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## Antinociceptive, anti-inflammatory and central nervous system depressant activities of crude methanol extract and its dichloromethane, chloroform and aqueous fractions of *Scurrula parasitica* (Linn.) leaves

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### Abstract

*Scurrula parasitica* (Linn.) has been used as traditional medicine against various diseases. Aim of the study was to evaluate analgesic, anti-inflammatory and CNS-depressant effects of methanol (MSPL), dichloromethane (DSPL), chloroform (CSPL) and aqueous (ASPL) extracts of *S. parasitica* leaves. Acetic acid-induced writhing and heat-induced tail immersion tests were used to evaluate analgesic effect while carrageenan-induced paw edema model was applied to find out anti-inflammatory potential at 100 and 200 mg/kg, b.w dose. The CNS-depressant effect of the extracts were evaluated by using open field and hole cross tests. All the extracts significantly ( $P^a < 0.01$ ,  $P^b < 0.001$ ) showed analgesic, anti-inflammatory and CNS depressant effects as dose dependant manner. Aqueous fraction showed maximum analgesic effect (74.54% inhibition of abdominal writhing and 90.78% elongation of tail withdrawal time) at 200mg/kg dose. The highest anti-inflammatory potential (92.10% inhibition of paw edema volume) was showed by the dichloromethane fraction at 200mg/kg dose. On the other hand, chloroform extract proved to have significant (96.16% and 95.01% inhibition of locomotion in open field and in hole cross test respectively) CNS-depressant activity after 120 min of oral administration of the extract at 200mg/kg dose. In conclusion, the methanol, dichloromethane, chloroform and aqueous extracts of the *S. parasitica* leaves possess marked analgesic, anti-inflammatory and CNS- depressant effects.

**Keywords:** *Scurrula parasitica*, analgesic, anti-inflammatory, CNS depressant effect

### Introduction

*Scurrula parasitica* (Linn.) is an herbaceous growing shrub of Loranthaceae family. It is a parasitic plant grown on *Dendrophthoe falcata* and on a wide range of hosts, including species of Apocynaceae, Euphorbiaceae, Fabaceae, Fagaceae, Lythraceae, Moraceae, Punicaceae, Rosaceae, Rutaceae, Sapindaceae, Theaceae, and Ulmaceae [1]. Traditionally it is used as diuretic, tranquilizing and hypotensive drug. In southwestern China, it has been used in ethno medicine as a shock therapy for the treatment of schizophrenia [2]. It has many pharmacological activities such as anticancer, immunomodulatory, antidiabetic, antioxidant, vasodilator and cytotoxic activity [3]. Many biologically active phytochemicals like lectins, viscotoxins, alkaloids, flavonoids, amines, terpenoids, quercetin, 4-O-acetylquercitrin, catechin, rutin, icaraside, aviculin, flavonoids, oleanolic acid, lupeol etc. have been identified from it [3, 4]. It is found in South East Asia, Malaysia, China, India and Australia [4].

Inflammation, associated with pain, is very important for proper functioning of the innate immune system. It protects from antigens and initiates immune responses [5]. Anxiety is responsible for obstacle of everyday life as it is involved with fear, worry as well as restlessness of a person. It, associated with psychological and physiological state, is marked by cognitive, somatic, emotional and behavioral factors which provoke a disagreeable emotion [6]. It is uncovered that the prolong use of synthetic drugs are harmful and very expensive for final development. These drugs have unpleasant side effects or toxic effects, and have failed to show a wide range of success against chronic diseases [7]. For these reasons, the medical practitioners and the patients are frequently choosing the herbal products having novel bioactive agent(s) [8, 9]. Inflammation and pain have long been treated with the traditional medicines, and often serve as a source of bioactive chemicals for the development of a new drug [10].

The present study was undertaken to investigate analgesic, anti-inflammatory and CNS- depressant activity of methanol, dichloromethane, chloroform and aqueous extracts of *S. parasitica* leaves in Swiss albino mice that may unveil the rationality of use of the plant as traditional medicines and potentiality of it in the herbal medicine.

## Materials and methods

### Plant materials

*S. parasitica* leaves were collected from Joypurhat, Bangladesh in June, 2013 and identified by an experts of the Bangladesh National Herbarium, Dhaka. Here a voucher specimen has also been retained with accession no. DACB-39434. The collected leaves were cleaned and dried for one week in electric oven, and pulverized into a coarse powder using a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark, and dry place until further analysis were taken.

### Extract preparation

Approximately, 500g of powdered material was placed in a clean, flat-bottomed glass container and soaked in methanol and similarly 500g of the powder was soaked in distilled water. Both of the containers with their contents were sealed and kept for 5 days. Then extraction was carried out by using an Ultrasonic Sound Bath accompanied by sonication (40 minutes). The entire mixture, then, underwent a coarse filtration by a piece of clean and white cotton material. Then the extracts were filtered through Whitman filter paper (Bibby RE200, Sterilin Ltd., UK) and were concentrated to obtain the methanol (13g) and aqueous (13g) extracts. Methanol extract was divided into two portions. One portion (2g) was poured into a glass vial to be tested as crude methanol extract, whereas the second portion (11g) was dissolved in 100 mL of methanol, and partitioned successively with chloroform and dichloromethane. The fractions were then concentrated using a rotary evaporator to obtain chloroform fraction (3.25g), and petroleum ether fraction (5.65g). This process rendered a gummy concentrated reddish black color. The gummy extracts were transferred to a closed container for further use and storage.

### Drugs and chemicals

Methanol, dichloromethane and chloroform were used as solvent for the extraction of the leaves and acetic acid was used in writhing test. These chemicals were purchased from Merck, Germany. Tween-80 was also collected from Merck, Germany. Ibuprofen, diazepam and morphine sulfate (MS) were collected from Square Pharmaceuticals Ltd. Bangladesh. All the chemicals used in these investigations were of analytical reagent grade.

### Animals

Swiss albino mice of either sex (25-30g) were purchased from the animal research branch of International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). Then the mice were kept in standard environmental conditions (25.0 ± 2.0°C & 55-65% relative humidity and 12h light/dark cycle) for four weeks to acclimate and fed ICDDR, B formulated rodent food and water *ad libitum*. The study protocol was approved by Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee of the University. The set of rules followed for animal experiment were approved by the institutional animal ethical committee

and handled in accordance with international guidelines for care and use of laboratory animals [11].

## Analgesic activity

### Acetic acid-induced writhing test

This test was performed using a method described by Koster [12]. Briefly, the animals were divided into ten groups (n=6). The first group received vehicle (1% tween 80 in water, p.o.). The second group received morphine sulfate at 10 mg/kg body weight while animals of group three to ten received MSPL, DSPL, CSPL and ASPL at 100 and 200mg/kg body weight. After 30 minutes of vehicle, standard drug and extracts administration, 0.6% v/v acetic acid was administered into the peritoneum of each animal. The writhing response was determined for 20 minutes after a latency period of 5 minutes. The Percentage reduction of abdominal constriction indicates the percentage protection against it which was taken as an index of analgesia. It was calculated as:

$$\% \text{ Inhibition} = [(N_c - N_t) / N_c] \times 100$$

Where,  $N_c$  = number of writhing of the control group,  $N_t$  = number of writhing of the treated group

### Tail immersion test

Tail immersion test was done by the method described by Toma [13]. According to the method, vehicle (1% tween 80 in water, p.o.), morphine sulfate (10mg/kg, p.o.), MSPL, DSPL, CSPL and ASPL were administered to the mice of respective groups 30 min before the experiment. About 3 cm tail of each mouse was immersed in warm water (55 ± 1°C) and reaction time (tail deflect time) was taken by a stop watch during drug treatment (0 min) and at 30, 60, 90, and 120 min of extract administration. To avoid tail tissue damage, 10s cut off period was maintained. The results were calculated as a percentage of the maximal possible effect (%MPE), which was calculated by using the following formula:

$$\% \text{MPE} = [(\text{Post drug latency} - \text{pre drug latency}) / (\text{Cut off period} - \text{pre drug latency})] \times 100$$

## Anti-inflammatory activity

### Carrageenan induced paw edema test

Anti-inflammatory activity was evaluated by carrageenan-induced paw edema model [14]. Swiss albino mice (25-30g) of both sexes were divided into ten groups of six animals in each. The test groups received 100 and 200 mg/kg, b.w of the extract MSPL, DSPL, CSPL and ASPL. The reference group received ibuprofen (10 mg/kg b.w.) while the control group received vehicle (1% tween 80 in water, b.w.). After 30 min, 100µl 1% carrageenan suspension was injected into the sub-plantar tissue of the left hind paw of each animal. The paw volume was measured before and after carrageenan injection at 1h, 2h, 3h and 4h using a Plethysmometer 7150 (Ugo Basile, Italy). The extent of reduction of paw volume revealed the ability of inflammation reduction and inhibition of inflammation was calculated by the following formula: % Inhibition =  $[(V_0 - V_s) / V_0] \times 100$

Where,  $V_0$  is the average paw edema volume of the control group,  $V_s$  is average paw edema volume of the treatment and standard group.

## CNS depression activity

### Open field test

CNS depression effect was evaluated by spontaneous locomotor activity of experimental mice which was described by Gupta *et al.* [15]. Sixty albino mice were divided into ten

groups ( $n = 6$ ). Before 30min of the experiment, vehicle (1% tween 80 in water, b.w.), diazepam (2mg/kg, b.w.), MSPL (100 and 200mg/kg, b.w.), DSPL (100 and 200 mg/kg, b.w.), CSPL (100 and 200 mg/kg, b.w.) and ASPL (100 and 200 mg/kg, b.w.) were administered to the respective group. The animals were placed on the floor of an open field (100 cm×100 cm×40 cm) having 64 squares of black and white color. The number of squares moved by each animal was counted for 5 min at 0, 30, 60, 90 and 120 min during the study period. Number of movement reduced by extracts revealed the extant of depressant, and the percentage inhibition of square movement was calculated at 120 min by the following formula:

$$\% \text{ Inhibition} = [(N_0 - N_s) / N_0] \times 100$$

Where,  $N_0$  is the average number of square traveled by control group,  $N_s$  is the average number of square traveled by treatment or standard group.

### Hole cross test

This test was performed by a method which was described by Takagi *et al.* [16]. Shortly, sixty albino mice were divided into ten groups ( $n = 6$ ). As like open field test, vehicle (1% tween 80 in water, b.w.), diazepam (2 mg/kg, b.w.), and the extracts (MSPL, DSPL, CSPL and ASPL) were administered to the respective group. After 30 minutes of treatment, passes of mice was counted for 5 minutes at 0, 30, 60, 90 and 120 min. The box is 30cm long, 20cm wide and 14 cm high. It has a 3cm diameter hole at 7.5 cm height in the center of the cage. CNS depressant activity was assessed by the reduced number

of passages of mice through the hole and percentage protection of movement was calculated at 120 min by the following formula:

$$\% \text{ Inhibition} = [(N_0 - N_s) / N_0] \times 100$$

Where,  $N_0$  is the average number of passage by control group,  $N_s$  is the average number of passage by treatment or standard group.

### Statistical analysis

All the values were expressed as the mean±SEM (Standard Error Mean) of three replicate experiments ( $n = 6$  mice per group). The analyses were performed by using SPSS statistical package for WINDOWS (version 15.0; SPSS Inc, Chicago) and  $P^b < 0.01$ ,  $P^a < 0.001$  were considered to be statistically significant compared to vehicle control group. Statistical significance (p) calculated by ANOVA followed by Dunnett's test.

## Results

### Analgesic activity

#### Acetic acid induced writhing method

All the extracts had reduced writhing response significantly ( $P^a < 0.001$ ) in dose dependant fashion. Standard drug morphine sulfate (10mg/kg) showed 83.84% inhibition followed by ASPL which showed 74.54% inhibition at 200mg/kg, b.w. The order of writhing inhibition of the extracts and the standard drug was MS>ASPL> CSPL> DSPL> MSPL (Table1).

**Table 1:** Analgesic effect of *S. parasitica* leaf extracts and morphine sulfate in acetic acid-induced writhing test.

Sample	Dose mg/kg, b.w.	Writhing number	Percent inhibition of writhing
Control(vehicle)	0.1ml/mice	47.53±6.21	00
MS	10mg/kg	7.68±1.15 <sup>a</sup>	83.84
MSPL	100	25.60±4.40 <sup>a</sup>	38.35
	200	17.55±3.25 <sup>a</sup>	60.69
DSPL	100	20.32±3.50 <sup>a</sup>	48.72
	200	14.22±2.10 <sup>a</sup>	64.94
CSPL	100	15.65±2.21 <sup>a</sup>	55.33
	200	10.58±2.43 <sup>a</sup>	69.85
ASPL	100	17.10±3.33 <sup>a</sup>	65.91
	200	12.50±2.11 <sup>a</sup>	74.54

Each value is presented as mean ± SEM ( $n = 6$ );  $p^a < 0.001$  compared with the control group. ANOVA followed by Dunnett's test is done in SPSS version 15.0.

### Tail immersion method

In the radiant heat tail-immersion test, the tail-withdrawal reflex time of the mice was significantly ( $p^a < 0.001$ ) elongated as dose dependent manner after administration of the extracts.

ASPL, among the extracts, exhibited potent effect. MPE of it was 25.17%, 43.47%, 60.59% and 90.78% at 1h, 2h, 3h and 4h, respectively. Order of tail withdrawal reflex time is MS>ASPL> CSPL> DSPL> MSPL (Table 2).

**Table 2:** Analgesic effect of *S. parasitica* leaves extracts and morphine sulfate in tail immersion test.

Sample	Dose mg/kg, b.w.	Response time (s) (%MPE)				
		0 min	30 min	60 min	90 min	120 min
Vehicle	0.1ml/mice	2.51±0.12	2.55±0.32	2.80 ±0.45	3.05±0.52	3.18±0.20
MS	10	2.55±0.21	4.48±0.33 <sup>a</sup> (24.89)	6.80±0.22 <sup>a</sup> (53.62)	7.80±0.36 <sup>a</sup> (67.40)	9.95 ± 0.75 <sup>a</sup> (96.22)
MSPL	100	2.62±0.27	2.79±0.10 (1.90)	3.56 ± 0.14 <sup>a</sup> (6.66)	4.13±0.12 <sup>a</sup> (13.03)	5.12±0.15 <sup>a</sup> (26.28)
	200	2.33±0.15	3.12±0.19 <sup>b</sup> (6.39)	4.52±0.42 <sup>a</sup> (20.57)	5.06±0.16 <sup>a</sup> (26.81)	6.28±0.23 <sup>a</sup> (43.80)
DSPL	100	2.31±0.16	2.83±0.16 (2.44)	3.72±0.23 <sup>a</sup> (8.98)	4.19±0.21 <sup>a</sup> (13.92)	5.72±0.38 <sup>a</sup> (35.34)
	200	2.55±0.18	3.11±0.36 <sup>b</sup> (6.25)	4.85±0.26 <sup>a</sup> (25.36)	5.28±0.20 <sup>a</sup> (30.07)	6.77±0.57 <sup>a</sup> (51.20)
CSPL	100	2.35±0.47	2.57±0.12 (2.99)	4.12±0.26 <sup>a</sup> (14.78)	5.16±0.70 <sup>a</sup> (28.29)	6.30±0.44 <sup>a</sup> (44.10)
	200	2.68±0.35	3.57±0.20 <sup>a</sup> (12.51)	4.92±0.34 <sup>a</sup> (26.37)	6.27± 0.20 <sup>a</sup> (44.74)	7.08±1.12 <sup>a</sup> (55.89)
ASPL	100	2.65±0.30	3.15±0.27 <sup>b</sup> (6.80)	4.87 ±0.25 <sup>a</sup> (25.65)	6.03± 0.42 <sup>a</sup> (41.18)	7.37±0.45 <sup>a</sup> (60.27)
	200	2.43±0.43	4.5±0.15 <sup>a</sup> (25.17)	6.10±0.35 <sup>a</sup> (43.47)	7.34 ±0.31 <sup>a</sup> (60.59)	9.39±0.57 <sup>a</sup> (90.78)

Each value is presented as mean ± SEM ( $n = 6$ );  $p^b < 0.01$ ,  $p^a < 0.001$  compared with the vehicle control group. ANOVA followed by Dunnett's test is done in SPSS version 15.0

**Anti-inflammatory activity**

The paw edema volume (mL) of the vehicle control group was a steady level during the study period. But administration of ibuprofen or extracts significantly reduced the volume according to dose size. Among the four extracts, MSPL

exhibited maximum effect, and inhibition of edema volume was 51.80%, 68.29%, 81.70% and 92.10% at 1h, 2h, 3h and 4h, respectively at 200mg/kg, b.w. dose. Order of the edema volume inhibition was ibuprofen >DSPL>MSPL>CSPL>ASPL (Table 3).

**Table 3:** Effect of p.o. administration of *S. parasitica* leaves extracts on carrageenan-induced paw edema.

Sample	Dose mg/kg	Mean edema volume in mL (Percent inhibition)				
		0 h	1 h	2 h	3 h	4 h
Control	0.1ml	0.85±0.01	0.83±0.04	0.82±0.02	0.82±0.03	0.76±0.02
Ibuprofen	10	0.83±0.02	0.47±0.02 <sup>a</sup> (43.37)	0.24±0.01 <sup>a</sup> (70.73)	0.11±0.01 <sup>a</sup> (86.58)	0.05±0.01 <sup>a</sup> (93.42)
MSPL	100	0.78±0.02	0.62±0.03 <sup>b</sup> (25.30)	0.45±0.03 <sup>a</sup> (45.12)	0.36±0.02 <sup>a</sup> (56.09)	0.21±0.01 <sup>a</sup> (72.36)
	200	0.75±0.03	0.56±0.02 <sup>b</sup> (32.53)	0.37±0.02 <sup>a</sup> (54.87)	0.22±0.03 <sup>a</sup> (73.17)	0.10±0.02 <sup>a</sup> (86.84)
DSPL	100	0.76±0.01	0.55±0.03 <sup>b</sup> (33.73)	0.34±0.01 <sup>a</sup> (58.53)	0.27±0.01 <sup>a</sup> (67.07)	0.15±0.03 <sup>a</sup> (80.26)
	200	0.79±0.02	0.40±0.02 <sup>a</sup> (51.80)	0.26±0.02 <sup>a</sup> (68.29)	0.15±0.01 <sup>a</sup> (81.70)	0.06±0.02 <sup>a</sup> (92.10)
CSPL	100	0.84±0.02	0.68±0.04 <sup>b</sup> (18.07)	0.52±0.03 <sup>a</sup> (36.58)	0.41±0.02 <sup>a</sup> (50.00)	0.28±0.02 <sup>a</sup> (63.15)
	200	0.83±0.03	0.65±0.03 <sup>b</sup> (21.68)	0.48±0.03 <sup>a</sup> (41.46)	0.34±0.03 <sup>a</sup> (58.53)	0.23±0.02 <sup>a</sup> (69.73)
ASPL	100	0.77±0.02	0.74±0.02 (10.84)	0.53±0.03 <sup>b</sup> (35.36)	0.47±0.03 <sup>a</sup> (42.68)	0.38±0.02 <sup>a</sup> (50.00)
	200	0.79±0.03	0.71±0.04 <sup>b</sup> (14.45)	0.48±0.03 <sup>a</sup> (41.46)	0.42±0.02 <sup>a</sup> (48.78)	0.34±0.01 <sup>a</sup> (55.26)

Each value is presented as mean ± SEM (n = 6); <sup>b</sup> $p < 0.01$ , <sup>a</sup> $p < 0.001$  compared with the vehicle control group. ANOVA followed by Dunnett's test is done in SPSS version 15.0

**CNS depression activity****Open field test**

Mice movement was significantly ( $P < 0.001$ ) reduced due to the extracts administration. The effect was significant from second observation (30 min) and continued up to the fifth

observation (120 min) in dose dependant manner. At the fifth observation, MSPL, DSPL, CSPL and ASPL showed 91.14%, 93.51%, 96.16% and 86.96% at a dose of 200mg/kg,b.w, respectively. The order of inhibition was CSPL >DSPL>MSPL>ASPL (Table 4).

**Table 4:** CNS depressant activity of different fractions of *S. parasitica* leaves by open field test in mice.

Sample	Dose mg/kg, b.w.	Number of movements					% Inhibition of movements after 120 min
		0 min	30 min	60 min	90 min	120 min	
Control	0.1ml	176.20±12.65	167.41±9.15	160.73±9.11	152.56±8.40	135.47±7.88	--
Diazepam	2	178.45 ±10.12	73.18±8.12 <sup>a</sup>	15.28±2.02 <sup>a</sup>	5.25±2.17 <sup>a</sup>	4.29±0.65 <sup>a</sup>	96.83
MSPL	100	180.00±14.58	140.40±11.78 <sup>b</sup>	80.40±8.05 <sup>a</sup>	17.33±3.05 <sup>a</sup>	20.33±1.52 <sup>a</sup>	85.23
	200	173.66±13.57	130.18±10.64 <sup>a</sup>	66.33±7.25 <sup>a</sup>	13.58±2.01 <sup>a</sup>	12.66±0.57 <sup>a</sup>	91.14
DSPL	100	178.20±12.35	120.08±8.23 <sup>a</sup>	67.66±5.06 <sup>a</sup>	14.33±2.05 <sup>a</sup>	14.66±1.52 <sup>a</sup>	89.17
	200	181.05±13.78	97.60±9.40 <sup>a</sup>	44.66±5.40 <sup>a</sup>	10.20±2.10 <sup>a</sup>	8.78±0.57 <sup>a</sup>	93.51
CSPL	100	174.63±11.59	105.36±10.11 <sup>a</sup>	48.66±6.12 <sup>a</sup>	9.66±2.30 <sup>a</sup>	9.10±0.65 <sup>a</sup>	93.28
	200	175.66±13.31	80.41±7.56 <sup>a</sup>	17.33±5.50 <sup>a</sup>	6.66±2.51 <sup>a</sup>	5.23±0.57 <sup>a</sup>	96.16
ASPL	100	184.33±11.12	160.56±12.07	90.33±8.36 <sup>a</sup>	28.33±4.45 <sup>a</sup>	28.82±1.12 <sup>a</sup>	78.72
	200	177.66±16.91	146.36±13.45 <sup>b</sup>	49.33±6.47 <sup>a</sup>	17.66±1.72 <sup>a</sup>	17.66±1.52 <sup>a</sup>	86.96

Each value is presented as the mean ± SEM (n = 6); <sup>b</sup> $p < 0.01$ , <sup>a</sup> $p < 0.001$  compared with the vehicle control group. ANOVA followed by Dunnett's test is done in SPSS version 15.0

**Hole-cross test**

Here, the passes number was reduced significantly because of extract treatment. All the extracts showed significant reduction from the second (30min) to the fifth (120min) observation. At the fifth (120min) observation the standard

drug diazepam (2mg/kg,b.w.) gave 96.57% inhibition, and MSPL, DSPL, CSPL and ASPL (200mg/kg, b.w.) showed 89.70%, 90.44%, 95.01% and 81.22% inhibition. The order of hole-cross reduction was diazepam>CSPL > DSPL>MSPL>ASPL (Table 5).

**Table 5:** CNS depressant activity of different fractions of *S. parasitica* leaves by hole cross test in mice.

Sample	Dose mg/kg,b.w.	Number of movements					% Inhibition of movements after 120 min
		0 min	30 min	60 min	90 min	120 min	
Control	0.1ml	36.20±4.70	35.04±3.18	33.94±3.20	33.12±4.38	30.68±3.66	--
Diazepam	2	34.27±3.20	7.38±1.12 <sup>a</sup>	4.12±0.02 <sup>a</sup>	2.01±0.01 <sup>a</sup>	1.05±0.02 <sup>a</sup>	96.57
MSPL	100	38.07±6.39	29.33±6.11 <sup>b</sup>	16.33±5.03 <sup>a</sup>	9.66±1.78 <sup>a</sup>	6.33±0.37 <sup>a</sup>	79.36
	200	35.25±5.15	19.00±2.64 <sup>a</sup>	10.33±2.51 <sup>a</sup>	5.33±1.80 <sup>a</sup>	3.16±0.52 <sup>a</sup>	89.70
DSPL	100	37.46±3.40	28.66±4.04 <sup>b</sup>	18.66±2.52 <sup>a</sup>	9.52±1.16 <sup>a</sup>	5.45±0.54 <sup>a</sup>	82.23
	200	35.23±4.10	20.0±5.15 <sup>a</sup>	10.66±2.08 <sup>a</sup>	5.04±0.57 <sup>a</sup>	2.93±0.57 <sup>a</sup>	90.44
CSPL	100	38.60±6.20	26.0±5.21 <sup>a</sup>	14.66±2.16 <sup>a</sup>	6.45±0.36 <sup>a</sup>	3.12±0.15 <sup>a</sup>	89.83
	200	36.12±3.78	18.33±4.50 <sup>a</sup>	7.15±1.20 <sup>a</sup>	3.32±0.70 <sup>a</sup>	1.53±0.17 <sup>a</sup>	95.01
ASPL	100	35.77±5.48	32.0±4.18	22.00±2.32 <sup>a</sup>	15.66±1.51 <sup>a</sup>	9.65±0.45 <sup>a</sup>	68.54
	200	37.09±4.65	26.33±5.09 <sup>a</sup>	12.66±2.65 <sup>a</sup>	8.23±1.65 <sup>a</sup>	5.76±0.57 <sup>a</sup>	81.22

Each value is presented as mean ± SEM (n = 6); <sup>b</sup> $p < 0.01$ , <sup>a</sup> $p < 0.001$  compared with the vehicle control group. ANOVA followed by Dunnett's test is done in SPSS version 15.0

## Discussion

Pain is associated with many diseases and several natural products are used to relieve pain and inflammation. Abdominal writhing mediates via local peritoneal receptor. Intraperitoneal injection of acetic acid induces capillary permeability that promotes generation of pain sensitive prostaglandin specifically PGE2 and PGF2 $\alpha$ , and release of free arachidonic acid, an endogenous inflammatory substance, from tissue phospholipids by the action of cyclo-oxygenase (COX) enzyme [17]. Substance(s) inhibiting the writhing response may have analgesic effect preferably by inhibition of prostaglandin synthesis [18]. Non steroidal anti-inflammatory drugs (NSAIDs) inhibit COX in peripheral tissues, and therefore interfere with the pain sensation. The extracts could block the pain inducing endogenous substances similar to that of NSAIDs [17]. The tail immersion method can evaluate centrally acting analgesic properties of a drugs or chemicals. Heat induces pain sensation through opioid receptors. So, the agent that elongates reaction time in tail immersion test, acts through the opioid receptors and called centrally acting analgesics [19]. Thus, the significant ( $p^a < 0.001$ ) reduction of acetic acid-induced writhing (Table 1), and elongation of reaction time in tail immersion test (Table-2) indicates the peripherally and centrally acting analgesic effect of the extracts, respectively.

Carrageenan-induced paw edema is a convenient and well established method for evaluation of anti-inflammatory effect of any compound. Here the edema formation is mediated by two phases such initial phase and late phase. The initial phase is involved with non-phagocytic edema formation and lasts between one to two minutes. Here carrageenan induces release of inflammatory mediators such as histamine, serotonin and bradykinin that promotes vascular permeability. The second phase, predominant phase, remains up to five hours and, increased edema formation takes place because of prostaglandin, bradykinin, protease, lysosome-like substances, growth factors and neurogenic factors release [20]. During the inflammatory response, prostaglandin such as PGE2 and/or PGI2 production is facilitated through COX-2 activity [21]. Moreover, it (inflammation) involves cell migration, plasma exudation, and production of mediators like interleukin (IL)-1 $\beta$ , IL-6 and tumor necrosis factor-alpha (TNF- $\alpha$ ) [22]. The anti-inflammatory drugs such as aspirin and ibuprofen have been found to inhibit prostaglandin production that is the main reason of anti-inflammatory action in carrageenan-induced paw edema [23]. All the extracts (MSPL, DSPL, CSPL and ASPL) significantly ( $p^b < 0.01$ ,  $p^a < 0.001$ ) reduced paw edema volume in dose dependant manner (Table 3). From the results, it is suggested that the anti-inflammatory effect of the extracts on carrageenan-induced paw edema may act via the inhibition of NO production, along with the inhibitory activity of COX-2 [21].

Open field, hole cross and head deep tests (locomotor test) are widely applied to evaluate CNS depressant potential of an agent. These tests evaluates index of alertness and a reduction of it is an indicative of sedative or CNS depressant activity [24]. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the CNS [25]. Various drugs such as anxiolytic, muscle relaxant and sedative-hypnotic acts via the GABA. Sedative agents may give the CNS depressant effect through modification of the GABA system where modification may take place by potentiating postsynaptic inhibition of the GABA receptors and elevating the GABA-induced chloride conductance with simultaneous depression

of voltage activated Ca<sup>++</sup> currents [26]. Therefore, it is predictable that the extracts may act by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization leading to a reduction in the firing rate of critical neurons in the brain [25]. The locomotor testes have shown that all doses of the extracts significantly ( $p^b < 0.01$ ,  $p^a < 0.001$ ) reduced the frequency and the amplitude of movements in dose dependant manner from the second observation (30 min) and continued up to the fifth observation (120 min) period (Table 4 and Table 5) which may be due to the presence of compound(s) having CNS depressant potential.

Natural products like alkaloids, flavonoids and tannins are potent analgesic compounds. Flavanoids exert their effect through inhibition of prostaglandin synthase [27]. Various flavonoid derivatives including quercetin have inhibitory effect of arachidonic acid metabolizing enzymes (phospholipase A<sub>2</sub>, cyclooxygenase and lipoxigenase). Several phytochemicals like flavonoids, saponins and tannins etc have CNS depressant effect. Many flavonoids and neuroactive steroids are ligands for GABA<sub>A</sub> receptors in the central nervous system which suggests that they can act as benzodiazepine-like agents [24].

Literature review revealed that *S. parasitica* leaves extracts are rich of alkaloids, flavonoids, amines, terpenoids, quercetin, 4-O-acetylquercitrin, catechin, rutin oleanolic acid etc. bioactive compounds [3, 4]. These compounds are believed to be responsible for analgesic, anti-inflammatory and CNS-depressant effects of the extracts. However, there is no strict evidence about which substances are exactly responsible for these effects. So further studies are necessary to find out the key compound(s) responsible for these effects, and to identify the mechanism of action and the pharmacodynamics of these effects.

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