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SPASMOLYTIC ACTIVITY OF THE AQUEOUS ROOT EXTRACT OF *SOLANUM INCANUM*, SOLANACEAE

Ashenafi Assefa[1,*], Kelbessa Urga[1], Mulugeta Guta[1], Daniel Melaku[1], Walelign Mekonen[1], Martha Melesse[1], Asnakech Senbeta[1] and Tsegayae Kidanemariam[2]

ABSTRACT: *Solanum incanum* L. (Solanaceae) is an annual herb that is used in the traditional medicine of Ethiopia for treating stomach and intestinal disorders. The spasmolytic activity of aqueous root extract of *S. incanum* was assessed on contractions of isolated guinea pig ileum, induced by acetylcholine, and compared with the effect of atropine. The aqueous root extract of *S. incanum* inhibited the response to acetylcholine in a concentration-dependent manner (EC_{50}= 0.215 mg/ml) similar to atropine which indicated that the extract is a relaxant of guinea pig isolated ileum. In addition to its antispasmodic activity *in vitro*, the extract inhibited charcoal travel in mice intestine by 36.28, 51.45, 52.93 and 38.53 percent in doses of 50, 100, 200 and 400 mg/kg body weight, respectively. As the inhibition of contractile activity of the ileum is the base of the treatment of some gastrointestinal disorders such as colic, *S. incanum* may have clinical benefits for treatment of these conditions. Phytochemical screening of the root of the plant revealed the presence of alkaloids, saponins, tannins and flavonoids. The alkaloids in the plant might be responsible for the anti-cholinergic activities observed. Results of acute toxicity study showed that the mice did not show any sign of conventional toxicity when administered doses of up to 15,000 mg/kg body weight orally.

Key words/phrases: Anti-spasmodic; Crude extract; *Solanum incanum*.

INTRODUCTION

The use of traditional medicine has expanded globally and is gaining popularity. It has continued to be used not only for primary health care of the poor in the least developed nations, but also in countries where conventional medicine is predominant in the national health care system (Lanfranco, 1999). In Ethiopia, traditional remedies represent not only part of the struggle of the people to fulfill their essential drug needs but also they are integral components of their cultural beliefs and attitudes (Dawit Abebe and Ahadu Ayehu, 1993).

The plant *Solanum incanum* L. (Solanaceae) is a very common shrub in Ethiopia, found in left-over agricultural lands, roadsides and village yards. The shrub is known by different local names in Ethiopia such as *Embuay*

[1] Department of Drug Research, Ethiopian Health and Nutrition Research Institute, P.O. Box 1242 or 5456, Addis Ababa, Ethiopia
[2] Core Laboratory Services, Ethiopian Health and Nutrition Research Institute, P.O. Box 1242 or 5456, Addis Ababa, Ethiopia
* Author to whom all correspondence should be addressed. E-mail: ashyaega@yahoo.com

(Amharic) and *Hidi* (Oromifa). Different parts of the plant are traditionally employed for a number of ailments including leprosy and asthma (Dawit Abebe and Ahadu Ayehu, 1993). The fruits and fresh leaves are applied for topical ulcer; the root is used for syphilis, leprosy and gonorrhea. The fruit juice is used for boil dressing, antiamoebic, vermifuge and dysentery, and as intelligence booster (Chapman and Hall, 1977; Dawit Abebe and Ahadu Ayehu, 1993). It is used for the treatment of chest pains, pleurist, pneumonia, tooth ache and sore throat in Korean traditional medicine and to treat hepatitis in Taiwan (Lina *et al*., 2000). The plant is also widely used for the treatment of cutaneous mycotic infections and other pathological infections and was reported to effectively inhibit the growth of both Gram positive and Gram negative bacteria, yeasts, dermatophytes and some pathogens of agricultural products (Beaman-Mbaya and Mohammed, 1976). Soladonine isolated from a number of Solanum species is said to be anti-neoplastic (Lin *et al*., 1990). The berries of the plant have been reported to contain a number of alkaloids and other compounds including cytotoxic compounds such as incanumine, solalamargin, solasonin, solasodine, urosolic acid and its derivatives (Fukuhar and Kubo, 1991; Lin *et al*., 1990).

Despite the widespread use of this plant in traditional medicine, surprisingly little research has been carried out to examine its basic pharmacological properties. In this study, we report our observation on the effect of the aqueous extract of the roots of *S. incanum* on the contractile responses of guinea pig ileum (*in vitro*), inhibition of charcoal traverse on the intestine of mice (*in vivo*) and the acute toxicity study, so as to evaluate the traditional claim and its clinical benefits in gastrointestinal disorders.

MATERIALS AND METHODS

All the experiments were conducted at the Department of Drug Research, Ethiopian Health and Nutrition Research institute.

Plant material and extraction

The root of *S. incanum* was collected in January 2004 from Butajira (100 km, south west of Addis Ababa). A taxonomist identified the plant specimen and a voucher specimen was deposited in the herbarium of the Department of Drug Research, Ethiopian Health and Nutrition Research Institute. The roots of *S. incanum* were dried under shade, powdered and water extraction was made by maceration of the powder overnight in distilled water at room temperature, filtered and lyophilized to yield 18.23%. The desired concentration (w/v) of the aqueous extract was diluted in physiological solution.

Preliminary phytochemical analysis of the aqueous root extract of *S.*

incanum were made according to Harborne (1973) and Tona *et al*. (1998) to show the presence of alkaloids, saponins, flavonoids, and polyphenols.

Pharmacological tests

Isolated tissue preparations

Isolated guinea pig ileum preparation was conducted according to Gilani and Cobbin (1986), Gilani *et al*. (1994) and Socorro *et al*. (2002). Male albino guinea pigs weighing 250-350 g obtained from Ethiopian Health and Nutrition Research Institute were killed by a blow to the head. Abdomen was removed with a scissor and the ileum gradually removed. After discarding the terminal 2-3 cm from the ileocecal junction, 2 cm in length of the ileum were immediately cut and cleaned and mounted in 20 ml of Tyrode's solution [8g NaCl, 0.2g KCl, 0.2g $CaCl_2$, 1g $NaHCO_3$, 0.5g NaH_2PO_4, 1g Glucose, $MgCl_2$ made up to 1 liter with double distilled deionized water (pH 7.4)]. The solution was bubbled with O_2 and maintained at 37°C.

Initial tension was 1g and stabilization time was 60-120 min. Isotonic contractions were recorded by Grass Force Displacement Transducer FT-10 connected to Grass model 7E Polygraph. Acetylcholine was added before the extract and the preparation was incubated for about 10 minutes with the extract before assessing the response. The contractile effect of the test materials (extract and atropine) was assessed as a percentage of the maximum effects produced by contractions with addition of acetylcholine (100 mM) to the guinea pig ileum.

Charcoal traverse method

The experiment was conducted according to Srivastava and Bhatt (1993). Thirty albino mice of either sex weighing 20-25 g were obtained from Ethiopian Health and Nutrition Research Institute. The mice were randomly divided into six groups of five animals each and were deprived of food for 12h prior to the experiments. Animals in groups 1 to 4 were administered orally with *S. incanum* aqueous root extract water suspension in doses of 50, 100, 200 and 400 mg/kg (body weight, b.w.) respectively, while those in group 5 received normal saline. The sixth group received atropine sulphate (10 mg/kg, b.w.), the standard drug for comparison. Ten minutes after treatment, 1 ml of charcoal meal (5% deactivated charcoal in double distilled deionized water) was administered orally to each mouse. The animals were sacrificed 30 minutes later and the abdomen opened. The intestine from the pylorus to caecum was removed and stretched under a load of 5 g for 1 minute in order to obtain uniform elasticity for measurement. The length of the small intestine traversed by charcoal was

measured following the method of Mehta *et al*. (1973) and the result was expressed as percentage of the total length.

$$\% \text{ traversed by charcoal} = \frac{\text{Total intestinal length} - \text{Charcoal traverse}}{\text{Total intestinal length}} \times 100$$

Percent inhibition of charcoal travel was calculated as:

$$\% \text{ inhibition} = \frac{\%\ \text{Av. charcoal travel in control} - \%\ \text{Av. charcoal travel in treatment}}{\%\ \text{Av. charcoal travel in control}} \times 100$$

Acute toxicity study

Acute toxicity studies were performed on mice using the method of Weil (1952), Paula *et al*. (2003) and Feres *et al*. (2006). Albino mice weighing 20-25 g of either sex were divided into four groups (five mice per group). Each group received the dry aqueous root extract of *S. incanum* suspended in double distilled deionized water and administered orally in doses of 500, 1,000, 5,000, 10,000 or 15,000 mg/kg (body weight, b.w.), respectively. Signs of toxicity and mortality were observed at the first, second, fourth and six hour with no food intake. Mortality was assessed every 24h for two days. After that, the animals were observed for 14 days, with food and water intake *ad libitum*.

Reference drugs

The following drugs were used for the experiments: acetycholine bromide (Riedel-de Häen 64241), atropine sulphate (Sigma 101H0329), and charcoal (BDH 33032). Acetycholine was made up as 100 mM stock solution. All the drugs were freshly prepared prior to the experiment.

Statistical analysis

Mean and SEM values were calculated for each group of results and significance of differences between the means was calculated by paired t-test and/or by one-way analysis of variance (ANOVA). Differences were considered statistically significant when $p < 0.05$. Prohibit analysis of SPSS were used to determine ED_{50} values.

RESULTS

Isolated tissue preparations

The normal spasm of the ileum is found to be linear after stabilization time in all experiments (Fig. 1). The contractile response of the guinea pig ileum to the root aqueous extract of *S. incanum* is given in Fig. 2. The extracts induced relaxation of the tissue precontracted by acetylcholine in a dose-dependent manner, giving an EC_{50} value of 0.0215 mg/ml, with a 95%

confidence limit of 0.0019-0.074 mg/ml. The inhibitory effect of the extract was reversed after washing the tissue with Tyrode solution, showing a non-permanent attachment of the extract and the receptor. The antagonistic activity of the extract against acetylcholine was observed at 0.0005 mg/ml and reached maximum at 0.05 mg/ml. Atropine sulphate showed similar dose-dependent activity with a relative potency compared to the extract, with the following concentration range from minimum to maximum respectively, 0.000014 mg/ml, 0.000036 mg/ml, and 0.000072 mg/ml (Fig. 3). Atropine is a pure compound with known spasmolytic effect whereas the extract is a crude mix of a number of compounds and thus cannot be compared with the same level of concentration. Further work is being undertaken to fractionate the crude extract.

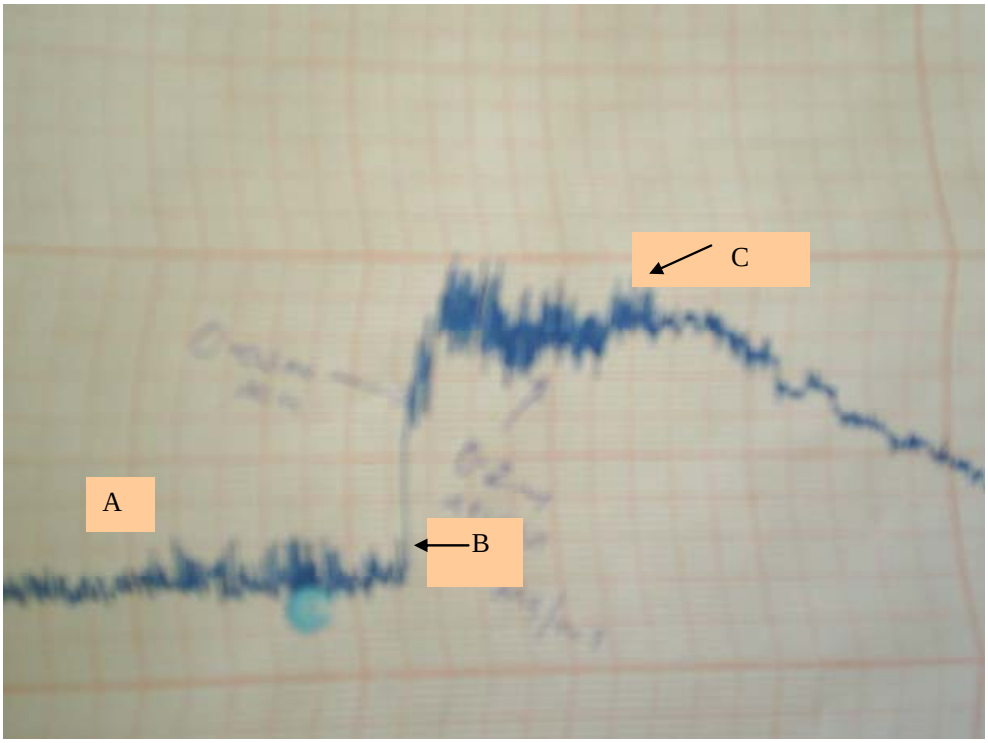

Fig. 1. Contraction of guinea pig ileum by acetylcholine and the subsequent relaxation of tissue with the addition of atropine. A: normal spasm, B: acetylcholine and C: atropine.

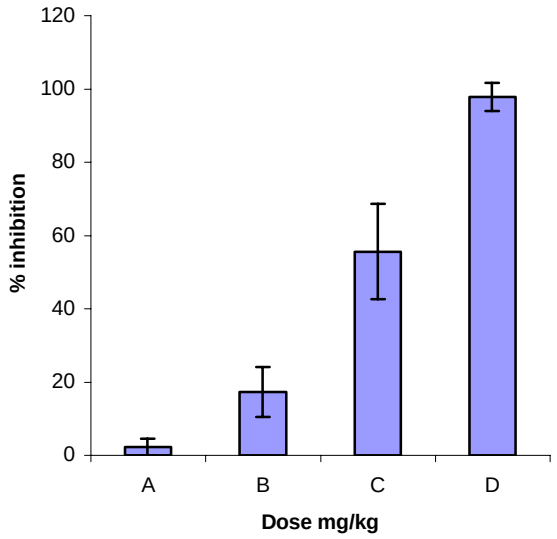

Fig. 2. Relaxation of guinea pig ileum by the aqueous root extract of *S. incanum* precontracted by acetylcholine. Activity starts at 0.0005 mg/ml and reaches maximum at a dose of 0.05 mg/ml. Data represent percentage of the maximum relaxation and given as mean ± SEM of three to five observations (A= 0.0005 mg/ml, B= 0.001 mg/ml, C= 0.0025 mg/ml and D= 0.05 mg/ml).

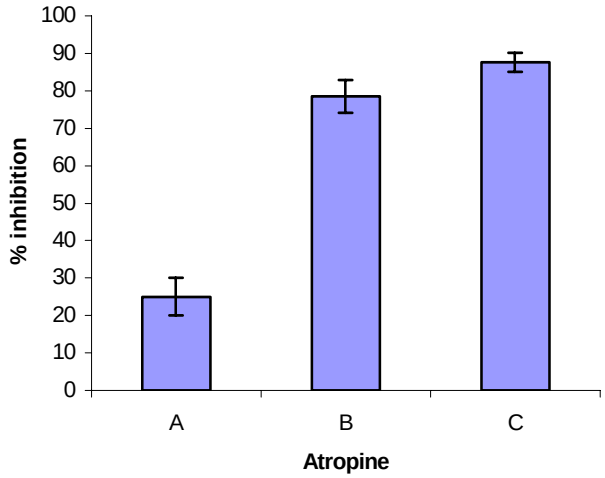

Fig. 3. Relaxation of guinea pig ileum by atropine precontracted by acetylcholine. Data represent percentage of the maximum relaxation and given as mean ± SEM of three observations (A= 0.000014 mg/ml, B= 0.000036 mg/ml, C= 0.000072 mg/ml).

Charcoal traverse

Table 1 shows inhibition of the root aqueous extract of *S. incanum* produced on the charcoal traversing the small intestine of mice. The extract inhibited charcoal travel by 36.28, 51.45 and 52.93 percent with concentrations of 50,100 and 200 mg/kg, respectively. However, 400 mg/kg only inhibited charcoal travel by only 38.5 percent, which was almost equivalent to the 50 mg/kg activity. Atropine showed a pronounced inhibition (73%) at 4 mg/ kg.

Table 1 Effect of the aqueous root extract of *S. incanum* on charcoal traversed through intestine in albino mice, each value represents mean ± SEM of five mice.

Product	Dose mg/kg	Length of intestine (cm) travelled by charcoal at 30 min.	% inhibition
Control	-	41.8 ± 0.55	_
Extract	50 mg/kg	25.9 ± 0.45	36.28
	100 mg/kg	19.3 ± 0.59	51.45
	200 mg/kg	19.5 ± 0.894	52.93
	400 mg/kg	26.1 ± 0.53	38.53
Atropine	4 mg/kg	10.7 ± 0.481	73.65

Acute toxicity study

In the oral acute toxicity study, no mortality and signs of toxicity was observed in all mice of both sexes, in graded doses of up to 15,000 mg/kg. The animals were observed for two weeks for any sign of toxicity, mortality or observable behavioral change; neither was observed in the study period mentioned. The extract was found to be too thick to be given through the oral gavages above 15,000 mg/ml.

DISCUSSION

The root aqueous extract of *S. incanum* has a dose-dependent spasmolytic activity in mice *in vitro* and concentration-dependent inhibition of charcoal traverse in guinea pigs *in vivo*. Using similar animals is recommended on such a study, however, using guinea pigs for the *in vitro* study is impractical. In addition to the experimental inconvenience and ethics, the required huge number of animals requires very long time and considerable finance and thus we resorted to using different animals for the two experiment groups.

The present results indicate that aqueous root extract of *S. incanum* induces concentration-dependent relaxation in guinea pig ileum precontracted by acetylcholine. The inhibitory effect of *S. incanum* on guinea pig ileal

smooth muscle stimulating agents was fully reversible in all cases following washout of the preparation, indicating that it does not cause tissue damage or tissue tolerance. *S. xanthocarpum* L. and *S. trilobatum* L. (other *Solanum* species) have also been reported to possess spasmolytic activities on smooth muscles (Govindan *et al*., 1999). The findings of the present study support the popular use of *S. incanum* in local folk medicine to treat gastrointestinal disturbances (Dawit Abebe and Ahadu Ayehu, 1993). In general, the study proved that the aqueous root extract of *S. incanum* had spasmolytic-like effect, which is similar to the anticholinergic drug atropine.

In the study of charcoal transit time, it was observed that under experimental condition, a normal animal would allow nearly complete intestinal transit of the test meal. However, when the extract was administered before the charcoal meal by oral gavages, the intestinal transit time was reduced to 36.28, 51.45, and 52.93% at doses of 50,100 and 200 mg/kg, respectively. However, the inhibition at a dose of 400 mg/kg was reduced to 38%. This finding is consistent with results of the *in vivo* study of Neblon, an antispasmodic drug (Srivastava and Bhatt, 1993) and extracts of *Epilobium* spp (Vitali *et al*., 2007).

The family Solanaceae contains solanaceous alkaloids. The principal alkaloids of the group are (-)-hyoscyamine, atropine, scopolamine (hyoscine) and the anhydride of atropine (apoatropine) and its stereoisomer, belladonnine. These are groups of ester alkaloids called tropane alkaloids. Atropine and scolpoamine are competitive with acetylcholine at the postganglionic synapse (muscarinic receptors M_2 and M_3) of the parasympathetic nervous system (Goyal, 1988; Tyler *et al*., 1988; Levey, 1993; Sadraei *et al*., 2003). Clinically useful effects obtained from blocking the muscarinic activity of acetylcholine are antispasmodic effect used principally to relieve spasm of the bowel in the treatment of spastic colitis, gastroenteritis and peptic ulcer. Thus, the root aqueous extract of *S. incanum* might contain one or more of the solanaceous alkaloids, which are responsible for the anti-cholinergic effect observed (Tyler *et al*., 1988).

Chemical screening has revealed the presence of alkaloids, saponins, tannins, flavonoids, and polyphenols. Previous studies of the plant and other *Solanum* species have reported the presence of different alkaloids, including solasodine and derivatives, which are known to be cytotoxic and poisonous (Lin *et al*., 1990; Mans *et al*., 2004). Sadraei *et al*. (2003), however, reported that plants that contained saponins and flavanoids rich extracts had a mild inhibitory action on guinea pig ileum. Therefore, the presence of saponins and flavonoids in *S. incanum* may add to the antispasmodic effect observed in the present study.

Results of the acute toxicity study of the aqueous root extract of *S. incanum* indicated the lethal dose (LD) to be above 15,000 mg/kg in contrast to the claimed toxicity of the plant. The low lethal dose observed in the present study may be attributed to the geographical variation, and/or the concentration of the toxic alkaloid solasodine which decreases with time and is low in the root compared with the berries and other aerial parts (El-Tayeb *et al*., 1997). Nonetheless, detailed toxicity study is warranted prior to recommendations for enhanced utilization of the plant.

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