ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 8, Issue 5, 2015

ISSN - 0974-2441

Research Article

ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF METHANOLIC SEED EXTRACT OF *STERCULIA VILLOSA* ROXB.

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Received: 24 June 2015, Revised and Accepted: 03 August 2015

ABSTRACT

Objective: To scientifically validate the analgesic and anti-inflammatory properties of methanolic seed extract of *Sterculia villosa* Roxb. (SVME) in animal models.

Methods: Analgesic activity of SVME was evaluated by the hot plate-induced pain model, acetic acid-induced abdominal writhing response, and formalin test in mice at the doses of 250 and 500 mg/kg. A carrageenan-induced paw edema model was also used to evaluate anti-inflammatory potential of SVME in rats at doses of 250 and 500 mg/kg.

Results: Phytochemical analysis revealed the presence of flavonoid, gums and carbohydrates, steroids, alkaloid, reducing sugar, and terpenoids in significant amounts. The SVME produced significant analgesic activity in the hot-plate test in mice at all the time points measured. Extracts of 250 and 500 mg/kg reduced dose-dependent acetic acid-induced writhing by 40.2% ($p<0.01$) and 59.8% ($p<0.001$), respectively. Significant inhibition of formalin-induced pain was also observed, with inhibition of 62.1% ($p<0.001$) and 66.7% ($p<0.001$) in the early phase at dosages of 250 and 500 mg/kg, respectively, and 64.4% ($p<0.01$) and 70.3% ($p<0.01$) in the late stage at these dosages. SVME pretreatments showed significant anti-inflammatory activity against carrageenan-induced edema at all the time points measured.

Conclusion: The results suggest that SVME possesses analgesic and anti-inflammatory activities. These findings provide support use of this plant in traditional medicine to treat pain and inflammation.

Keywords: *Sterculia villosa* Roxb., Analgesic, Anti-inflammatory, Carrageenan.

INTRODUCTION

Inflammation is a pathophysiological process mediated by a variety of signaling molecules, such as nitric oxide, prostaglandin, and tumor necrosis factor. These inflammatory mediators are produced by leukocytes, macrophages, and mast cells [1,2]. Pain and inflammation are associated with enhanced production of prostaglandins [3]. Non-steroidal anti-inflammatory drugs are widely used in the treatment of pain and inflammation, but unexpected bronchus-, gastric mucosa-, cardiac-, and kidney-related side effects have been reported [4]. Therefore, many researchers are interested in drugs of medicinal plant origin due to their wide range of pharmacological activities, including analgesic and anti-inflammatory potential, with minimum side effects [5].

Herbal drugs obtained from plants and herbs have long been used extensively to cure a variety of illness. The mechanism by which many of these drugs act remains unexplored scientifically. However, there is growing interest in evaluating the pharmacological basis of the medicinal activities of plants used in traditional systems of medicine.

Sterculia villosa Roxb. (Family-Sterculiaceae) is used by Indians as a traditional remedy for inflammation [6]. The white exudate of the *S. villosa* tree is used for throat infection, and a root infusion is taken as a food adjunct. The whole plant extract has been reported to be useful for skin diseases [7]. *S. villosa* is traditionally used, among other indications, for the treatment of inflammation and pain. Although various studies have been shown the medicinal values of this plant parts but anti-inflammatory and analgesic effects of seed extract have not been investigated yet. Therefore, as a part of our continuing studies on the various medicinal plants of Bangladesh [8,9] the present study was designed to explore the possible analgesic and anti-inflammatory potential of methanolic seed extract of *S. villosa* (SVME) in experimental animal models to justify as a new source of drug candidate.

METHODS

Plant material

Fresh seeds of *S. villosa* Roxb. were collected from Sitakundo Botanical Garden and Eco Park, Chittagong, Bangladesh. The plant was identified by experts at Bangladesh National Herbarium (BNH). The specimen was preserved in BNH, and it has been assigned the accession number of 38764 for further reference.

Extraction

The seeds were kept under sunshade for 5 days and then heated in an oven at 40°C for 24 hrs to be fully dried. After drying, they were ground thoroughly into powdered form with a mortar and pestle. The obtained powder was extracted via the method of cold extraction using 95% (v/v) methanol and then kept for 5 days with occasional shaking and stirring. The whole mixture was run in a coarse filtration through a piece of clean, white cotton material, followed by filtration through filter paper. The obtained filtrate (methanol extract) was evaporated by a rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 6 rpm, at 68°C. It rendered a concentrate of light black color that was designated as crude methanol extract. The extract was finally dried by a freeze dryer and preserved under −40°C. The yield of the extract was 11.35% (w/w).

Animals
Male and female Swiss-Albino mice weighing 25-35 g and Wistar rats of either sex with an average weight of 240-290 g were used for the experiment and maintained in the animal house of the Department of Pharmaceutical Sciences, North South University. The animals were housed in standard cages under standard environmental conditions, with a room temperature of 25±2°C, relative humidity of 55-65%, and a 12-12 hrs dark-light cycle. Standard food for rodents and water *ad libitum* were regularly provided.

Ethical approval
"Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed for the animal study. All experiments have been examined and approved by the Institutional Animal Care and Use Committee at the North South University, Bangladesh. Animal experiments were also performed in accordance with the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals [10].

Phytochemical screening
The SVME was qualitatively tested for the presence of chemical constituents by the method described previously [11,12]. The extract was tested for the presence or absence of tannins, flavonoids, saponins, gums and carbohydrates, steroids, alkaloids, reduced sugar, and terpenoids.

Analgesic activity by hot-plate test
The effect of SVME on nociceptive thermal pain was determined using a previously described hot-plate test [13,14]. The temperature was regulated at 55°C±1°C. Mice of either sex were divided into four groups consisting of five animals in each group. The mice in each group were placed in a beaker on the hot plate to observe the animal's response to an electrical heat-induced pain stimulus. Licking of the paws or jumping out of the beaker was taken as an indicator of the animal's response to the heat-induced pain stimulus. The time for each mouse to lick its paws or jump out of the beaker was measured as the reaction latency time (in seconds) prior to the administration of the extract, drug, or vehicle. After the basal reaction latency time was recorded, the mice were orally administered distilled water (10 ml/kg body weight), diclofenac (10 mg/kg), or SVME (250 and 500 mg/kg). 1 hr after the administration, the reaction latency time of each group of mice was evaluated again. Each animal in each group was assessed at 1 hr intervals thereafter for up to 4 hrs.

Analgesic activity by acetic acid-induced abdominal writhing test
To evaluate the effect of SVME on nociceptive visceral pain, the acetic acid-induced abdominal writhing test was used, as previously described [15-17]. The mice were divided into four groups with five mice in each group. The animals were treated with acetic acid intraperitoneally (0.6%, v/v in saline, 10 ml/kg) 1 hr after the oral administration of distilled water (10 ml/kg), diclofenc (10 mg/kg), or SVME (250 or 500 mg/kg). The animals were kept individually under a glass jar for observation. Each mouse in each group was observed, and the number of writhes during 15 minutes was counted, beginning 5 minutes after the acetic acid injection. The number of writhes in each treated group was compared to that of a control group in which diclofenac was used as a reference compound. The percentage inhibition of writhing was calculated using the following formula:

$$\%\text{Inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

Where, V_c and V_t represent the average number of writhing motions of the control and drug-treated mice, respectively.

Analgesic activity by formalin test
The formalin test was performed as previously described [18-20] to determine the effect of SVME on the neurogenic and inflammatory pain responses. The mice were divided into four groups, each containing five mice. They were orally administered distilled water (10 ml/kg), SVME (250 and 500 mg/kg), or diclofenac (10 mg/kg). 1 hr after the administration, formalin (20 µl of 1% solution) was injected subcutaneously under the plantar surface of the right hind paw of the mice. The time (in seconds) spent in licking and biting responses of the injected paw was taken as an indicator of the pain response and recorded for each mouse. The early phase was recorded during the first 5 minutes, and the late phase was recorded during the 20-30 minutes after the formalin injection. The percentage inhibition of formalin-induced pain was calculated using the following formula:

$$\%\text{ Inhibition} = \frac{\text{Reaction time (control) - Reaction time (treated)}}{\text{Reaction time (control)}} \times 100$$

Anti-inflammatory studies by carrageenan-induced paw edema
The anti-inflammatory activity of SVME was investigated on carrageenan-induced inflammation in rat paws following an established method [21]. The rats were randomly divided into four groups, each consisting of five animals. Distilled water (10 ml/kg body weight) as a vehicle, diclofenac (10 mg/kg) as the reference compound, and SVME (250 or 500 mg/kg) were administered orally to each group of rats. Edema was induced by injecting carrageenan (0.1 ml of 1%, w/v in saline) into the planter side of the right hind paw 1 hr after the oral administration of the various agents. The paw volume was measured before and 1, 2, 3, 6, and 9 hrs after carrageenan injection using a plethysmometer (Model 7141, UGO Basile, Italy). The percentage inhibition was calculated using the following formula:

$$\%\text{Inhibition of paw edema} = \frac{V_c - V_t}{V_c} \times 100$$

Where, V_c and V_t represent the average paw volume of the control and treated animal, respectively.

Statistical analysis
The experimental results are expressed as the mean±standard error of mean, with five animals in each group. Data were analyzed by a one-way analysis of variance followed by Dunnett's *t*-test for multiple comparisons. The results were considered statistically significant at $p<0.05$ compared to control group.

RESULTS AND DISCUSSION

Phytochemical analysis
The study was designed to scientifically validate the anti-inflammatory and analgesic activities of this traditional medicinal plant. Phytochemical screening of the SVME revealed the presence of flavonoid, gums or carbohydrates, steroids, alkaloid, reducing sugar, and terpenoids. Tannins and saponins were absent in SVME. The result of the phytochemical test has been summarized in Table 1. Flavonoids possess a variety of biological and pharmacological activities. Analgesic and anti-inflammatory activity have been observed in flavonoids [22-24]. Flavonoids can significantly inhibit a number of enzymes, some of which are involved in the inflammatory process [25]. They have been reported to inhibit

Table 1: Phytochemical analysis of the methanol extract of *S. villosa* seed

Phytochemicals	Presence
Tannins	–
Flavonoids	+++
Saponins	–
Gums and carbohydrates	+++
Steroids	++++
Alkaloids	+++
Reducing sugar	+++
Terpenoids	+++

Symbol (++++) indicates presence in high concentration, symbol (+++) indicates presence in moderate concentration, and (–) indicates absence of the respective phytochemical. *S. villosa: Sterculia villosa*

cyclooxygenase and 5-lipooxygenase pathways [26]. It is also reported that flavonoids such as rutin, quercetin, and luteolin produced significant antinociceptive and anti-inflammatory activities [27,28]. Studies have also demonstrated that alkaloids produced anti-inflammatory activity by inhibiting the action of arachidonic acid metabolism via cyclooxygenase and 5-lipoxygenase pathways [29,30]. Terpenoids have been shown to possess analgesic and anti-inflammatory effects [31-33]. The presence of flavonoids, alkaloids and terpenoids may be responsible for the anti-inflammatory and analgesic effects of SVME.

Analgesic activity by hot-plate test

The basal reaction latencies of the vehicle-, diclofenac-, 250 mg/kg SVME-, and 500 mg/kg SVME-treated groups were 8.30±0.63, 7.42±0.29, 7.68±0.21, and 7.40±0.38 seconds, respectively. After oral administration of SVME, significant inhibitory effects on thermal stimuli were observed from the first tested time point, 1 hr, for both dosage groups of mice. The reaction latency time was significantly increased at all the time points measured in the animals treated with SVME and diclofenac compared to that of the control. The maximum effects were observed at 3 hrs for both dosage groups of the SVME-treated group (Table 2). A nociceptive reaction toward the thermal stimuli in the hot-plate test is widely used to assess analgesic effects. The hot-plate test measures the complex feedback that occurs in response to a non-inflammatory, acute nociceptive input, and it is one of the models normally used for studying central nociceptive activity [34,35]. The latency time was longer in the SVME group than the control group in the hot-plate test. In addition, the antinociceptive effect against thermal pain lasted until 4 hrs after the administration of the SVME. This result suggests that the SVME possesses promising analgesic properties, which are probably mediated by a central inhibitory mechanism and may have potential benefit for the management of pain.

Analgesic activity by acetic acid-induced writhing test

The SVME showed significant inhibitory effects on the acetic acid-induced writhing response. At SVME dosages of 250 and 500 mg/k, the percentage inhibition values were 40.2% and 59.8%, respectively, ($p<0.01$ and $p<0.001$ compared to that of the control group, respectively), with inhibition occurring in a dose-dependent manner. The percentage inhibition value of diclofenac (10 mg/kg) was 67.8% ($p<0.001$ compared to that of the control group) (Fig. 1). Acetic acid-induced writhing in mice, attributed to visceral pain, is commonly used to evaluate peripherally active analgesics [36]. The acetic acid-induced writhing has been associated with increased levels of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ in peritoneal fluid, as well as lipoxygenase products [37]. The increase in prostaglandin levels within the peritoneal cavity enhances inflammatory pain by increasing capillary permeability [38]. Agents aimed at reducing writhing should exert analgesic activity preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition [39]. Therefore, the reduction in the number of writhes in the present study indicates that the SVME might exert analgesic activity at doses of 250 and 500 mg/kg ($p<0.01$ and $p<0.001$, respectively) by inhibiting the synthesis or action of prostaglandins.

Analgesic activity by formalin test

The effect of the SVME on formalin-induced pain in the mice is shown in Fig. 2. The extract significantly inhibited the pain response in the early phase: 62.1% at 250 mg/kg ($p<0.001$) and 66.7% at 500 mg/kg ($p<0.001$). In the late phase, the inhibitory responses were 64.4% ($p<0.01$) and 70.3% ($p<0.01$) at dosages of 250 and 500 mg/kg, respectively. The response duration was also significantly reduced by diclofenac in the early phase and late phase. The formalin-induced test in mice is a widely used model to evaluate the mild analgesic effect of drugs [40]. Two distinct phases are observed after formalin injection in the right hind paw of mice: An early phase (0-5 minutes) and a late phase (15-30 minutes). The early phase, named the neurogenic phase is likely to be due to the direct effect on peripheral nociceptors activating primary afferent fiber. Substance P, glutamate and bradykinin probably participate in this phase, which is thought to be non-inflammatory pain [41]. The late phase is due to the release of inflammatory mediators, such as histamine and prostaglandin [42,43]. Peripherally acting drugs inhibit the second phase [44] while centrally acting drugs inhibit both phase in generally [45]. In Fig. 2, the SVME suppressed both phase pain, but more significant effects showed in the second phase.

***In vivo* anti-inflammatory activity by carrageenan-induced rat paws edema**

The anti-inflammatory activity of the SVME is presented in Table 3. In the animals, the subcutaneous injection of carrageenan into the plantar produced local edema. The paw volume was not significantly different between the groups before injecting carrageenan (0.73±0.038, 0.69±0.035, 0.68±0.036, and 0.75±0.047 ml for the control, diclofenac, 250 mg/kg SVME, and 500 mg/kg SVME group, respectively). However, the paw edema volume was significantly decreased at all the time points measured in the animals pretreated with SVME and diclofenac compared to that of the control. The most significant ($p<0.001$) inhibition was 52.7% and 48.7%, which were observed at 9 hrs in the 250 mg/kg SVME and 500 mg/kg SVME groups, respectively. Carrageenan-induced

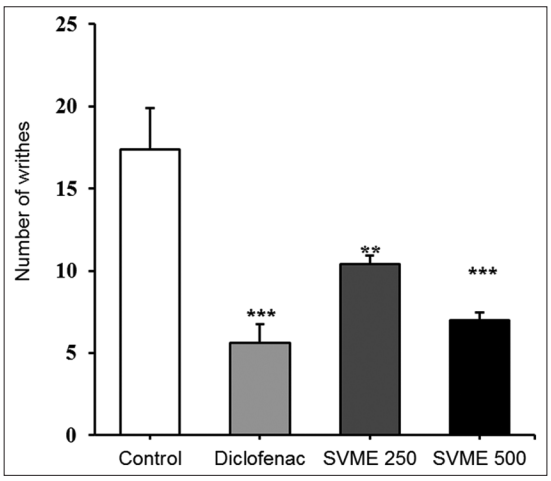

Fig. 1: Analgesic activity of *Sterculia villosa* seed extract in acetic acid-induced writhing response. The mean±standard error of mean, (n=5), Diclofenac (10 mg/kg, p.o.), methanolic seed extract of *Sterculia villosa* Roxb. (SVME) 250 (250 mg/kg, p.o.), and SVME 500 (500 mg/kg p.o.) were significantly different from vehicle control group (p<0.01 and ***p<0.001)**

Table 2: Effects of SVME on hot plate-induced pain in mice

Group	Dose (mg/kg)	Reaction latency (s)			
		1 hr	2 hrs	3 hrs	4 hrs
Control	0	7.42±0.419	7.50±0.389	7.50±0.447	7.18±0.328
Diclofenac	10	9.52±0.320**	10.58±0.381***	11.98±0.479***	13.38±0.271***
SVME	250	9.74±0.140***	12.40±0.257***	14.64±0.214***	13.12±0.334***
SVME	500	9.68±0.379**	12.26±0.186***	14.64±0.294***	12.60±0.303***

Values are expressed as mean±SEM, (n=5), where, **p<0.01 and ***p<0.001 as compared with the vehicle control group, SVME: Methanolic seed extract of *Sterculia villosa* Roxb., SEM: Standard error of mean

Table 3: Effects of SVME and diclofenac on carrageenan-induced hind paw edema in rats

Group	Dose (mg/kg)	Edema volume (ml)				
		1 hr	2 hrs	3 hrs	6 hrs	9 hrs
Control	0	1.32±0.019	1.58±0.018	1.79±0.032	1.69±0.021	1.58±0.020
Diclofenac	10	0.96±0.050 (27.0)**	1.34±0.036 (15.1)***	1.05±0.045 (41.5)***	0.88±0.021 (48.0)***	0.77±0.027 (51.4)***
SVME	250	1.10±0.081 (16.8)*	1.27±0.040 (19.6)***	1.02±0.036 (43.0)***	0.90±0.024 (47.0)***	0.75±0.020 (52.7)***
SVME	500	1.01±0.043 (23.5)**	1.27±0.039 (19.9)***	1.02±0.037 (42.8)***	0.90±0.018 (46.8)***	0.81±0.023 (48.7)***

Values are expressed as mean±SEM, (n=5). The percentage inhibition of edema is indicated in parenthesis, where *p<0.05, **p<0.01 and ***p<0.001 as compared with the vehicle control group, SVME: Methanolic seed extract of *Sterculia villosa* Roxb., SEM: Standard error of mean

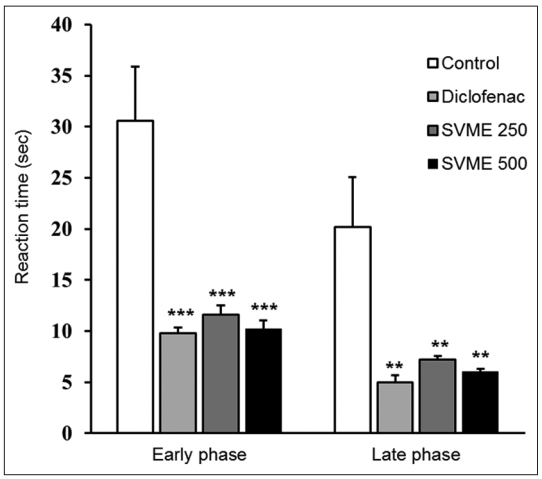

Fig. 2: Analgesic activity of *Sterculia villosa* seed extract in formalin-induced pain. The mean±standard error of mean, (n=5), Diclofenac (10 mg/kg, p.o.), methanolic seed extract of *Sterculia villosa* Roxb. (SVME) 250 (250 mg/kg, p.o.), and SVME 500 (500 mg/kg p.o.) were significantly different from vehicle control group (p<0.01 and ***p<0.001)**

inflammation is one of the most widely used experimental models for evaluating the anti-inflammatory response of compounds or natural products [46]. The edema induced by a carrageenan injection involves three phases of chemical mediator release in an orderly sequence [47]. The first phase (the first 90 minutes) involves the release of histamine and serotonin, and it is characterized by increased vascular permeability. The second phase (90-150 minutes) is mediated by the release of bradykinin, an important chemical mediator of both pain and inflammation. The release of prostaglandins and cyclooxygenases products takes place in the third phase, after 180 minutes [48]. In the present study, SVME notably inhibited the edema induced by the carrageenan injection in all three phases. The percentage inhibition of edema was highest at 9 hrs of the study at both doses of SVME. The anti-edema effect of SVME was highest in the third phase, suggesting that SVME might have inhibitory effects on the pathways involved in the synthesis or release of prostaglandins.

CONCLUSION

In this study, we found that SVME exhibited anti-inflammatory and analgesic effects on various animal models. We conclude that the central and peripheral analgesic and anti-inflammatory activity of the SVME may be due to the presence of flavonoids, alkaloids, or terpenoids. Our study supports the use of *S. villosa* Roxb. as the analgesic and anti-inflammatory agent. A further detailed study is ongoing to characterize which compounds are responsible for the anti-inflammatory and analgesic activity of the SVME.

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