

ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF METHANOL EXTRACT OF *VALLARIS SOLANACEA* LEAVES

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ABSTRACT

Objective: The present communication attempts to evaluate the Anti-inflammatory and Analgesic activities of methanol extract of *Vallisneria spiralis* leaves belongs to the family: Apocynaceae, which is widely distributed in India.

Methods: Methanol extract of *Vallisneria spiralis* at doses of 250mg/kg and 500mg/kg were tested for their anti-inflammatory activity against carrageenan induced paw edema and formalin induced arthritis models in rats and analgesic activity was tested against acetic acid induced writhing (in mice) models and the two phases of formalin induced paw licking models of pain.

Results: The results indicate that the methanol extracts at the doses of 250 and 500mg/kg body weight significantly inhibited, the carrageenan induced paw edema and formalin induced arthritis models of inflammation, similarly the extract at the doses of 250 and 500mg/kg significantly inhibited the acetic acid induced writhing (in mice) models and the two phases of formalin induced paw licking models of pain.

Conclusion: This study confirms that the methanol extract of *Vallisneria spiralis* has good analgesic and anti-inflammatory property. Further studies are necessary to isolate the compounds from the methanol extract of *Vallisneria spiralis*, responsible for the activity.

Keywords: *Vallisneria spiralis*, analgesic, inflammation, extracts, rats and mice.

INTRODUCTION

Vallisneria spiralis (Roth) Kuntze (family: Apocynaceae), local name: Agarmoni; Bread flower, is a tall climbing shrub which is locally used as medicinal plant. It is native in India and Burma and also found in Bangladesh. It is traditionally used for sores and wounds. [1] We have previously reported the analgesic [2] and anti-inflammatory activities [3] of the ethanol extract of this plant. Therefore in the present study we aim to investigate whether the methanol extract of *Vallisneria spiralis* also has analgesic and anti-inflammatory activities. Since different solvent extracts of a particular plant may have different biological activities due to differences in chemical composition. Furthermore we seek to investigate whether the methanol extract has comparable or higher analgesic and anti-inflammatory activities than the ethanolic extract. So, that the extract with the higher activity will be further subjected to bioguided fractionation.

MATERIALS AND METHODS

Animals

Albino rats of either sex weighing 150-200gm were used for this study. They were bred and housed in the department of Pharmacology, college of Pharmaceutical Sciences, Shri Guru Ram Rai Institute of Technology and Sciences, Dehradun, India. The animals were maintained under standard environmental conditions of Temperature, illumination, light and dark cycle.

They were also fed with mouse cubes (Bendel feeds) and water was provided ad libitum. Ethical clearance for handling the animals and the procedures used in the study was obtained from the institutional animal ethical committee.

Plant material

As in the previous study the plant used for this study was collected from local areas of Dehradun, forest of Santla Devi Temple, near the road side and identification of plant was done on the bases of morphological characters. The collected plant material was authenticated by Dr S.K Srivastava (Scientist-"D"/HOD), Botanical Survey of India, Dehradun Voucher specimen of the plant has been deposited in the herbarium of the institute.

Plant extraction

The fresh leaves of *Vallisneria spiralis* were air-dried and subsequently reduced to powdery form. 500 g of the powdery sample was exhaustively extracted with 5 L of methanol by maceration. The solvent was evaporated in a water bath maintained at 45 °C to give a dark brown solid extract weighing 30 g. The extract was stored at 4°C until it was reconstituted in normal saline for pharmacological studies.

Analgesic activity

Formalin induced paw licking in Rats

The method [4] was used for this study. Animals were divided into four groups denoted as Control group, Positive control group and Test group I and Test group II consisting 06 mice in each group. Control group received orally 0.1ml of 1% suspension in sodium CMC at the dose of 10 ml/kg body weight and Positive control group received orally indomethacin at the dose of 100mg/kg body weight. Test group I and Test group II were treated with test

Sample orally at the dose of 250 and 500 mg/kg body weight. 0.2 ml of 3 % formalin was injected into the dorsal surface of the left hind paw of rats 1 h after oral administration of the extracts. The time spent by each animal in licking the injected paw was observed for 5 min. (from 0- 5min post formalin injection) and 10 min (from 20-30 min post formalin injection). The mean of the licking time was determined and compared with the mean for the control group.

Acetic Acid induced writhing test in mice

The analgesic activity of the sample was studied using acetic acid induced writhing model in mice [5]. In this study animals were grouped as in the formalin induced paw licking method in rats. Control group received orally 0.1ml of 1% suspension in sodium CMC at the dose of 10 ml/kg body weight and Positive control group received orally Indomethacin at the dose of 100mg/kg body weight. Test group I and Test group II were treated with test sample orally at the dose of 250 and 500mg/kg body weight. A thirty minutes interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (1%w/v) was administered intra-peritoneally to each of the animals of a group. After an interval of 5 minutes was given for

absorption of acetic acid and number writhing was counted for 15 minutes. The percentage inhibition was calculated using the formula

$$\% \text{ Inhibition} = \frac{\text{Mean no. of writhing (control)} - \text{Mean no. of writhing (drugs)}}{\text{Mean no. of writhing (control)}}$$

Mean no. of writhing (control)

Anti-inflammatory studies

Carrageenan induced paw edema

Pedal inflammation was produced in rats according to the carrageenan induced paw edema method [6]. 24 albino rats were divided into four groups of six each and fasted overnight for 18 hrs with water. Next day the animals were weighted and numbered. A mark was made on the right hind paw just beyond tibio-tarsal junction, so that every time the paw was dipped in the mercury (Hg) column up to the fixed mark to ensure constant paw volume. The initial paw volume was noted of each rat by mercury (Hg) displacement method. 0.1 ml of 1 % carrageenan was injected into the right hind paw of each rat under the sub plantar aponeurosis. The animals were treated orally with extracts (250 and 500 mg/kg), indomethacin (100mg/kg) or Saline (10 ml/kg) 1 h before carrageenan injection. The paw volume of each rat after carrageenin injection after one hour, two hour, three hour and five hour was recorded by mercury displacement in plethysmograph.

This volume is called final volume. The anti-inflammatory activity of the extract was measured by its potential to prevent edema caused by carrageenan as against the control group which was given the vehicle only. Mean paw edema was calculated for each group as average of paw volume of individual rats belonging to that group. Since, the mean was subjected to positive and negative fluctuations hence; standard error for each group was also calculated.

Standard error (S.E) = standard deviation

$$\sqrt{n}$$

Where, n = number of rats in each group

Percent inhibition of paw edema was calculated according to the following formula:

$$\% \text{ Inhibition} = \frac{a-b}{a} \times 100$$

a- is the mean paw inflammation volume of control.

b- is the mean paw inflammation volume of test

Formalin induced arthritis

In this study arthritis was produced by sub aponeurotic injection of 0.1 ml of 2 % formaldehyde in the right hind paw of the rat on the first and the third day [7]. The animals were treated daily with different doses of the extract (250 and 500mg/kg), saline (10 ml/kg) or indomethacin (100mg/kg) for 10 days. The daily changes in paw size were also measured (as in the carrageenan test) by mercury displacement in plethysmograph.

Statistical Analysis

All values are Mean \pm SEM. Statistical analysis was by the student's t test. Values with $p < 0.05$ were considered as being statistically significant.

RESULTS

Analgesic studies

Formalin-induced paw licking

The methanol extract of *Vallis solanacea* at the doses of 250 and 500 mg/kg significantly inhibited the two phases of the formalin test. The summary of the result are shown in Table 1.

Table 1: Effects of the methanol extract of *Vallis solanacea* leaves on Formalin-Induced Paw Licking Test.

Group	No. of animal	Dose (mg/kg orally)	Licking time(sec) ^a	
			Early phase	Late phase
Control Sodium CMC vehicle	6	10ml/kg	131.6 \pm 5.8	89.6 \pm 5.8
Indomethacin	6	100mg/kg	68.2 \pm 5.4**	27.2 \pm 5.5***
Extract I	6	250mg/kg	64.0 \pm 3.7**	35.6 \pm 7.9**
Extract II	6	500mg/kg	52.8 \pm 4.6**	26.2 \pm 2.4***

^aEach value is the mean \pm S.E.M. of 6 rats

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.01$ compared with control; student's t-test.

Table 2: Effect of the methanol extract of *Vallis solanacea* leaves on Acetic acid induced writhing in mice.

Group	No. of animal	Dose (mg/kg b.wt.)	Writhing count
Control Sodium CMC vehicle	6	10ml/kg	16.0 \pm 0.37
indomethacin	6	100mg/kg	7.0 \pm 0.9**
Extract I	6	250mg/kg	10.0 \pm 0.37*
Extract II	6	500mg/kg	7.9 \pm 0.26*

^aEach value is the mean \pm S.E.M. of 6 rats

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.01$ compared with control; student's t-test.

Tables 3: Effects of methanol extract of *Vallis solanacea* leaves on Carrageenan-induced paw edema.

Group	No. of animal	Dose (mg/kg b.wt.)	Mean increase in paw volume		Inhibition (%)	
			3hr	4hr	3hr	4hr
Control Sodium CMC vehicle	6	10ml/kg	3.6 \pm 0.60	3.4 \pm 0.75	---	---
Indomethacin	6	100mg/kg	1.6 \pm 0.24*	0.4 \pm 0.45*	55.6	88.9
Extract I	6	250mg/kg	0.8 \pm 0.20**	0.8 \pm 0.20*	77.8	77.8
Extract II	6	500mg/kg	0.6 \pm 0.24**	0.2 \pm 0.2*	83.3	94.4

^aEach value is the mean \pm S.E.M. of 6 rats

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.01$ compared with control; student's t-test.

Acetic acid induced writhing

The methanol extract of *Vallisaria solanacea* at the doses of 250 and 500 mg/kg significantly decreased the number of writhing from 10.0 ± 0.37 to 7.9 ± 0.26 . The activity of the higher dose of the extract (500 mg/kg) was comparable to that of indomethacin. The summary of the results are shown in Table 2.

Anti-inflammatory studies

Carrageenan induced paw edema

The methanol extracts of *Vallisaria solanacea* at the doses of 250 and 500 mg/kg significantly reduced the carrageenan induced edema. The paw size was reduced from 3.6 ± 0.60 to 0.6 ± 0.24 mm after 3 h of carrageenan administration. Likewise the extract significantly decreased the paw edema after 5 h of carrageenan injection. The results are shown in Table 3.

Formalin-induced Arthritis

The extracts (250-500 mg/kg) significantly ($P < 0.05$) inhibited paw edema formation in the animals as from the 4th day of formalin injection and extract administration. The results were shown in Table 4.

Table 4: Effects of methanol extract of *Vallisaria solanacea* leaves on Formalin-induced arthritis in rats.

Group	Dose (mg/kg b.wt.)	Mean increase in paw volume	Inhibition (%)
Control	10ml/kg	0.14 ± 0.05	----
Sodium CMC vehicle			
indomethacin	100mg/kg	$0.10 \pm 0.01^*$	28.8
Extract I	250mg/kg	0.11 ± 0.04	21.4
Extract II	500mg/kg	$0.10 \pm 0.01^*$	28.8

^aEach value is the mean \pm S.E.M. of 6 rats

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.01$ compared with control; student's t-test.

DISCUSSION AND CONCLUSION

The results obtained from the present study shows that the methanol extract of *Vallisaria solanacea* has analgesic and anti-inflammatory activities. The extracts significantly inhibited the licking time in the two phases of the formalin test as well as the characteristic writhing observed following intraperitoneal injection of acetic acid. These two tests (formalin induced paw licking and acetic acid induced writhing) are used for detecting specific activities of drugs that may have analgesic activities. Drugs that inhibit the first phase of the formalin test have the ability to alleviate neurogenic pain while those drugs that inhibit the second phase of the test have the ability to inhibit inflammatory pain [8]. The acetic acid induced writhing test on the other hand is a model of visceral pain that is useful for analgesic drug development except that it gives false positives for muscle relaxant and sedatives [9], [10] Since the extract of *V. solanacea* produced significant effects in all the models of pain used in this study it show that the extract at high doses has strong analgesic activity. The anti-inflammatory activity of the methanol extract of *V. solanacea* was investigated using two models namely; carrageenan, and formalin (arthritis) models which represent acute and chronic forms of inflammation[11] The extract dose dependently inhibited both two types of inflammation although it was the higher doses of the extracts that were highly effective

against both the two types of inflammation Above extract at concentration of 500mg/kg was found to inhibit 94.4% of edema measured after 4 hrs of injecting 0.1ml of 1% (w/v) of carrageenin, which was comparable to indomethacin (100mg/kg) taken as standard, showing 88.9% of edema inhibition after 4 hrs. A marked decrease in mean paw edema was observed in both the extract treated groups observed at 1hr, 2hr, and 3hr and 4hr favoring it was more explicit in the higher dose (500mg/kg) group.

Hence it can be interpreted from the above results that extract at 500mg/kg concentration has good anti-inflammatory activity. In conclusion, this study further confirms the anti inflammatory and analgesic activities of *Vallisaria solanacea*. The focus of further study is to use bio guided fractionation to determine the active components in this extract and to determine the mechanism of action of the active components.

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