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Anti-inflammatory, analgesic and antipyretic activities of *Physalis minima* Linn

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Abstract

In our present investigation, the crude methanol extract and chloroform fraction of the whole plant of *Physalis minima* Linn (Solanaceae) was investigated for anti-inflammatory, analgesic and antipyretic activities in NMRI mice and Wistar rats of either sex at 200 and 400 mg/kg, respectively. Various established *in-vivo* model's were used during the study. Both crude extract and chloroform fraction showed marked anti-inflammatory and analgesic activities as compared to a control at tested doses. The antipyretic potential of the crude extract and chloroform were insignificant in the Brewer's yeast fever model. Therefore, the whole plant of *Physalis minima* Linn could be considered as a potential candidate for bioactivity-guided isolation of natural anti-inflammatory and analgesic agents.

Keywords: Physalis minima, *anti-inflammatory, analgesic, antipyretic*

Introduction

Medicinal plants have the potential to provide compounds of novel and complex structures that are capable of interacting with biological systems. The research into plants with alleged folklore use as anti-inflammatory and pain relievers should therefore be considered as a fruitful and logical strategy in the search of new anti-inflammatory and analgesic drugs. Although a large number of synthetic clinically useful anti-inflammatory and analgesic drugs are available in the market, but the search for new effective drugs with meaningful safety profile remains vital.

Physalis minima Linn (Solanaceae) is a small herbaceous annual plant grown as weed in crop fields. The plant has a bitter taste and is used as tonic, diuretic, laxative, applied in inflammations, enlargement of the spleen, ascites, and as a helpful remedy in ulceration of the bladder. In the traditional system of medicine, the plant is extensively used for the treatment of cancers because of cytotoxic activity and the leaves are crushed and applied over snakebite site [1,2]. Fruits of this plant are used to cure spleen disorders [3]. Alcohol extract of leaves and callus of plant also showed significant antimicrobial activity [4]. Similarly, oral dosing of the *Physalis minima* Linn caused infertility in female albino rats and showed significant antigonorrhoeal activity [5,6]. The abortifacient activity of Physalin-x isolated from *P. minima* in female albino rats is also reported [7].

The plants of genus Physalis have been demonstrated diverse biological and pharmacological activities and these including anti-inflammatory, quinone reductase induction, immunomodulatory, antitumor, antioxidant, anticarcinogenic, and hypoglycemic activities [8–14]. Phytochemical studies of the plant indicating

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the presence of steroidal lactones, physalins including leishmanicidal physalins and withanolides [11,15].

Materials and methods

Animals

NMRI mice (22–28 g) and Wistar rats (180–200 g) of either sex were obtained from the animal house facility of H.E.J. Research Institute of Chemistry, University of Karachi, Karachi, Pakistan. Animals were housed 10 per cage under standard environmental condition with 12 h light–dark cycle and free access to food and water. Ethical principles established in 1979 for laboratory animals in the service of mankind, Lyons, France were followed.

Plant material

Physalis minima as a whole plant was collected from Swat, N.W.F.P (Pakistan) during the month of June 2007. Authentication of the plant material was done by the Botany department of the Jehanzeb college swat and a voucher specimen was deposited there.

Extraction

The shade dried plant material was chopped into small pieces and the plant material (10 Kg) was soaked in methanol with occasional shaking, at room temperature. After 15 days, the methanol soluble materials were filtered off. The filtrate was concentrated under vacuum at low temperature (40°C) using rotary evaporator. A crude extract (207 g) was obtained. The crude methanol extract (207 g) was suspended in distilled water (500 mL) and partitioned with chloroform (3 × 500 mL) to offered chloroform fraction (49 g).

Carrageenan-induced oedema

The carrageenan induced hind paw edema test was conducted according to the method of [16]. Acute inflammation was produced by subplantar injection of 0.1 mL of 1% suspension of carrageenan with 2% gum acacia in normal saline, in the right hind paw of the rats, 1 h after oral administration of the drugs. The paw volume was measured plethysmometrically (Ugo Basile, Italy) at '0' and 3' h after the carrageenan injection. Aspirin 100 mg/kg, p.o. suspended in 2% gum acacia was used as the standard drug. The percent inhibition of the inflammation was determined by applying statistics on raw data followed by calculation of percent inhibition for each group by comparison with control group and the following formula was used: $\% I = 1 - (dt/dc) \times 100$ where "dt" is the difference in paw volume in the drug treated group and "dc" the difference in paw volume in the control group. "I" stands for inhibition [17].

Cotton pellet-induced granuloma

Wistar albino rats (170–200 g) of either sex were divided into groups of 6 animals in each group. Cotton pellets weighing 30 ± 1 mg were autoclaved and implanted subcutaneously into both sides of the groin region of each rat [18]. The crude methanol extract and chloroform fraction at concentration of 200 and 400 mg/kg was administered to animals for 7 days. Standard drug, aspirin at a dose of 5 mg/kg was administered for same the period. On the 8th day the animals were sacrificed and the pellets together with the granuloma tissues were carefully removed, dried in an oven at 60°C, weighed and compared with control. The percent inhibition of the inflammation was determined by applying statistics on raw data followed by calculation of percent inhibition for each group by comparison with control group.

Acetic acid-induced abdominal constrictions test

The prescreened animals were divided into groups. The abdominal constrictions test in mice was carried out using the standard method [19]. The constriction were induced by intraperitoneal injection of 1.0% acetic acid (v/v, 0.1 mL/10 g body weight). Two different doses (200 and 200 mg/kg) of crude methanol extract and chloroform fraction were administered to mice each, 60 min before chemical stimulus. Aspirin (100 mg/kg) as a positive control was administered 30 min prior to acetic acid injection. The number of muscular contractions was counted over a period of 10 min after acetic acid injection. The number of constriction in each treated group was compared with control (Saline treated group) and has been represented as percent inhibition of the abdominal constrictions. The percent inhibition of the abdominal constrictions was determined by applying statistics on raw data followed by calculation of percent inhibition for each group by comparison with control group.

Formalin test

The method used in our study was similar to that described previously [20]. Twenty microliter of 5% formalin was injected subcutaneously into the right hind paw of mice. The time (in seconds) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for 5 min after formalin injection (early phase) and 20–30 min after formalin injection (late phase). The tested compounds crude methanol extract and chloroform fraction (200 and 400 mg/kg, p.o.) was administered 60 min before formalin injection. Aspirin (10 mg/kg, i.p.) was administered 30 min before formalin injection. Control group received the same volume of saline by oral

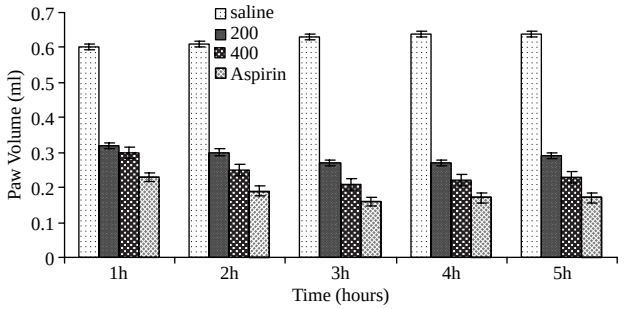

Figure 1. Anti-inflammatory effect of the crude methanol extract of the *Physalis minima* Linn in carrageenan induced hind paw edema in mice at 200 and 400 mg/kg. Difference of means of edema volume (mL) between control and treatment values at different doses ± S.E.M. *P* value was calculated using ANOVA followed by Dunnet's test for multiple comparisons. Values of $p < 0.05$ were considered significant in all cases.

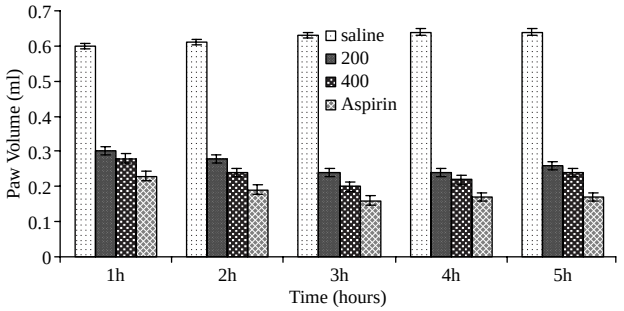

Figure 2. Anti-inflammatory effect of the chloroform fraction of the *Physalis minima* Linn in carrageenan induced hind paw edema in mice at 200 and 400 mg/kg. Difference of means of edema volume (mL) between control and treatment values at different doses ± S.E.M. *P* value was calculated using ANOVA followed by Dunnet's test for multiple comparisons. Values of $p < 0.05$ were considered significant in all cases.

administration. The percent inhibition was determined by applying statistics on raw data followed by calculation of percent inhibition for each group by comparison with control group.

Antipyretic assay

Fever was induced by injecting 15% suspension of Brewer's yeast (*Saccharomyces cerevisiae*), following a standard method [21]. A thermister probe was inserted 3–4 cm deep into the rectum, after fastened the tail, to record the basal rectal temperature. The animals were then given a subcutaneous injection of 10 mL/kg of 15% w/v Brewer's yeast suspended in 0.5% w/v methylcellulose solution and at 19 h after yeast injection, the rectal temperature of the rats were recorded. Immediately the crude extract and chloroform fraction were administered at doses of 200 and 400 mg/kg. Paracetamol was used as standard drug. Rectal temperature of all the rats was recorded at 19 h, immediately before extract or vehicle or paracetamol administration, and again at 1 h interval upto 23 h, after yeast injection.

Statistical analysis

Values are expressed in mean ± S.E.M for six animals in each group. *P* value was calculated using ANOVA followed by Dunnet's test for multiple comparisons. Values of $p < 0.05$ were considered significant in all cases.

Results

Effect of carrageenan induced hind paw edema response

The results of carrageenan induced hind paw edema test of the crude methanol extract and chloroform fraction of the *Physalis minima* are presented in Figures 1 and 2 and Table I. Both the crude extract and chloroform fraction displayed a significant ($p < 0.05$) activity at tested doses (200 and 400 mg/kg). Highest inhibition of paw edema was observed at third hour of the experiment (66%) and (68%) at 400 mg/kg for crude extract and chloroform fraction respectively.

Effect of cotton pellet induced granuloma response

Regarding the results of Cotton pellet induced granuloma as shown in Table II, crude methanol extract and chloroform fraction of the *Physalis minima* showed significant ($p < 0.05$) effect in rats. In a dose dependent manner at 400 mg/kg, the crude methanol extract and chloroform fraction reduced granuloma (48%) and (62%) respectively.

Table I. Inhibition of paw edema (%) of the crude methanol extract and chloroform fraction of the *Physalis minima* Linn in carrageenan-induced hind paw edema in mice at 200 and 400 mg/kg, i.p. Values of $p < 0.05$ were considered significant in all cases.

		% Inhibition of paw edema				
Group	Dose (mg/kg, p.o.)	1 h	2 h	3 h	4 h	5 h
Crude extract	200	46.67%	50.81%	57.14%	57.81%	54.69%
	400	50.00%	59.01%	66.67%	65.63%	64.04%
Chloroform fraction	200	50.00%	54.10%	61.90%	61.90%	59.37%
	400	53.33%	60.66%	68.25%	64.06%	62.50%
Aspirin	100	61.66%	68.85%	74.60%	73.44%	73.44%
	–					

Table II. Anti-inflammatory effect of the crude extract and chloroform fraction of the *Physalis minima* Linn in Cotton pellet induced granuloma in rats at 200 and 400 mg/kg, i.p. Values of $p < 0.05$ were considered significant in all cases.

Group	Dose (mg/kg, p.o.)	Weight of the Cotton pellet (mg)	Inhibition (%)
Saline	5 mL/kg	65.25 ± 1.5	–
Crude extract	200	38.78 ± 1.7	40.57%
	400	33.80 ± 1.2	48.20%
Chloroform fraction	200	32.22 ± 0.9	50.62%
	400	25.44 ± 1.2	61.82%
Aspirin	100	19.70 ± 0.9	69.80%

Values are expressed in mean ± S.E.M. for six animals in each group. *P* value was calculated using ANOVA followed by Dunnet's test for multiple comparisons. Values of $p < 0.05$ were considered significant in all cases.

Table III. Effect of the crude extract and chloroform fraction of the *Physalis minima* Linn in abdominal constriction induced by acetic acid in mice at 200 and 400 mg/kg, i.p.

Group	Dose (mg/kg)	No. of abdominal constriction movements (Counts/20 min)	Protection (%)
Saline	10 mL/kg	70 ± 0.75	–
Crude extract	200	44.45 ± 0.03	55.89%
	400	29.33 ± 0.01	67.57%
Chloroform fraction	200	31.22 ± 0.05	60.03%
	400	21.06 ± 0.33	73.03%
Aspirin	100	15.33 ± 0.55	80.37%
	–		

Results of the study were expressed as mean ± SEM. *P* value was calculated using ANOVA followed by Dunnet's test for multiple comparisons. Values of $p < 0.05$ were considered significant in all cases.

Effect of acetic acid-induced abdominal constrictions test response

As presented in Table III, both the crude methanol extract and chloroform fraction along with the standard, significantly ($p < 0.05$) decreased the number of constriction against control. In a dose dependent manner at 400 mg/kg, the crude extract and chloroform fraction reduced the number of constriction (52%) and (38%) respectively.

Effect of formalin test response

In case of formalin induced pain effect of the extracts of plant, both the crude extract and chloroform fraction offered significantly ($p < 0.05$) effect in the late phase. As presented in Table IV, the crude methanol extract demonstrated (51%) and chloroform fraction (31%) activity in dose dependent way in the late phase.

Effect of anti-pyretic activity response

In case of antipyretic assay, the crude extract and chloroform fraction of *Physalis minima* expressed insignificant activity as shown in Table V. Significant activity was recorded for the standard drug with respect to control.

Discussion

Carrageenan-induced paw edema as an *in vivo* model of inflammation has been frequently used to assess the antiedematous effect of natural products, exhibits a high degree of reproducibility [16]. There are two phases of carrageenan-induced inflammatory reaction and the injection of carrageenan into the rat paw induces the liberation of bradykinin, which later induces the biosynthesis of prostaglandin and other autacoids, which are responsible for the formation of the inflammatory exudates [22].

The crude extract (58%) and chloroform fraction (62%) of *Physalis minima* significantly inhibited the carrageenan induced pay edema in rats at 400 mg/kg. The significant ameliorative activity of the extracts and standard drug observed in the present study may be due to inhibition of the mediators of inflammation such as histamine, serotonin and prostaglandin. The abdominal constriction test is generally used for screening of antinociceptive effects of natural products [19,23].

In the cotton pellet-induced granuloma formation, both the crude extract and chloroform fraction showed a trend towards an effect but it did not achieve statistical significance in early phase while more effective in the late phase. Aspirin, a non-steroidal anti-inflammatory agent has been shown

Table IV. Effect of the crude extract and chloroform fraction of the *Physalis minima* Linn on the formalin test in mice at 200 and 400 mg/kg, i.p.

Group	Dose (mg/kg)	Early phase (0–5 min)	Protection (%)	Late phase (15–40 min)	Protection (%)
Saline	10 mL/kg	74.4 ± 0.9	–	87.65 ± 1.3	–
Crude extract	200	65.40 ± 0.8	12.06%	42.85 ± 1.5	51.11%
	400	64.43 ± 1.6	13.40%	35.50 ± 2.7	59.49%
Chloroform fraction	200	62.75 ± 1.2	15.65%	29.70 ± 1.3	66.11%
	400	58.95 ± 0.9	20.77%	22.40 ± 0.8	74.44%
Aspirin	10	51.34 ± 1.2	31%	12.65 ± 0.9	85.56%

Results of the study were expressed as mean ± SEM. *P* value was calculated using ANOVA followed by Dunnet's test for multiple comparisons. Values of $p < 0.05$ were considered significant in all cases.

Table V. Antipyretic effect of the crude extract and chloroform fraction of the *Physalis minima* Linn in rats at 200 and 400 mg/kg, i.p.

Group	Dose (mg/kg)	Rectal temperature (°C)					
		0 h	1 h	2 h	3 h	4 h	5 h
Saline	5 mL/kg	37.7 ± 0.9	37.6 ± 0.9	37.4 ± 1.2	37.5 ± 1.1	37.3 ± 0.9	37.6 ± 1.1
Crude extract	100	37.5 ± 1.1	37.7 ± 2.17	37.6 ± 0.9	37.8 ± 1.1	37.6 ± 1.4	37.8 ± 1.5
	200	37.7 ± 0.9	37.7 ± 1.1	37.8 ± 1.2	37.8 ± 1.2	37.4 ± 1.3	37.7 ± 1.5
Chloroform fraction	100	37.4 ± 0.9	37.7 ± 1.2	37.6 ± 1.3	37.5 ± 0.9	37.7 ± 1.2	37.8 ± 1.2
	200	37.7 ± 1.4	37.6 ± 2.1	37.6 ± 1.2	37.7 ± 2.1	37.8 ± 1.4	37.7 ± 0.9
Paracetamol	150	37.6 ± 0.9	36.8 ± 0.9	36.2 ± 0.9	36.3 ± 0.9	36.3 ± 1.1	36.4 ± 1.1

Results of the study were expressed as mean ± SEM. *P* value was calculated using ANOVA followed by Dunnet's test for multiple comparisons. Values of $p < 0.05$ were considered significant in all cases.

effect like that while the steroidal anti-inflammatory drugs have a strong inhibition on both phases [24]. This effect showed the ability of the tested compounds in reducing the number of fibroblasts, and synthesis of collagen and mucopolysaccharide, which are natural proliferative events of granulation tissue formation.

Acetic acid causes pain by liberating endogenous substances and many others that excite pain nerve endings [25]. Aspirin like other NSAIDs can inhibit number of writhes by inhibition of cycoloxygenase enzyme in peripheral tissues, thus interfering with the mechanism of transuduction in primary afferent nocciceptors by blocking the effect or the synthesis and/or release of inflammatory mediators [26]. The significant effect of the crude extract and chloroform fraction could probably be due to the blockade of the effect or the release of endogenous substances that excite pain nerve endings like NSAIDs.

Formalin-induced paw pain is a well established *in-vivo* model for analgesic study. It is suggested that the earlier phase of formalin induced pain reflects the direct effect of formalin on nociceptors, whereas the late phase reflects inflammatory pain which appears to be attributable to prostaglandin synthesis [20,27]. Our results show hat the crude methanol extract and chloroform fraction exerts significant inhibitory effect on nociceptive response of the late phase of the chemical and inflammatory pain model in formalin test. The formalin test may be a more useful model of clinical pain in which the first phase seems to be due to direct chemical stimulation of nociceptors whereas the second phase is dependent on peripheral inflammation and changes in central processing [27]. It has been suggested that substance P and bradykinin participate in the early phase, while histamine, serotonin, prostaglandins, nitric oxide and bradykinin are involved in the late phase of the formalin test [27]. This evidence suggests that peripheral inflammatory processes are involved in the second phase. The inhibitory effect of the extract on nociceptive response in the late phases of formalin test suggests that the anti-nociceptive effect of the crude extract and chloroform fraction could be due to its peripheral action.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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