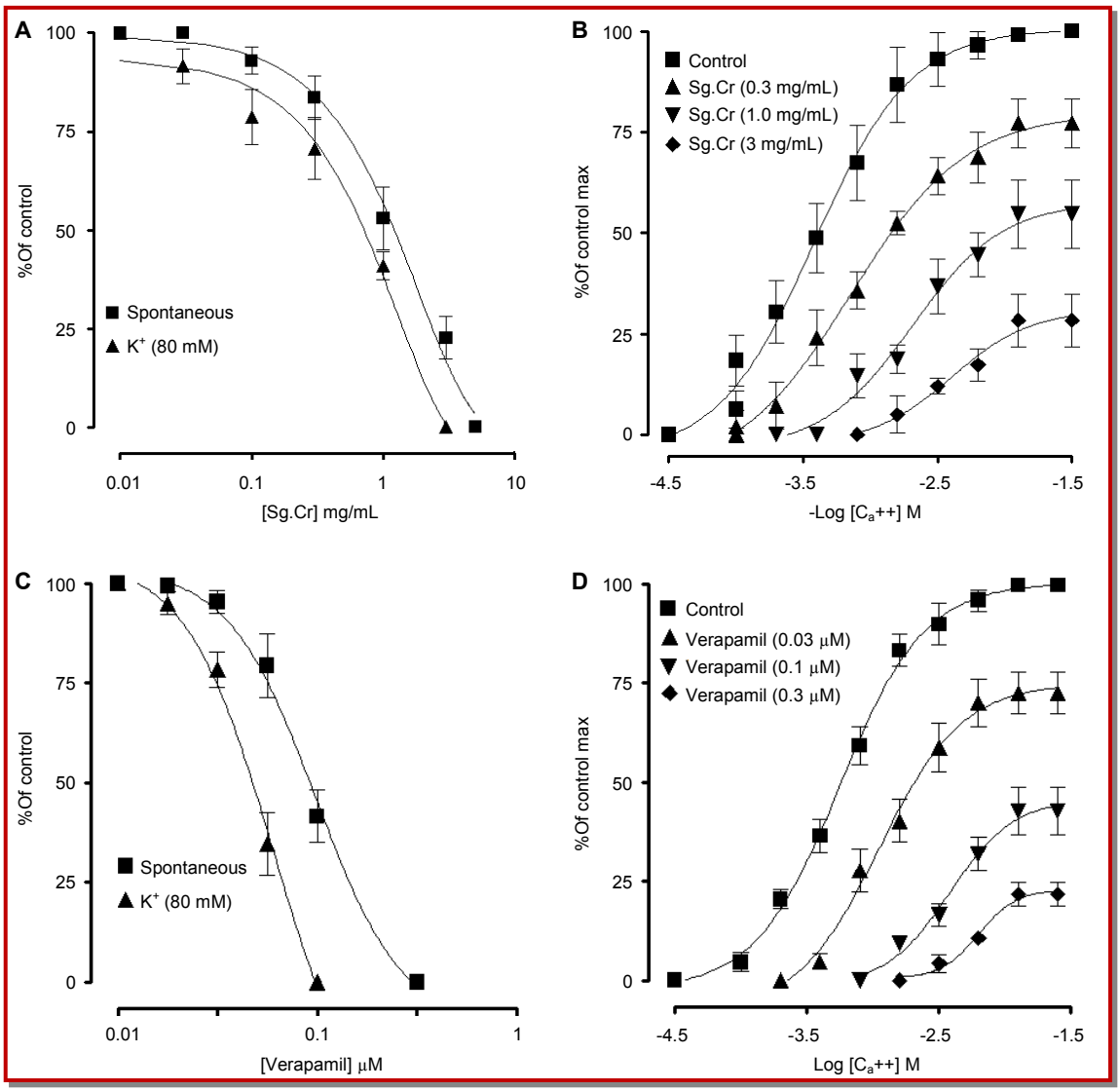

Figure 2: The concentration response curves (CRCs) of the crude extract of S. guttatum (A) and verapamil (C) on spontaneous and high K+ (80 mM)-induced contractions in isolated rabbit jejunum preparations. The CRCs on the right panel represents calcium curves in the absence and presence of the crude extract (B) and verapamil (D) showing dose-dependent rightward shift and suppression of maximum response in the calcium curves

calcium entry blocking effect (Godfraind et al., 1986). Thus the inhibition of high K^+ (80 mM)-induced contraction of rabbit jejunum by the crude extract of *S. guttatum* may reflects inhibitory effect on the Ca^{++} entry through voltage-dependant Ca^{++} channels. This hypothesis was further strengthened when pre-incubation of the jejunal preparations with the extract (0.3-3 mg/mL) caused a rightward displacement in the Ca^{++} concentrations-response curves (Figure 2C), similar to that caused by verapamil (Figure 2D) (Fleckenstein, 1977). The rightward displacement may be attributed to the presence of phytochemicals like alkaloids and tannins as these types of compounds in medicinal plants has been shown to have calcium entry blocking activity (Kai et al., 1998; Hwang et al., 2001).

The results of the current study suggest that the *S. guttatum* extract possesses antispasmodic activity. It is possible to suggest here that the antispasmodic activity of the extract may be due to calcium entry blocking effect, which is considered useful as antispasmodic (Brunton, 1996; Khan et al., 2013). Therefore, the presence of calcium channel antagonizing constituents in the crude extract of *S. guttatum* may be the possible mechanism for its spasmolytic activity. Additionally, early studies have reported that, Ca^{++}channel blocker are also known having role in managing diarrhea (Brunton, 1996), therefore, further study of the extract will be interested to explore anti-diarrheal activity of this plant.

In acute toxicity study, the crude extract of *S. guttatum* was found safe in mice up to the dose of 3 g/kg. After 24 hours no mortality or any other apparent behavioral

abnormalities were observed.

We provided here the first pharmacological evidence that the crude extract of *S. guttatum* possesses spasmolytic effect, which is mediated through the presence of Ca^{++} entry blocking constituent(s) and the extract was found safe up to the dose of 3 g/kg. Further insight into the chemical constituents and molecular characterization of the effect will be worthwhile.

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