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Study of analgesic activities of the plant *Spilanthes acmella* var; *Oleracea* in experimental animals models

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ABSTRACT

The analgesic activity of extracts of the plant *Spilanthes acmella* Var;*oleracea* was studied in albino rats of wistar strain. The extraction of *Spilanthes acmella* Var;*oleracea* were carried out using distilled water and ethanol. Ethanolic extract was fractionated using ethyl acetate, diethyl ether, petroleum ether and butanol extracts in succession. In the present studies aqueous extract of *Spilanthes acmella* Var;*oleracea* was evaluated for its peripheral activity of acetic acid induced writhing response in albino mice, central analgesic activity by tail flick method in albino rats. It was found that the aqueous extract of *Spilanthes acmella* Var;*oleracea* has very good peripheral analgesic activity and significant central analgesic activity in comparison to that of aspirin and pethidine respectively. The activity was found to be more significant on acetic-acid induced model (P<0.001) than the tail flick model (P<0.001) and thus it appear that the test drug inhibits predominantly the peripheral pain mechanism.

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KEYWORDS

Writhing;
Tail flick;
Analgesic;
Flavanoids;
SPAO.

INTRODUCTION

Spilathes acmella var;*oleracea*(SPAO) is an indigenous herb belonging to the family Asteraceae (Kannada-Vana mugali, Sanskrit-Akalkar, Hindi-Ukara, pokarmul, Telegu-Maratitige). It is grown as an annual herb throughout the tropics. It is commonest plant in the South canara district of Karnataka. It is also distributed in Nepal, Bangladesh, Malaysia, Indonesia, Srilanka and Brazil^[1].

It has conical small yellow flowers. The whole plant possesses medicinal properties. The flower heads were too chewed to relieve toothache and crushed plant used in rheumatism. The leaves are also eaten raw or as a

vegetable by many tribes of India. In the form of tincture in the doses of 10 to 30 minims is used as a powerful stimulant and sialogogue. It is a popular remedy for children who stammer. It is used to treat inflammation of the periosteum of the jaw and application has speedy effect in relieving pain and swelling. SPAO is generally known as toothache plant^[2].

MATERIALS AND METHODS

Preparation of aqueous extract

The plant was authenticated by Dr. Gopalkrishna Bhat, Department of Botany, Poornapragna College,

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TABLE 1 : Effect of various Aqueous extract of the plant *SPA O* on tail flick latency in rats

Treatment	Dose mg/kg	Tail flick latency in Sec (Mean \pm SEM) in at time (min)				
		0 min	30 min	60 min	90 min	120 min
Control	---	2.38 \pm 0.006	2.40 \pm 0.007	2.35 \pm 0.005	2.32 \pm 0.006	2.34 \pm 0.003
Pethidine	05	2.51 \pm 0.019	4.87 \pm 0.021**	7.5 \pm 0.061**	8.37 \pm 0.018**	5.13 \pm 0.045**
Aqueous extract	100	2.61 \pm 0.019	4.88 \pm 0.066**	5.37 \pm 0.044**	6.18 \pm 0.068**	4.64 \pm 0.053**
Aqueous extract	200	2.43 \pm 0.028	5.19 \pm 0.010**	6.19 \pm 0.067**	6.73 \pm 0.064**	5.24 \pm 0.0067**
Aqueous extract	400	2.74 \pm 0.044	5.61 \pm 0.016**	6.85 \pm 0.025**	7.84 \pm 0.034**	5.43 \pm 0.097**
Ethanol extract	100	2.62 \pm 0.011	4.88 \pm 0.066**	5.37 \pm 0.044**	6.18 \pm 0.068**	4.64 \pm 0.053**
Ethanol extract	200	2.99 \pm 0.500	4.36 \pm 0.020**	5.23 \pm 0.010**	6.3 \pm 0.018**	4.16 \pm 0.017**
Ethanol Extract	400	2.5 \pm 0.008	4.63 \pm 0.021**	5.73 \pm 0.040**	7.2 \pm 0.014**	4.81 \pm 0.017**
Ethyl acetate extract	100	2.48 \pm 0.017	2.81 \pm 0.009**	3.75 \pm 0.018**	4.93 \pm 0.020**	3.10 \pm 0.015**
Ethyl acetate extract	200	2.33 \pm 0.009	3.74 \pm 0.024**	4.25 \pm 0.012**	6.15 \pm 0.014**	3.74 \pm 0.019**
Ethyl acetate extract	400	2.73 \pm 0.014	3.58 \pm 0.03**	5.63 \pm 0.023**	7.02 \pm 0.027**	3.68 \pm 0.047**
Diethyl ether extract	100	2.78 \pm 0.023	3.11 \pm 0.015**	3.45 \pm 0.019**	3.65 \pm 0.025**	3.11 \pm 0.024**
Diethyl ether extract	200	2.6 \pm 0.016	3.23 \pm 0.017**	4.12 \pm 0.030**	4.92 \pm 0.021**	2.76 \pm 0.017**
Diethyl ether extract	400	2.66 \pm 0.019	4.70 \pm 0.015**	5.74 \pm 0.018**	5.74 \pm 0.018**	3.28 \pm 0.032**
Petroleum ether extract	100	2.25 \pm 0.016	2.89 \pm 0.014**	3.10 \pm 0.031**	3.58 \pm 0.028**	2.67 \pm 0.020**
Petroleum ether extract	200	2.58 \pm 0.020	3.11 \pm 0.034**	3.56 \pm 0.015**	4.33 \pm 0.022**	2.96 \pm 0.043**
Petroleum ether extract	400	2.4 \pm 0.024	3.15 \pm 0.040**	4.05 \pm 0.027**	4.65 \pm 0.022**	3.47 \pm 0.026**
Butanol extract	100	2.27 \pm 0.026	2.86 \pm 0.024**	2.97 \pm 0.021**	3.58 \pm 0.029**	3.07 \pm 0.021**
Butanol extract	200	2.68 \pm 0.026	2.92 \pm 0.021**	3.45 \pm 0.031**	4.04 \pm 0.040**	3.15 \pm 0.019**
Butanol extract	400	2.74 \pm 0.024	3.15 \pm 0.033**	3.57 \pm 0.032**	4.51 \pm 0.021**	3.50 \pm 0.033**

All the values are expressed as mean \pm SEM (n=6), **P<0.01 significant compared to control

Udupi, and Karnataka. Fresh aerial parts of *SPA O* were collected from Mangalore. The plant parts were cleaned dried under shade and powdered by a mechanical grinder. The dried plants were powdered (500gm) and soaked in distilled water and kept aside for 2 days. After 2 days, aqueous layer was decanted off and allowed to evaporated under the flame to a syrupy consistency and then evaporate to dryness (120gm). The extract of *SPA O* was administered as a suspension (2% acacia) to the animals.

Preparation of ethanolic extract

The dried plants were powdered (2Kg) and soaked in ethanol (95%) and kept aside for 4 days, the ethanolic layer was decanted off. The process was repeated for four times. The solvent from the total extract was distilled off and the concentrate was evaporated on a water bath to a syrupy consistency and then evaporated to dryness (250gm).

Fractionation of ethanolic extract

The ethanolic extract (160 gm) was divided in to

four equal portions. Each portion was suspended in distilled water (500ml) and then extract with petroleum ether (60-80°C, 8X500 ml), Diethylether (8x500ml), Butanol (8x500ml) and Ethyl acetate (8x500ml in succession. The entire fractions were freed of solvent by distillation. The ethanolic extract was thus fractioned in to Petroleum ether (60-80°C) soluble fraction (5gm), Diethyl ether soluble fraction (8gm), butanol soluble (10gm) and ethyl acetate soluble fraction (7gm).

Phytochemical studies

Freshly prepared *SPA O* extract was subjected to photochemical screening tests for the detection of various constituents using by standard procedure^[3].

Selection of animals

Albino rats of wistar strain (150-200gm) and Swiss albino mice (25-30gm) of either sex were produced from the central animal house of the institute. They were housed in standard polypropylene cages and kept under controlled room temperature (24 \pm 2°C; relative humidity 60-70%) in a 12hour light-dark cycle. The

TABLE 2 : Effect of extract of the plant SPAO on acetic acid induced writhes in mice

Treatment	Dose mg/kg	Number of writhing mean \pm SEM	% Inhibition
Control (1% Tween)	-----	80.66 \pm 2.12	-----
Aspirin	300	21.33 \pm 0.71**	73.55
Aqueous extract	100	33.83 \pm 0.749**	58.05
Aqueous extract	200	27.83 \pm 0.600**	65.49
Aqueous extract	400	22.83 \pm 0.542**	71.69
Ethanol extract	100	35.66 \pm 0.066**	55.78
Ethanol extract	200	31.50 \pm 0.56**	60.94
Ethanol extract	400	24.66 \pm 0.66**	69.42
Ethyl acetate extract	100	41.33 \pm 0.042**	48.76
Ethyl acetate extract	200	37.83 \pm 0.47**	53.09
Ethyl acetate extract	400	30.66 \pm 0.88**	61.98
Diethyl ether extract	100	46.66 \pm 0.71**	42.15
Diethyl ether extract	200	41.33 \pm 0.88**	48.76
Diethyl ether extract	400	34.33 \pm 0.88**	57.43
Petroleum ether extract	100	54.66 \pm 1.382**	32.23
Petroleum ether extract	200	48.50 \pm 0.885**	39.87
Petroleum ether extract	400	40.83 \pm 0.909**	49.38
Butanol extract	100	57.83 \pm 1.70**	28.30
Butanol extract	200	55.66 \pm 0.128**	30.99
Butanol extract	400	50.16 \pm 0.94**	37.81

rats were given a standard laboratory diet and water ad libitum. Food was withdrawn 12h before and during the experimental hours. The experimental protocols were approved by the institutional animal ethics committee.

Acute toxicity study

The preliminary pharmacological studies were conducted to assess the acute pharmacological effect of LD₅₀ of the drug extracts. The acute toxicity study was carried out in albino rats by "up and down method (OECD guidelines 425)"^[4].

Statistical analysis

The results were analyzed for statistical significance using one-way ANOVA followed by Dun net's test. A P value < 0.001 was considered as significant.

Tail flick method

The pre screened animals were dividing in to groups. Pethidine 5mg/kg acted as the standard drug. The drugs were administered intra-peritoneally. The tail flick latency was assessed by the analgesiometer. The strength

of the current passing through the naked nicrome wire was kept constant at 6 Amps. The distance between the heat source and the tail skin was 1.5 cm. The site of application of the radiant heat in the tail was maintained at 2.5 cm, measured from the root of the tail. The cut-off reaction time was fixed at 10 sec to avoid tissue damage^[7].

Acetic acid- induced writhing test

The prescreened animals were divided in to six groups. Aspirin in doses of 50, 100, and 150 mg/kg. Suspended in (2%) gum acacia was used as the standard drug. The drugs were autoclaved at 121°C for 30 min⁵ (the compounds were assumed to be heat stable) and administered subcutaneously. Writhing was induced 30 min later by intra-peritoneal injection of 10ml/kg of 0.3% acetic acid in distilled water^[6]. The number of writhes was counted for 30 min immediately after the acetic acid injection. The percentage of protection was calculated.

RESULTS

The LD₅₀ study reveals that no adverse effect or mortality was found in albino rats up to 3000/kg.p.o. of SPAO during the 24hr. observation period ,hence 1/10 th of LD₅₀ i.e 300mg/kg body weight was taken as therapeutic dose to study pharmacological activity.

The preliminary phytochemical screening of the ethanolic extract of the plant SPAO revealed the presence of spilanthal, fixed oils, flavanoids, resins, yellow coloring matter, glucose, extractive materials were found.

Analgesic activity suggested that treatment of rats with the aqueous, ethanolic, ethyl acetate, diethyl ether, petroleum ether, butanol, extract of the plant SPAO at a dose of 100,200and400 mg/kg body weight significantly increased (P<0.001) the tail flick latency compared to control. The tail flick latency was maximum (90 min) at a dose of 400mg/kg body weight.

Treatment of mice with the aqueous, ethanolic, ethylacetate, diethyl ether, petroleum ether, butanol extract of the plant SPAO at a dose of 100, 200 and 400 mg/kg body weight produced a significant reduction (P<0.001) in writhes induced by acetic acid compared to control .However it exhibited dose dependent analgesic activity.

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DISCUSSION

The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is thought to involve local peritoneal receptors. The number of writhing movements during a 30 min observation in the control group was 80.66 ± 2.12 which corresponds with the findings of other workers^[8,9]. In the tail flick model, the test drug in different doses increased the pain threshold significantly during the period of observation and this indicates the involvement of a higher center.

The results of the present study demonstrated that significant analgesic activity in acetic acid induced writhing and tail flick models. However the analgesic activity of SPAO was found to be more significant on the acetic-induced model ($P < 0.001$) than the tail flick model ($P < 0.01$) and thus it appears that the test drug inhibits predominantly the peripheral pain mechanism.

On preliminary phytochemical screening the alcoholic extract of SPAO was found to contain flavanoid compound. Flavanoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception^[10].

Hence the presence of flavanoids may be contributory to the analgesic activities of extraction of SPAO. Further studies is required to revealed to may reveal the exact mechanism of action responsible for the analgesic activities of SPAO.

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