Pharmacological Evaluation of SJ-200, a Polyherbal Formulation for its Antispasmodic Activity.

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Summary

SJ-200, a Polyherbal formulation contains active constituents of various plants which were proved individually in the earlier research work effective for their antispasmodic activity. So in the present study we have evaluated SJ-200 for antispasmodic activity in gastrointestinal spasm on various smooth muscles in vitro and intestinal transit rate in vivo. SJ-200 inhibited spontaneous contraction of rabbit jejunum and also acetyl choline and barium chloride induced contraction dose dependently, 5-HT and acetyl choline induced contraction of rat fundus inhibited dose dependently. SJ-200 also inhibited acetyl choline and calcium chloride induced contraction or rat colon dose dependently. Oral administration of SJ-200 dose-dependently reduced intestinal transit in mice when compared to atropine at 0.1mg/kg i.p. SJ-200 at (300 mg/kg) protected mice against diarrhoea induced by castor oil significantly as compared to control and standard loperamide at a dose of 5 mg/kg orally. In the present study it is concluded that SJ-200 inhibits the contraction produced by various spasmogens like acetylcholine, barium chloride, histamine, serotonin and calcium chloride. This suggests that the activity of SJ-200 is non-specific to any spasmogen.

KEY WORDS: Antispasmodic, SJ-200, Spasmogens, intestinal transit, smooth muscles, nonspecific

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Introduction

Spasm is a sudden violent involuntary muscular contraction or a transitory constriction of a passage. Various gastrointestinal disorders like abdominal pain, flatulence, colic, diarrhea, constipation, bloating, cramping, Irritable bowel syndrome are due to abnormalities in motor function disturbance [1,2,3]. Majority of the above symptoms are due to spasm. No single drug has proven to be effective in treating the said condition. Majority of the population in developing countries remains dependent on medicinal plants for health care. Based on this fact, scientists and WHO are focusing attention on medicinal plants, because of great potential that these plants carry in combating various diseases. Ayurveda, an Indian system of medicine cited several plants, which are useful in various gastrointestinal disorders without any side effect. SJ-200, an polyherbal formulation contains Zingiber officinale Roscoe, Zingiberaceae (rhizome), Apium graveolens L., Apiaceae (fruit) and Foeniculum vulgare Mill., Apiaceae (fruit). All these plants have been used to treat various gastrointestinal disorders like abdominal pain, flatulence and colic [4,5,6,7,8,9,10,11,12,13]. The present study has been carried out using SJ-200 to evaluate its antispasmodic activity on various smooth muscles in vitro, intestinal transit rate and antidiarroheal activity in vivo.

Methods

Plant materials

Apium graveolens, Foeniculum vulgare and Zingiber officinale were procured from a local supplier and identified by Dr. Kannan, Botanist, The Himalaya Drug Company, Bangalore. Samples were retained for reference purpose at the R & D herbarium.

Experimental animals

Study was performed by using healthy guinea pigs, wistar rats, New Zealand white rabbits of average weight and either sex for invitro studies. Albino mice were used for invivo studies. They were maintained on synthetic pelleted feed (Lipton India Ltd., Mumbai, India) and water ad libitum. Approval for the use of the animals was obtained from the Institutional Animals Ethics committee constituted for the purpose.

Method

Healthy, Guinea pigs (weighing 300-500 g), Wistar Rats weighing 150-200 g and rabbits of either sex were fasted 24 h before the study. Then the animals were sacrificed to isolate the ileum, fundus, colon and jejunum respectively. In case of rats, ether was used as anaesthetic agent, until death. The guinea pigs and rabbits were sacrificed by stunning or exsanguination as per CPCSEA recommended guidelines.

The ***in vitro*** method was performed using guinea pig ileum, rat fundus, rat colon and rabbit jejunum. Guinea pig ileum was used to study the effect of SJ-200 on calcium chloride induced contractions, rat fundus was used to study the effect of SJ-200 on 5-HT and acetyl choline induced contractions, Rat colon was used for Acetyl choline, $Cacl_2$ induced contraction, whereas rabbit jejunum was used for studying the Effect of SJ-200 on pendular movement and acetyl choline and barium chloride induced contraction of Rabbit intestine.

Effect of SJ-200 on rat fundus preparation

A rat fundus preparation was set up according to the method described by Vane [14]. Both ends of the fundal strip were tied with the thread and were mounted in an organ bath containing Kreb's solution at 37^{o}C and tissue was well aerated. 1 gm. lad was applied to allow the preparation to equilibrate for 30 min. Contractions of muscle preparation were recorded using isotonic transducer connected to single channel student physiograph. Antispasmodic activity of SJ-200 was evaluated by its ability to inhibit the spasm induced by a sub maximal concentration of Acetylcholine chloride ($56X10^{-7}$M) and 5-HT creatinine sulphate ($13X10^{-7}$M) respectively. The IC_{50} (50% inhibitory concentration) was calculated graphically.

Effect of SJ-200 on rat colon preparation

A rat colon preparation was set up according to the method described by Kulkarni [15]. A 3 cm long tissue was mounted in an organ bath containing modified Ringer solution (pH 7.4) maintained at 25^{o}C and well aerated. The preparation was allowed to equilibrate for 45-min under-500mg tension. Contraction of the muscle preparation was recorded by an isotonic fine movement transducer connected to a single channel student physiograph. Antispasmodic activity of SJ-200 was evaluated by its ability to inhibit spasm induced by

a sub maximal concentration of Acetylcholine chloride ($14X10^{-7}M$) and Calcium chloride ($14.4X10^{-2}M$). The IC_{50} (Inhibitory concentration) was calculated graphically.

Effect of SJ-200 on rabbit jejunum

A rabbit jejunum preparation was set up according to the method described by Burn [16]. About 2-3 cm in length jejunum free from mesenteric attachments was used. Upper portion of the lumen was tied to isotonic fine ,movement transducer and the lower end to the tissue holder. The preparation was mounted in an organ bath of 25ml capacity filled with Tyrode's solution at 37^0C and aerated well. 1 gm. weight load was applied and the tissue was allowed to stabilize for 30 min. at 37^oC. Muscle responses were recorded using isotonic fine movement transducer connected to single channel student physiograph. The effect of SJ-200 was studied by its ability to inhibit spasm induced by the sub maximal concentration of Acetylcholine chloride ($17.6X10^{-8}M$) and Barium chloride ($153X10^{-6}M$) considering sub maximal concentration as 100% contraction and also on the spontaneous contraction. The IC_{50} (50% Inhibitory concentration) was calculated graphically. Rhythmic contraction, which is in a regular manner, was recorded on a student Physiograph. The effect of SJ-200 on this pendular movement was also evaluated.

Calcium antagonism in Guinea pig Ileum

A Guinea pig ileum preparation was set up as described by Magnus [17] and for evaluating Ca^{++} antagonism activity of SJ-200, the method described by Van den Broucke[18] was followed.About 2-3 cm long lumen was suspended in an organ bath of 25 ml capacity containing depolarizing solution to make the muscle membrane permeable for Ca^{2+} ions. Composition of salt solution is gm / lt: NaCl 1.58; $NaHCO_3$ 1.26; KCl 7.46; $MgCl_2$ $6H_2O$ 0.25; Glucose 1.98. The preparation was aerated and allowed to stabilize for 30 min before starting the experiment. In order to remove intra and extra cellular Ca^{2+}, the longitudinal muscle strip was washed during one hour with the depolarizing solution. Contraction of the tissue preparation to different doses of calcium chloride was recorded using isotonic fine movement transducer with a tension of 0.5 g, connected to student physiograph. Dose-response curves were obtained with calcium chloride. Then SJ-200 was added to the organ bath in increasing concentration. The effect of SJ-200 to antagonize Ca^{2+} ($14.4 X 10^{-2}M$) concentrations in the Guinea pig ileum was evaluated.

Effect of SJ-200 in intestinal transit time in mice

The passage of an orally administered charcoal meal through the gastrointestinal tract in mice is used as parameter for evaluating intestinal motility in an invivo test

The evaluation was carried out according to the procedure given in Vogel [19]; Janssen and Jageneau [20]. Albino mice (20-25 gm) were randomly divided into 5 groups of 6 mice each. The animals were starved for 24 hrs prior to the experiment but were allowed access to water. One group of animals was given 20 ml/ kg of normal saline orally, while remaining 3 groups received orally SJ-200 at a doses of 50, 100, 200 mg / kg. The last group received atropine (0.1mg / Kg.). After 60 min. of drug administration through oral route (15 min after drug administration through i.p) charcoal meal (0.2 ml of a 4% suspension of charcoal in 2% carboxymethylcellulose solution) was administered to each animal orally. The animals were killed 30 min later and the abdomen was opened. Percentage distance (from pylorus to caecum) traveled by the charcoal plug in both the extract and normal saline treated groups were determined. As the intestines of the mice used were all of similar length, it was considered justifiable to use the distance travelled by the charcoal meal as an index of intestinal transit. In this way, the intestinal transit was measured for different groups of mice.

Effect of SJ-200 on castor oil induced Diarrhoea.

The antidiarrhoeal activity of SJ-200 was determined in albino mice, as described by Amos [21]. Albino mice of either sex (18-22 gm) were used for the experiment. The mice were fasted for 18 hr with water ad libitum.The animals were randomly divided into 6 groups of 6 mice each. The animals in group one received normal saline (30 ml /kg: i.p), while those animals in group II, III, IV, V received the SJ-200 (doses of 50, 100, 200 and 300 mg /kg orally) respectively. The last group received loperamide (5 mg /kg P.o). After 60 min of drug treatment, castor oil (0.2ml /mouse) was administered orally. The animals were placed in individual cages over clean filter paper. After 3 hr. of oil challenge, mouse cages were inspected (by observer unaware of the particular treatment) for the presence of characteristic diarrhoea droppings, their absence was recorded as a protection from diarrhoea and the percentage protection was calculated.

Statistical analysis

Data were expressed as Mean ± SEM. Significance was assessed by Student's 't' test or ANOVA followed by Dunnet's test. The minimum level of significance was fixed at $p<0.05$.

Results

Effect on rabbit jejunum:

Fig. 1a and 1b shows dose dependent inhibition of SJ-200 on the contractile response of rabbit jejunum to acetylcholine (10^{-7}M) and barium chloride (153 X 10^{-6} M). The IC_{50} (50% inhibitory concentration) against acetylcholine and barium chloride induced contraction on rabbit jejunum was 33.0 mcg/ml and 18.9 mcg/ml respectively.

SJ-200 inhibited the tone of spontaneously contracting intestine in a concentration dependent manner thereby confirming the antispasmodic activity of SJ-200 on rabbit jejunum. SJ-200 contains potent spasmolytic components which is evident in the spontaneous contracting preparation of rabbit jejunum.

Effect on rat colon:

Fig 2a. shows the dose dependent inhibition of SJ-200 on the contractile response of rat colon to calcium chloride (14.4 X 10^{-2}M). The IC_{50} (50% inhibitory concentration) against acetylcholine (14 X 10^{-7}M) and calcium choride induced contractions were 25.5 and 29.5 mcg/ml respectively. The antagonism displayed towards the spasmogens acetylcholine, calcium chloride were concentration dependent and competitive.

Effect on rat fundus:

Fig. 2b and 3a shows, SJ-200 significantly inhibited the serotonin (13 X 10^{-7}M) and acetylcholine (56 X 10^{-7}M) induced contraction of rat fundus smooth muscle preparation and the inhibition was dose dependent and competitive. The IC_{50} (50% inhibitory concentration) against serotonin and acetylcholine induced contraction on rat fundus were found to be 11.82 and 14.72 mcg/ml respectively.

Calcium antagonism:

Fig 3b. shows the effect of SJ-200 at doses of 10-60 mcg/ml caused a moderate spasmolytic effect in isolated guinea pig ileum suspended in a depolarizing solution and the inhibition was dose dependent and reversible.

Effect on Intestinal transit time in mice:

The results of charcoal meal test showed that SJ-200 caused a significant decrease in gut motility when compared to normal saline. At 50-200 mg/kg po, SJ-200 dose dependently reduced intestinal transit time in mice. The effect on this parameter was significant at a dose of 50, 100, 200 mg/kg po of SJ-200. The mean percentile inhibition was 67.24 ± 4.77%, 54.85 ± 6.082%, 50.17± 6.7045% respectively whereas atropine at 0.1mg/kg i.p produced 46.68 ± 7.912 % inhibition.

Effect on castor oil induced diarrhoea:

SJ-200 at (300 mg/kg) and loperamide (5mg/kg) protected mice against diarrhoea induced by castor oil significantly as compared to control

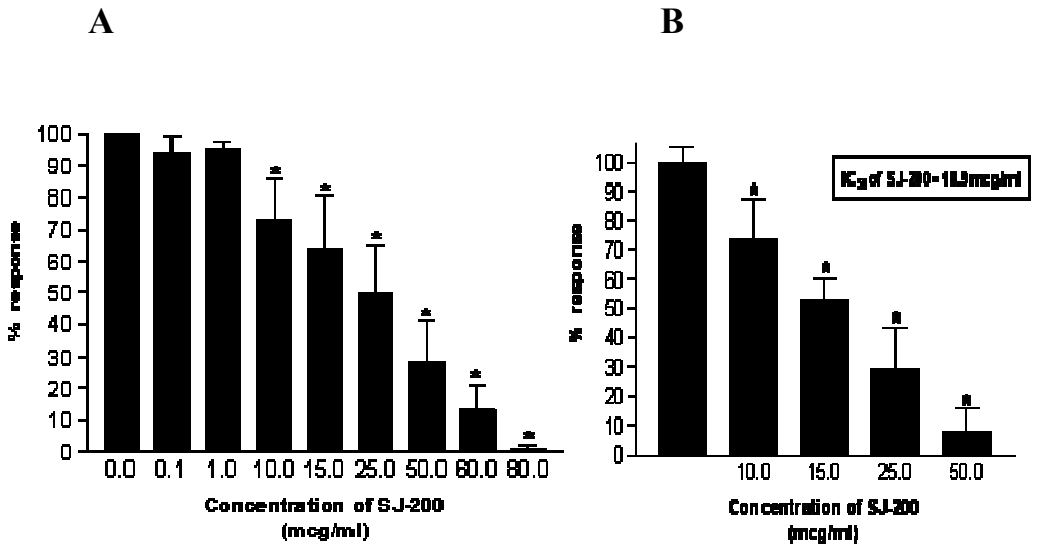

Fig 1. Effect of SJ-200 on Ach (17.6 X 10^{-8}M) induced contractions on rabbit jejunum [A] and $Bacl_2$(153 X 10^{-6}M) induced contractions on rabbit jejunum [B].Values expressed as mean ±SEM. *P<0.05 Vs Control.

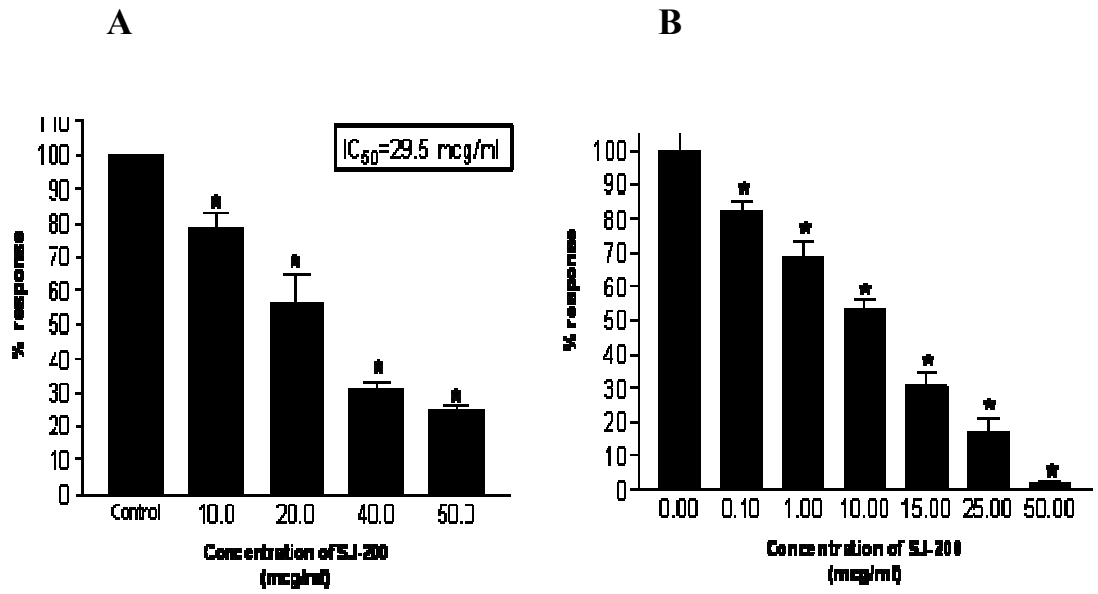

Fig 2 Effect of SJ-200 on $Cacl_2$(14.4X 10^{-2}M) induced contractions on rat colon [A] and 5-HT (13 X 10^{-7}M) induced contractions on rat fundus respectively. Values expressed as mean ±SEM. *P<0.05 Vs Control.

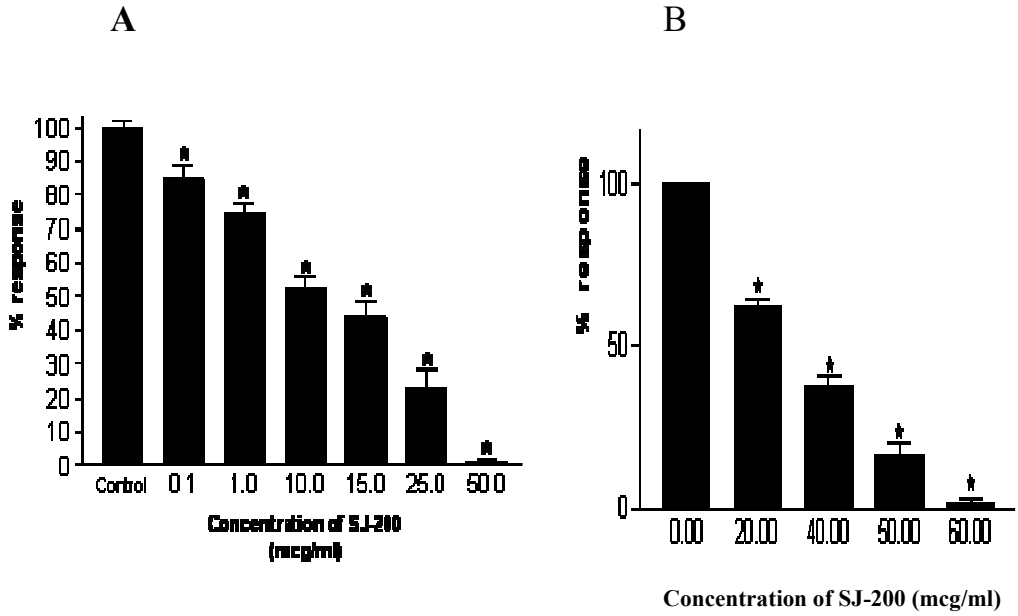

Fig 3. Effect of SJ-200 on Ach (56 X 10^{-7}M) induced contractions on rat fundus and $Cacl_2$ (14.4 X 10-2M) induced contractions on guinea pig ileum respectively. Values expressed as mean ±SEM. *P<0.05 Vs Control.

Discussion

The use of various spasmogens, with different mechanisms to cause intestinal contraction, can provide information on the pharmacological basis of antispasmodic activity of SJ-200. SJ-200 did not show any agonistic property like acetyl choline, Histamine on various smooth muscles preparations. Acetycholine and histamine are known to act on the smooth muscle cells of the guinea pig ileum, interfering with specific receptors for these spasmogens [22]. The mechanism of Ba^{2+}, causing contraction was not well understood. Acetyl choline-induced spasms are due to muscarinic M_3 receptor activation, which is a characteristic of vagal stimulation in the body. So mostly all endogenous colic pain like biliary, gastrointestinal, ureter arise due to such acetyl choline-induced spasms. Histamine-induced spasms are mediated by H_1 receptor activation which is characteristic of allergy producing substances leading to abdominal pain e.g. lead poisoning, uremia, excessive gastric acid or even bile secretion. Barium chloride-induced spasms are not mediated by any receptor but they are mediated by increased Ca^{++} channel entry due to spasmogen or increased phosphodiaesterase activity leading to calcium channel activation.

Since the availability of calcium is a basic determinant of muscle contraction, possible calcium antagonism by SJ-200 was also studied.

Inhibition of smooth muscle contractility may be the result of two different actions. A neurotropic one as inhibition of neurotransmitter release from nerve terminals, or blockade of specific membrane receptor sites. Musculotropic action may be the result of:

■stabilization of the muscle membrane

■Interference with the availability of Ca^{2+} at a step or steps in the contraction sequence subsequent to membrane activation.

■Interference with the normal function of the regulatory proteins involved in contraction and relaxation e.g. troponin, tropomysin.

■Inhibition of the actinomycin ATP-ase and subsequently inhibition of chemomechanical transduction.

The results indicate that antispasmodic activity is caused by a direct and indirect action on the smooth muscle. It is suggested that muscle relaxant effect results from a decrease of the Ca^{2+} availability for muscle contraction by (1) blocking the release of intracellular

bound Ca^{2+} and (2) prevention of the extracellular bound Ca^{2+} influx in the smooth muscle cell. Inhibition of the nerve action potential in the postganglionic nerve action potential in the postganglionic nerve fibre is proposed to be the indirect action of antispasmodic activity. SJ-200 decreases intestinal transit by inhibiting the gastrointestinal motility. The finding that SJ-200 decreased peristaltic movement in the charcoal meal study corroborated with some of the results of *invitro* studies. 5-HT is involved in the pathogenesis of various type of secretory diarrhoea including irritable bowel syndrome (IBS) rationalizes the use of SJ-200 in the management of IBS.This observation supports the use of SJ-200 in diarrhoea, which is one of the symptom seen in IBS. The results of the present study indicate that SJ-200 possesses potent antispasmodic properties on the smooth muscles of the gastrointestinal tract. All contractions induced by various spasmogens with different pharmacological mechanisms to cause contractions were reduced or blocked and the blockade was reversible. Hence, the antispasmodic activity is nonspecific in nature [18].

Conclusion

All the above findings suggest that SJ-200 is a non-specific antispasmodic, which can be used in the treatment of various spasmodic disorders of gastrointestinal tract and other viscera. The present study confirms the antispasmodic activity of the said constituents using modern pharmacodynamic experiments. Further studies are required to find the biochemical and molecular mechanism of action of SJ-200.

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