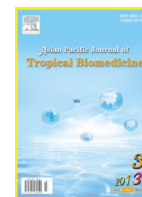




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In vivo screening of essential oils of *Skimmia laureola* leaves for antinociceptive and antipyretic activity

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PEER REVIEW

Peer reviewer

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Comments

Generally, the study is a very potential one and is based on the rationalization traditional therapies. It is a good finding and could be useful addition for the journal. Therefore I recommend for publication.

(Details on Page 206)

ABSTRACT

Objective: To study the screening of essential oils of *Skimmia laureola* leaves (SLO) for acute toxicity, antinociceptive, antipyretic and anticonvulsant activities in various animal models. **Methods:** SLO were extracted using modified Clevenger type apparatus. Acute toxicity test was used in mice to observe its safety level. Antinociceptive activity of SLO was evaluated in acetic acid induced writhing and hot plate tests. Yeast induced hyperthermic mice and pentylenetetrazole induced convulsive mice were used for the assessment of its antipyretic and anticonvulsant profile respectively. **Results:** Substantial safety was observed for SLO in acute toxicity test. SLO showed a high significant activity in acetic acid induced writhing test in a dose dependent manner with maximum pain attenuation of 68.48% at 200 mg/kg *i.p.* However, it did not produce any relief in thermal induced pain at test doses. When challenged against pyrexia evoked by yeast, SLO manifested marked amelioration in hyperthermic mice, dose dependently. Maximum anti-hyperthermic activity (75%) was observed at 200 mg/kg *i.p.* after 4 h of drug administration. Nevertheless, SLO had no effect on seizures control and mortality caused by pentylenetetrazole. **Conclusions:** *In vivo* studies of SLO showed prominent antinociceptive and antipyretic activities with ample safety profile and thus provided pharmacological base for the traditional uses of the plant in various painful conditions and pyrexia. Additional detail studies are required to ascertain its clinical application.

KEYWORDS

Skimmia laureola leaves, Essential oils, Antinociceptive, Antipyretic activity

1. Introduction

Skimmia laureola (*S. laureola*) locally named as Nazar Panra belongs to family Rutaceae. In Pakistan, it is common in the Hazara region, Murree Hills, Kashmir, upper Swat and Shangla^[1,2]. Therapeutic values of the plant have been recognized in various cultural of the world. It has been used for the treatment of cold, fever and headache. Smoke of leaves and twig smoke are used as demon repellent^[1]. In the form of smoke, dry leaves have been employed for nasal tract clearness. Additionally, leaves of the plant have been recommended for multiple purposes including relieve coughs^[3], insecticides, and pesticides^[5]. Leaves are commercially harvested and used in food as flavoring agent,

in traditional healing and cultural practices, being made into garlands and considered sacred^[4]. Essential oils extracted from the plant showed profound anthelmintic activity^[6].

Phytochemically, fatty acid ester and quinoline alkaloid have been isolated from the plant with significant inhibition on tyrosinase^[10]. Lupene derivatives, chromones, coumarines and triterpine have also been reported^[11–13]. Three quinoline alkaloids (3-hydroxy, 2, 2, 6-trimethyl-3, 4, 5, 6-tetrahydro-2H-pyranof[3, 2-c] quinoline 5-one, ribalinine and methyl isoplatydesmine) were isolated from the aerial parts of the plant with prominent inhibition against acetylcholinesterase. Additional, methyl isoplatydesmine showed spasmolytic activity possibly mediated through the blockade of voltage-dependent Ca²⁺ channels^[14].

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Keeping in mind the outstanding ethno-pharmacological and phytochemical background of *S. laureola*, the current project was designed to extract the essential oils from the leaves of the plant followed by acute toxicity test, antinociceptive, antipyretic, and anticonvulsant activities in various animal models.

2. Materials and methods

2.1. Chemicals

Paracetamol (Tianjin Bofa Pharmaceutical Co., Lit., China), diclofenac sodium (Suzhou Ausun Chemical Co., Lit., China), acetic acid, Brewer's yeast (Merck Germany), tramadol[®] (Seale Pakistan Ltd.), and pentylenetetrazole (PTZ) (Sigma Chemical Co., St Louis, USA) were prepared.

2.2. Essential oil extraction

For the extraction of essential oil from the leaves of *S. laureola*, a modified Clevenger type apparatus was used. Briefly, the leaves of the plant were thoroughly washed, cut into small pieces, placed in distillation flask and subjected to hydro-steam distillation for about 4 h. The steam and vaporized oil were condensed into liquid by a vertical condenser and collected in measuring tube. The volatile oil being immiscible and lighter than water, separated out as an upper layer. The oil was then separated from water and collected in small bottles, dried with anhydrous sodium sulphate. The bottles were sealed, labeled and stored in light resistant vials at 4–6 °C for further use in various experiments^[15].

2.3. Animals

NMRI mice were used in various experiments. They were fed with standard laboratory food and water *ad libitum*. Animals were kept under standard condition of temperature and light. Before the start of experiment, animals were acclimatized with laboratory conditions. All the protocols were approved by Ethics Committee of HEJ Research Institute Karachi. The rulings of the institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council were maintained during all the experiments performed^[16].

2.3. Acute toxicity test

Acute toxicity test of essential oils from the leaves of *S. laureola* (SLO) was performed in NMRI mice ($n=6$) of either sex to evaluate any possible toxicity. The animals were tested by administering different doses (500, 1000, 1500 and 2000 mg/kg) of SLO, while the control group received normal saline (10 mL/kg). All the groups were observed mortality during 24 h^[17].

2.4. Antinociceptive activity

2.4.1. Acetic acid induced writhing test

The analgesic activity was carried out using NMRI mice (18–22 g) of either sex. Animals were divided into five groups ($n=6$). Group I and II were injected with normal saline (10 mL/kg, *i.p.*) and ibuprofen (150 mg/kg, *i.p.*), while remaining groups were treated with SLO (20, 100 and 200 mg/kg, *i.p.*). After the

above treatments, animals were injected *i.p.* with acetic acid (1%). The abdominal constriction (writhing) was counted for 10 min after 5 min of acetic acid injection^[18,19].

2.4.2. Hot plate test

NMRI mice of either sex ($n=6$) weighing (18–22) g were acclimatized to laboratory conditions for 1 h before the start of experiment with food and water available *ad libitum*. Animals were then subjected to pre-testing on hot plate (Havard apparatus) maintained at (55.0±0.1) °C. Animals having latency time greater than 15 seconds on hot plate during pre-testing were rejected (latency time). All the animals were grouped each of six. Group I was treated with saline (10 mL/kg), Group II was treated with tramadol[®] (30 mg/kg *i.p.*). Group III, IV and V were treated with 50, 100 and 200 mg/kg SLO, *i.p.* respectively. After 30 min of treatment, the animals were placed on hot plate, and the latency time [time for which mouse remains on the hot plate at (55.0±0.1) °C without licking or flicking of hind limb or jumping] was measured in seconds. In order to prevent the tissue damage, a cut-off time of 30 seconds was imposed for all animals^[20]. Percentage of analgesia was calculated using the following formula:

$$\text{Analgesia (\%)} = (\text{Test latency} - \text{Control latency}) / (\text{Cut-off time} - \text{Control latency}) \times 100$$

2.5. Antipyretic test

The antipyretic activity was evaluated for SLO using NMRI mice (25–30 g) of either sex. The animals were divided into five groups with each of six. All groups were fasted overnight but allowed free accesses to drinking water. Group I received saline (10 mL/kg) as a negative control, Group II received paracetamol (150 mg/kg) as a standard drug, while the remaining Groups III, IV and V received 50, 100 and 200 mg/kg *i.p.* SLO respectively. Normal temperature was recorded using digital thermometer, and then pyrexia was induced in all mice using 20% aqueous suspension of Brewer's yeast (10 mL/kg *s.c.*). Rectal temperature was recorded after 24 h and corresponding groups were injected with above doses accordingly^[21]. After drugs administration, rectal temperature was again recorded periodically at 1, 2, 3, 4 and 5 h of drugs administration. The percentage of reduction in pyrexia was calculated by the following formula:

$$\text{Reduction (\%)} = (T_a - T_b) / T_b \times 100$$

Where T_a represents normal body temperature; T_b , temperature after yeast injection.

2.6. Pentylenetetrazole induced anticonvulsant test

The animals were divided into groups of at least six animals. Group I was treated with normal saline (10 mL/kg) as a control, Group II was treated with diazepam (5 mg/kg, *i.p.*), while remaining groups were treated with SLO (50, 100 and 200 mg/kg *i.p.*). After 30 min of treatment, all the animals were injected PTZ 80 mg/kg *s.c.* Each animal was observed for onset and mortality^[15].

2.7. Statistical analysis

Results were expressed as mean±SEM. One-way ANOVA was used for comparison test of significant differences

among groups followed by Dunnet's multiple comparison post test. A level of significance ($P < 0.05$ or $P < 0.01$) was considered for each test.

3. Results

3.1. Acute toxicity test

SLO was found substantially safe in acute toxicity test. As shown in Table 1, gross behaviour changes were not observed even at all test doses (500, 1000, 1500 and 2000 mg/kg *i.p.*). All mice survived at 500 and 1000 mg/kg. However, 16.67% and 66.67% mortality was noted at 1500 and 2000 mg/kg *i.p.* respectively.

Table 1

Effect of essential oil of *S. laureola* in acute toxicity test.

Treatment	Dose	Gross behaviour effect	No. of animal died (n=6)	Mortality (%)
Normal saline	10 mL/kg	No change	0	–
	500 mg/kg	No change	0	–
SLO	1000 mg/kg	No change	0	–
	1500 mg/kg	No change	1	16.67
	2000 mg/kg	No change	4	66.67

3.2. Antinociceptive activity

3.2.1. Effect of SLO in acetic acid induced writhing test

Results of SLO in acetic acid induced writhing test are presented in Table 2. Profound amelioration of abdominal constriction induced in acetic acid pain model was observed in a dose dependent manner. Maximum pain attenuation (68.48%) is noted at 200 mg/kg *i.p.* (Figure 1). Diclofenac was more prominent in its antinociceptive activity and showed 84.45% pain reversal at 30 mg/kg *i.p.*

Table 2

Effect of the essential oils of *S. laureola* in acetic acid-induced writhing in mice.

Group	Dose	No of writhes
Saline	10 mL/kg	72.35±2.90
SLO	50 mg/kg	48.10±2.35
	100 mg/kg	35.74±3.10*
	200 mg/kg	22.80±2.40**
Diclofenac	10 mg/kg	11.25±2.50**

Values were expressed as mean±SEM (n=6). *: $P < 0.05$, **: $P < 0.01$ indicated statistically significant values from control.

Table 4

Effect of essential oil of *S. laureola* at 50, 100 and 200 mg/kg *i.p.* in yeast induced pyrexia.

Drugs	Dose	Rectal temperature (°C)						
		Normal (A)	After yeast (B)	1 h (C1)	2 h (C2)	3 h (C3)	4 h (C4)	5 h (C5)
Saline	10 mL/kg	37.10±0.17	38.44±0.30	38.49±0.15	38.65±0.10	38.80±0.15	38.68±0.17	38.55±0.21
PRA	150 mg/kg	37.00±0.15	39.33±0.10	38.08±0.12**	37.58±0.09**	37.20±0.04**	37.22±0.06**	37.27±0.12**
	50 mg/kg	37.12±0.17	39.60±0.22	39.39±0.12	39.30±0.02	39.10±0.15*	38.79±0.19*	38.90±0.16*
SLO	100 mg/kg	37.05±0.10	39.05±0.14	38.81±0.11	38.52±0.09**	38.±1.55**	38.00±0.15**	38.03±0.45*
	200 mg/kg	37.00±0.09	39.76±0.23	39.34±0.55*	38.95±0.25**	38.07±0.35**	37.69±0.10**	37.77±0.45**

Values were expressed as mean±SEM (n=6). *: $P < 0.05$, **: $P < 0.01$ indicated statistically significant values from control. PRA: Paracetamol.

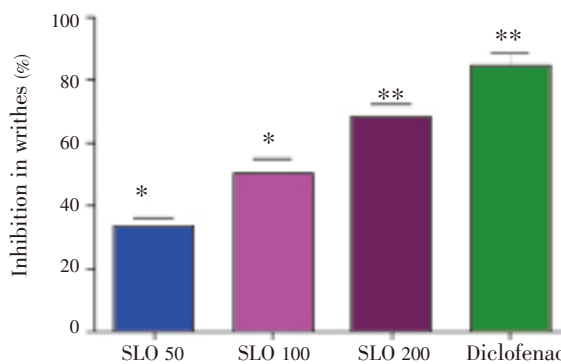


Figure 1. Protection (%) of essential oil of *S. laureola* in acetic acid induced writhing test.

3.2.2. Effect of SLO in hot plate test

The results of SLO in hot plate test are shown in Table 3. The effect was not significant even at the maximum test 200 mg/kg *i.p.* However, tramadol^R used a standard drug, showed profound activity in hot plate test.

Table 3

Effect of essential oil of *S. laureola* in hot plate test.

Groups	Dose	Latency time (min)			
		0	60	90	120
Saline	10 mL/kg	9.17±0.09	9.16±0.05	9.19±0.45	9.12±0.88
SLO	50 mg/kg	9.21±0.50	9.20±0.55	9.45±0.40	9.22±0.33
	100 mg/kg	9.25±0.70	9.28±0.33	10.11±0.66	9.90±0.44
	200 mg/kg	9.29±0.76	10.34±0.45	10.90±0.77	10.20±0.55
	Tramadol ^R	30 mg/kg	9.20±0.02	25.88±0.06*	25.80±0.07*

Values were expressed as mean±SEM (n=6). *: $P < 0.001$ indicated statistically significant values from control.

3.2.3. Effect of SLO in yeast induced hyperthermic mice

SLO manifested marked antipyretic activity in yeast induced hyperthermic mice as shown in Table 4. It showed profound protection (%) in a dose dependent manner (Figure 2). Maximum protection (80.03%) was observed at 200 mg/kg *i.p.* (Figure 2c) after 4 h of treatment.

3.2.4. Effect of SLO in PTZ induced anticonvulsant test

The results of PTZ induced convulsion test are illustrated in Table 5. The results of pentylenetetrazole induced convulsion test are illustrated in Table 5. As shown in Table 5, SLO did not exhibit any protection from seizures and mortality at test doses (50, 100 and 200 mg/kg *i.p.*). However, diazepam (7.5 mg/kg *i.p.*) being the standard drug, exhibited marked protection from both seizures and mortality.

Table 5

Effect of essential oil of *S. laureola* on duration and inhibition of seizures and mortality in acute s.c. PTZ-induced seizure model in mice.

Groups	Dose	Onset of jerks (seconds)	Rearing & falling (seconds)	Hind limb tonic extension (seconds)	Mortality protection (%)
PTZ	90 mg/kg	210±35	465±65	850±65	0
	50 mg/kg	210±43	500±55	887±82	0
SLO	100 mg/kg	217±65	514±35	889±58	0
	200 mg/kg	225±43	510±40	890±80	0
Saline	0.5 mL/kg	–	–	–	100
Diazepam	7.5 mg/kg	850±23	0	0	100

SLO and diazepam were injected *i.p.* 45 min before the administration of 90 mg/kg of s.c. PTZ. Values were expressed as mean±SEM for the duration of tonic seizures ($n=6$).

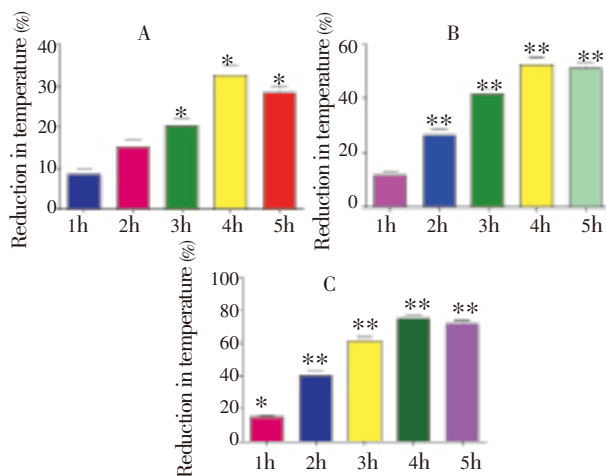


Figure 2. Protection (%) of SLO in yeast induced hyperthermic mice at A: 50 mg/kg, B: 100 mg/kg, C: 200 mg/kg during various assessment times (1–5 h).

Values are presented as mean±SEM for pyrexia control ($n=6$).

4. Discussion

The present study demonstrated strong antinociceptive (peripherally acting) and antipyretic activities of SLO with substantial safety profile in acute toxicity test. However, it did not show any protection against seizures.

Acetic acid induced writhing test has been primarily used by various research groups for the assessment of antinociceptive of natural compounds worldwide[22,23]. Acetic acid caused the release of different endogenous noxious mediators such as bradykinin, serotonin, histamine, and substance P[24,25]. The resulting pain is symbolized by contraction of the abdominal muscle accompanied by an extension of the forelimbs and body elongation. Peripheral nociceptive fibers are sensitive to both narcotics analgesic and non-steroid anti-inflammatory drugs[18,20]. SLO showed marked reduction in the abdominal constriction provoked by the acetic acid in a dose dependent manner. Consequently, one possible mechanism of antinociceptive activity of SLO could be due to the blockade of the effect or the release of endogenous substances (arachidonic acid metabolites) that excite pain nerve endings.

Hot plate test is principally a spinal reflex and used to test

supra-spinal analgesic activity of test articles. For that reason, this test is generally employed for the assessment of centrally acting analgesic drugs, like morphine, while peripheral anti-nociceptive agents are found to be inactive on thermal stimulus[18]. When SLO was subjected to the challenge of hot plate test, it did not show any thermal pain attenuation at test doses. Thus, it could be assumed that the antinociceptive action of SLO was purely mediated through blockade of peripheral pain stimulant without the any involvement of central action.

Fever is the primary feature of disease since time immemorial. The febrile response is coordinated by the central nervous system through endocrine, neurological, immunological and behavioural mechanisms. The initiation, manifestations and regulation of the febrile response are dependent on the pyrogenic and anti-pyrogenic properties of various exogenous and endogenous substances. Medical experts believe that fever is based on consistent rise in body temperature above normal daily fluctuations originating in combination with an elevated thermoregulatory set point[21,28]. These neurons are sensitive not only to changes in blood temperature but also to cold and warm receptors located in skin and muscle and thus maintain an appropriate balance between the heat production and loss[29].

SLO exhibited profound antipyretic activity against yeast evoked hyperthermic mice when administered intraperitoneally during various assessment times (1–5 h). Pyrexia amelioration was in a dose dependent manner and remained significant up to 5th hour of drug administration. The nonsteroidal antiinflammatory drugs (NSAID) are the drug of choice for the control of febrile in routine practice. Over a period of time after thorough investigation, it is believed that NSAID inhibits prostaglandins synthesis via cyclooxygenase pathway[30]. For that reason, SLO might contain active principle(s) that exhibited inhibitory action on cyclooxygenase. As a result, they produced antipyretic activity by preventing the formation of prostaglandins or by increasing the concentration of body's own antipyretic components[22].

The intraperitoneal administration of SLO did not show any protection from seizures and mortality at various test doses in PTZ induced convulsion test. Consequently, it suggests the absence of such components could exhibit anticonvulsant activity.

In short, SLO showed prominent peripheral antinociceptive and antipyretic activity in animal studies without any anticonvulsant effect. SLO exhibited substantial safety profile in acute toxicity test. Our findings are consistent with the ethnopharmacological uses of the plant in various painful conditions and as antipyretic and thus offered an alternate natural therapeutic modality with considerable safety profile. However, detail studies on the efficacy, potency and safety aspects will guaranteed its clinical utility.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Synthetic agents used in the current treatment system are partly healing the diseased condition while largely affecting several other organs. In this condition, traditional therapeutic system could be ideal. Here a traditional therapy is validated in established experimental protocols.

Research frontiers

This research article is based on the preliminary studies on the analgesic and antipyretic potential of essential oil of a viola species. However, mechanism of the said activity is still required to be evaluated.

Related reports

There is not any related report on this particular activity of the plant in literature. So it is first time reported.

Innovations and breakthroughs

In the preliminary stage, it is a very important study that showed yet another natural healing agent for the treatment of very common human agony like pain.

Applications

It could be now used as analgesic and antipyretic in the traditional system of treatment.

Peer review

Generally, the study is a very potential one and is based on the rationalization traditional therapies. It is a good finding and could be useful addition for the journal. So I recommend for publication.

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