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Antipyretic and Antinociceptive Profile of Leaves of *Skimmia laureola*

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Abstract: The main objective of the current finding was to evaluate the possible antipyretic and analgesic potential of crude ethanolic extract of the leaves of *Skimmia laureola* (SLE). The antipyretic potential was investigated using brewer's yeast hyperthermia paradigm. The antinociceptive effect was assessed through acetic acid (1 %) induced writhing test and tail immersion test. Various classes of phytoconstituents were determined quantitatively.The test samples significantly ($P< 0.05$) attenuated the induced pyrexia in a dose dependant manner. The percent antipyretic effect of SLE at the dose of 300, 200 and 100 mg/kg was 72.31, 52.84 and 29% respectively at the third hour and remained significant till the fifth hour. Regarding the acetic acid induced writing test the maximum protection was observed at 300 mg/kg followed by 200 and 100 mg/kg having the percent protection effect as 72.45, 58.34 and 24.23 respectively. No antinociceptive effect was observed in tail immersion test. The presence of alkaloids (12.50±0.09 mg/g), sterols (81.30±0.61 mg/g) saponins (20.93±0.06 mg/ g), tannins (26.83±0.12mg/g), phenolic compounds (10.33±0.66 mg/g) and flavoniods (12.58±0.66 mg/ g) was found in SLE.The present study strongly supports the antipyretic and analgesic folklore of *Skimmia laureola*. The leaves of this plant are a rich source of alkaloids, seroles, saponins, tannins, phenolic compounds and flavoniods.

Key words: Antipyretic · Antinociceptive · Andbrewer's Yeast

INDRODUCTION

*Skimmia laureola*belongs to family Rutaceae.It grows at altitude of 5500-10500 feet under shady condition in forest. It is common in the Hazara region, Murree Hills, Kashmir, Upper Swat and Upper Dir [1,2]. In Nathiagally the plant is growing gregariously around the tract leading to Mukshpuri top. *S. laureola* is consideredan important medicinal and sacred plants.Leaves are used traditionally in local ailments. When crushed, the leaves give a musky odor due to the presence of a poisonous compound skimnianine [2]. Traditionally, burning smoke from the dried leaves and branches is demon repellent. Medicinally they are used for relief in cough [3]. Leaves are commercially harvested and are used in food as flavoring agent, in traditional healing and cultural practices, being made into garlands and considered sacred.Crushed leaves with wheat flour are used as anthelmintic for livestock [1]. The smoke from the leaves is also used for clearing the nasal tract. It is also used as cold remedy, antipyretic and analgesic, used as insecticides and pesticides [4]. In the present study, *Skimmia laureola*wastested for its antipyretic, antinociceptive, and its phytoconstitutes.

MATERIALS AND METHODS

Plant Materials: Fresh collection of *Skimmia laureola* was undertaken from Patrak (Upper Dir). Plant materials were cleaned, washed and garbled. Some fresh specimen were pressed, dried, mounted on herbarium sheets, given voucher number Bot. 8815 and kept in the herbarium of Department of Botany, University of Peshawar, Pakistan for ready references. The dried plant parts were then pulverized into powder with electric grinder. These powders was then extracted with ethanol and n-hexane, according to standard method [5-8], the crude ethanolic extract was used for evaluation of antipyretic and antinociceptive effects.

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Chemicals: Paracetamole, Diclofenac sodium (Suzhou Ausun Chemical Co, Lit.,China), Acetic acid, Brewer's yeast (Merck Germany) and Carrageenan (Sigma Lambda, USA). Sterile normal saline was used in all experiments as control while extract was prepared in normal saline.

Animals: BALB/c mice of either sex were used in all experiments. Animals were purchased from the Pharmacology Section of the Department of Pharmacy, University of Peshawar, Peshawar. The animals were maintained in standard laboratory conditions (25°C and light/dark cycles i.e. 12/12 h and were fed up with standard food and water. The experimental protocols were approved by the ethical committee of the university of Peshawar [9].

Acute Toxicity: The acute toxicity study was carried out for the crude ethanolic extract of leaves (SLE) of *Skimmia laureola*. The study was carried out using BALB/c mice weighing 20-25 g of either sex. The animals were randomly distributed into four groups each of six animals. The animals were acclimatized to the laboratory conditions before the commencement of experiment. All the animals were deprived from food overnight, the control group received normal saline and the remaining II-IV groups were treated with 500, 1000 and 2000 mg/kg body weight respectively with crude ethanolic extract of leaves. The animals were observed continuously for the first 4 hrs and then for the next 24 [10-12].

Antipyretic Activity: The antipyretic activity of SLE was evaluated using BALB/c mice (25 - 30 g) of either sex. The animals were acclimatized to the laboratory condition before the start of experiment; all the animals were divided in five groups each of six. All groups were fasted overnight while allowed free accesses to drinking water. Groups I and II received saline and paracetamol as control and standard drug while the remaining groups received 100, 200 and 300 mg/kg of SLE respectively. Normal temperature was recorded using digital thermometer before injecting 20% aqueous suspension of Brewer's yeast (10 ml/kg s.). After 18 hrs, rectal temperature was recorded and corresponding groups were injected with the doses mentioned. Rectal temperature was recorded periodically at 1, 2, 3, 4 and 5 hrs of the drugs administration [13,14]. The percent reduction in pyrexia was calculated by using the following formula.

$$\text{Percent reduction} = (B - Cn)/(B - A) \times 100$$

Where, A (normal rectal temperature), B (temperature after yeast injection) and Cn is body temperature after 1, 2, 3, 4 and 5 hrs[15].

Antinociceptive Activity: The SLE (100, 200 and 300 mg) was tested for its antinociceptive effect using acetic acid induced writhing and tail immersion paradigm. Animals were divided into five groups. Group I was injected with normal saline (10 ml/kg i.p) as control while Group II was injected with standard drug diclofenac sodium (10 mg/ kg i.p) and the remaining three groups were injected with 100, 200 and 300mg/kg i.p. SLE. After 30 min of saline, diclofenac sodium and plant extract injection, the animals were treated i.p. with 1% acetic acid. The writhing was counted after 5 min of acetic acid injection. The number of abdominal constrictions (writhing) was counted for 10 min [16].

Quantitative Chemical Analysis: Quantitative analysis of the *S. laureola* leaf was carried out to determine alkaloids, sterols, tannins, saponins, phenols and flavonoids quantitatively. Alkaloids in the leaves of *S. laureola was* quantitatively determined following the method ofVan Buren and Robinson [17]. Saponin quantity was determined by method of Huang *et al*. [18]. Percent tannins in the respective samples were determined following McDonald *et al*, Percentage of sterol was determined by following Chang *et al.* [20]. The percent phenol concentration of ethanolic extracts was determined followingMuhammad and Saeed [21]. Total flavonoid contents were determined by following aluminum chloride colorimetric method of Khan *et al.* [22,23].

Statistics and Interpretation: The data were analysed using ANOVA (Graphpad prism5, Germany, Germany). *P* Values < 0.05 were regarded as significant. *P* Values >0.05 were regarded as non-significant [18].

RESULTS AND DISCUSSION

Acute Toxicity: In the present study ethanolic extract of *S.laureola*leafatdoses of 500, 1000 and 2000 mg/kg body weight was evaluated for toxicological effects, using mice as test animals. No mortality or morbidity was observed for the first four hours and then even for the next 24 hours, showing that the plant is safe for further *in*-vivo work (Table 1).

Table 1: Acute toxicity test of leaves of *Skimmia laureola* in mice, assisted for 24 hrs

Group	Dose (mg/Kg)	Dead	Survived	Gross effect
Saline	10	-	All	-
SLE	500	-	All	-
	1000	-	All	-
	2000	-	All	-

Table 2: Antipyretic effect of ethanolic extract of *Skimmia laureola* leaf (100, 200 and 300 mg/kg i.p.) and pracetamol (150 mg/kg)

		Initial rectal temperature (C°)		Rectal temperature (C°) after administration of drug				
Treatment	Dose mg/kg	Normal (A)	After 24 h (B)	1 h (C1)	2 h (C2)	3 h (C3)	4 h (C4)	5 h (C5)
Normal saline	10 mL	36.66±0.21	38.93±0.23	38.81±0.12	38.88±0.13	38.88 ± 0.22	38. 78 ± 0.18	38.75 ± 0.25
Paracetamol	150	37.10± 0.08	38.8± 0.04	37.51± 0.01	37.46± 0.03	37.32± 0.02	37.35± 0.28	37.45± 0.04
SLE	100	37.10± 0.17	38.37±.0.06	38.10 ± 0.44	38.13± 0.12	38.00±0.10	38.00±0.10	38.00±0.10
	200	36.87± 0.31	38.63± 0.05	38.07± 0.38	37.83*± 0.15	37.70* ± 0.17	37.73*± 0.15	37.8* ± 0.17
	300	37.17± 0.31	38.47± 0.21	37.98* ± 0.44	37.93*± 0.23	37.53**± 0.35	37.60**± 0.10	37.63**± 0.15

Data presented as mean ± SEM

Antipyretic Effect: The SLE was screened for its antipyretic agent (Table 2). The percent antipyretic effect of SLE at the dose of 300, 200 and 100 mg/kg was 72.31, 52.84 and 29% at the third hour and remained significant (P<0.01) till the fifth hour (Figure 1). At low dose no promising antipyretic activity was observed. At a dose of 200 mg/kg, significant (P<0.01) reduction in body temperature was observed from second hour to fifth hour while at a dose of 300mg/kg, fall in body temperature was observed at the first hour, after which highly significant hypothermia was recorded till the end of experiment. The positive control drug (paracetamol, 150 mg/kg) showed a highly significant (P<0.001), the antipyretic effect of SLE was not higher than paracetamol, while in comparison with negative control it was significant (P<0.01) as shown in figure 1.

Antinociceptive Effect: SLE significantly (P<0.001) and at a dose dependant manner blocked the acetic acid induced writhing in mice. The maximum effect was observed at 300 mg/kg followed by 200 and 100 mg/kg having the percent protection effect as 72.45, 58.34 and 24.23 respectively as shown in figure 2. The antinociceptive effect of diclofenac sodium (10 mg/kg) was better than SLE. No antinociceptive effect was observed in tail immersion model.

Phytochemical Profile: The results revealed the presence of bioactive constituents in leaves comprising of alkaloids (12.50±0.09 mg/g), sterols (81.30±0.61 mg/g) saponins (20.93±0.06 mg/ g), tannins (26.83±0.12mg/g), phenols (10.33±0.66 mg/g) and flavonoids (12.58±0.66 mg/ g) in the ethanolic extract of the leaves.

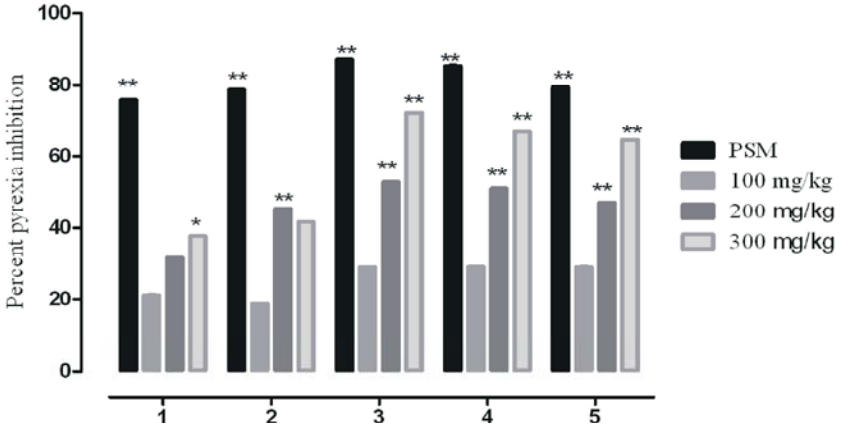

Fig. 1: Antipyretic effect of SLE in mice. Bar presents the percent inhibition of pyrexia after 1,2,3,4 and 5h of the treatment with paracetamol (150mg/kg) and SLE (100, 200 and 300 mg/kg). The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. *P<0.05, **P<0.01

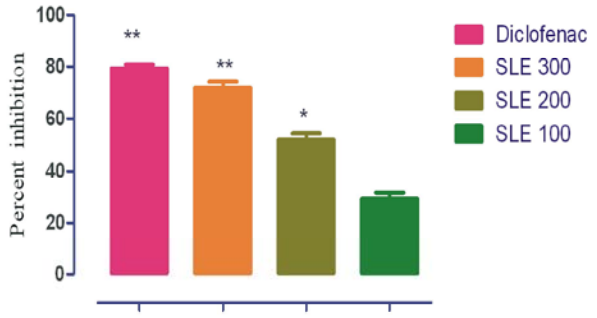

Fig. 2: Percent analgesic activity of SLE (100, 200 and 300mg/kg) in acetic acid induce pain model. Bars present as mean±S.E.M. of percent analgesia for group of six animals. The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. *P<0.05, **P<0.01.

Pyrexia or hyperthermia is a secondary blow of malignancy, infectivity or other ill situations. Barkatullah and Muhammad[24]. Body temperature is regulated by very narrow range between heat gain and heat loss and set a point at which body temperature is maintained, is controlled by the hypothalamus. Elevation of this set point causes pyrexia and various synthetic drugs like paracetamol are then used to normalize body temperature [25]. Hyperthermia due to brewer's yeast intake is well known and convenient method for assessment of antipyretic potential of medicinal plants or other agents[26]. At higher doses, its efficacy was found to almost paracetamol. Hesperidin and coumarinolignans isolated from *Zanthoxylum*species (Rutaceae), have been reported as antipyretic, analgesic and anti-inflammatory agent[27,28]. As *S.laureola* belongs to this family, these compounds might also be present in this plant, which were responsible for the antipyretic effect of SLE. Like the other antipyretic drugs, SLE might be inhibitory to prostaglandin- biosynthesis and this may be the possible mechanism of its antipyretic action.

Acetic acid induced writhing test is very simples and well validated procedure for testing of any substances for its antinociceptive effect. Acetic acid induced test is conducted for evaluation of peripheral antinociceptive effect while the tail immersion conducted for central antinociceptive effect. The intraperitoneal injection of acetic acid produces an abdominal writhing response due to sensitization of chemo-sensitive nociceptors by prostaglandins. Increase level of protanoids as well as lipoxygenase products have been found in the peritoneal fluid after the injection of the acetic acid. The analgesic effect of SLE may therefore be due to either its action on visceral receptors sensitive to acetic acid or due to inhibition of the production of prostaglandins [29]. The SLE was proved as peripheral analgesic rather central one because no effect was observed in tail immersion test.

Quantitative analysis for bioactive constituents like alkaloids, sterol, saponins, tannins, phenols and flavoniods were also carried out in leaves and bark of *Skimmialareola*. It is obvious from both the qualitative and quantitative analysis, that this plant is a rich source of bioactive substances, which might be helpful in combating diseases.

It is very interesting that SLE is antipyretic and analgesic at the same time; because in most of the case pyrexia is coexist with body aches. It is common perception among the public that natural products are same and free of side effects, so further work is needed to find the exact mode of action as antipyretic and antinociceptive. The isolation of bioactive constitutes may help in finding a new safe and effective antipyretic and antinociceptive molecule.

CONCLUSION

The present study strongly supports the use of leave of *Skimmia laureola*in the management of fever and pain.

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