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In vivo study on analgesic, gastrointestinal tract (GIT) motility, and anti-termite potential of methanolic extract of *Sarcococca saligna* (D. Don) Muell. fruits

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ABSTRACT

The current paper summarizes the evaluation of the alcoholic extract of *Sarcococca saligna* (D. Don) Mull. fruits for acute toxicity, analgesic, GIT motility modulation, and anti-termite properties. The extract was evaluated for analgesic activity using acetic acid-induced writhing model while charcoal meal model was adopted for GIT motility estimation in mice. The fruit extract exhibited significant analgesic and GIT motility potential at 700, 1000 mg/kg *i.p.* in comparison to standard drug (Diclofenac sodium). The extract also showed good anti-termite activity. The extract was also evaluated for toxicological effects which showed that the fruit extract is safe for the consumption of mice at 1000 mg/kg *i.p.*

1. Introduction

Sarcococca saligna (D. Don) Muell. is a small shrub with scaly buds belonging to the family Buxaceae, which comprises of four genera and about a hundred species. It is widely distributed in the northern areas of Pakistan at an altitude of 5000–9000 ft (Ahmad et al., 2010). The aqueous extract of this plant is reported to be used as an antipyretic and a calmative agent (Qureshi et al., 2007). The Buxaceae family plants are rich sources of bioactive secondary metabolites encompassing terpenoids. Some phytochemicals isolated from this family has been reported for cholinesterase inhibition, antibacterial and anti-leishmanial activities. Moreover, the family Buxaceae is among the top twenty significant alkaloid-containing plant families, having four genera, *Sarcococca, Pashysandra, Styloceras* and *Buxus* (Hansen et al., 2007). Plants and their extracts belonging to the genus *Sarcococca* are most-commonly used in South Asia and China as folk medicines for relieving pain, malaria and other parasitic and skin diseases (Atta-ur-Rahman et al., 1997). Steroidal alkaloids of this genus have been reported for anti-leishmanial (Devkota et al., 2007a, 2007b; Choudhary et al., 2010), antifungal (Moghaddam et al., 2010), antiplasmodial (Devkota et al., 2007a, 2007b), cholinesterase inhibitor (Atta-ur-Rahman et al., 2004) and antibacterial (Atta-ur-Rahman et al., 1998) activities.

Based on the reported literature of *S. saligna* and other plants belonging to this family, the fruits of *S. saligna* were evaluated for possible pharmacological/biological activities.

2. Experimental

2.1. Collection of plant material and extraction

The fruit sample of *S. saligna* was collected from the hilly areas of Abbottabad, Pakistan. The samples were identified by Prof. Dr. Farrukh Hussain from the Department of Botany, University of Peshawar,

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Table 1
Analgesic activity of crude methanolic extract of *S. saligna* fruits.

S. No.	Treatment	Dose mL or mg/kg	No. of writhing/(10 min) (Mean ± SEM)
1	Normal saline	10	52.50 ± 4.28
2	Crude methanolic extract	500	48.33 ± 1.78*
		700	39.83 ± 1.22**
		1000	31.67 ± 1.24***
3	Diclofenac sodium (Standard)	10	19.50 ± 1.02***

Values are reported as mean ± S.E.M. (n = 6). ANOVA was used and Dunnett's test was applied on the data to find the level of significance comparing to negative control.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Table 2
Effect of crude methanolic extract of *S. Saligna* fruits on GIT Motility.

Treatment	Dose mg/Kg	Mean total length of intestine (cm)	Mean charcoal movement (cm)	% GIT motility
Normal saline	10	61.00 ± 2.182	33.21 ± 2.781	54.44
Methanolic extract of *S. saligna* fruits	500	55.00 ± 2.131	20.58 ± 2.658***	37.41
	700	52.00 ± 1.915	16.98 ± 2.245***	32.62
	1000	59.00 ± 2.357	08.35 ± 2.305***	14.15
Castor oil	0.1	55.00 ± 1.255	08.05 ± 1.355***	09.05

*** $P < 0.001$.

Pakistan, and were submitted in the herbarium of the same Department with Voucher No. Bot(PUP)577.

The fruit materials were shade-dried, cut into chunks and grounded to powder form. The pulverized fruits (1.5 Kg) were soaked in methanol (commercial grade) for five days (twice), at 25 °C with occasional shaking. Subsequently, the crude suspension was filtered. The filtrate was concentrated on a rotary evaporator, at reduced pressure and 40 °C. After multiple iterations of filtration step, 200 g of blackish crude methanolic extract was obtained.

2.2. Animals used in the experiments

BALB/C mice of both gender, age 4–5 weeks and mean weight of 20–25 g, were used in the experiments. The mice were procured from the Animal Research Branch, National Institutes of Health (NIH), Pakistan. A period of one week was given to the animals in standard laboratory conditions for acclimatization after their purchase, during which they were supplied with formulated rodent food and water *ad libitum*. The criteria set by the Animal Ethics Committee (Drummond, 2009), were adhered to.

2.3. Acetic acid-induced writhing

The analgesic activity of the crude methanolic extract was performed as per standard procedure (Bashir et al., 2013). The BALB/C mice of both sexes weighing 20–22 g each were included and distributed into five groups (n = 6). The Group I and II were treated as a negative and a positive control, respectively. The Group I was given a normal saline (10 mL/Kg/bw), whereas the Group II was given Diclofenac sodium (10 mg/kg/bw). The animals were not provided with food any supply, 2 h before the start of each experiment. The Group-III (500 mg/kg/bw), IV (700 mg/kg/bw) and V (1000 mg/kg/bw) were given the crude extract in the mentioned amounts. After 30 min of the crude extract treatments, 1% acetic acid was injected intra peritoneally (*i.p.*). Abdominal writhes (contractions) were counted for the next 10 min, after 5 min of acetic acid injection. For the percent analgesic effect following equation was used:

$$\%\text{Analgesic effect} = 100 - \frac{\text{No. of writhing in tested animals}}{\text{No. of writhing in control animals}} \text{ X } 100$$

2.4. Gastrointestinal tract (GIT) motility

The crude methanolic extract was screened for its possible effect on GIT motility. The BALB/C mice of 20–25 g weight were selected and were distributed into five groups. The Group-I and II was used as a ositive and negative controls, respectively. The Group-I was administrated with normal saline (10 mL/Kg), however, Group-II was treated with castor oil (as a standard drug) 0.1 mL/Kg. Moreover, the Group-III, IV and V were i.p. injected with crude extract at a dose of 500, 700 and 1000 mg/Kg/b.w.; later after 15 min of this treatment, a dose of 0.3 mL aqueous charcoal suspension was orally administered to each moue. Subsequently, after 30 min of charcoal treatment the animals were killed by cervical dislocation. In small intestine, flux of charcoal was observed and percent GIT motility was calculated by the following formula:

$$\text{Percent GIT motility} = \frac{\text{Distance covered}}{\text{Total length of intenstine}} \times 100$$

2.5. Anti-termite activity

The extract was analyzed to check the ant-termite potential using an established standard procedure (Salihah et al., 1993). A sterilized blotting paper was cut according to the size of sterilized Petri plates of uniform size. The test sample was prepared as 2.0 mg/mL in methanol. The blotting papers were dipped in the test sample and excess solvent was removed by holding it for sometimes, subsequently, papers were placed in the Petri plates. The excess solvent was evaporated by keeping the Petri plates overnight. The twenty five termites were shifted to each Petri plate and were observed after 24 h, till all the termites were dead. Methanol alone was also used as a negative control. The experiments

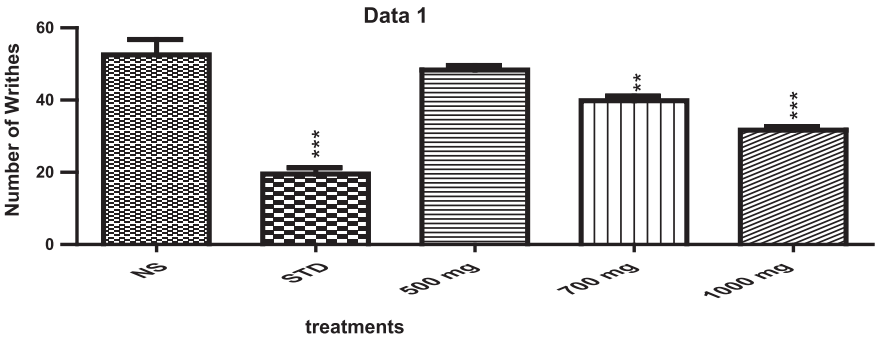

Fig. 1. Percent analgesic activity of analgesic activity of crude methanolic extract of *S. saligna* fruits (500, 700 and 1000 mg/kg) in acetic acid induce pain model.