Vol. 7/2 (2007) 229 - 233

JOURNAL OF NATURAL REMEDIES

Effect of leaf galls of *Piper nigrum* Linn. against carageenan induced inflammation in Albino rats.

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Abstract

Objective: To evaluate the anti-inflammatory activity of leaf galls of *Piper nigrum* Linn. against the carageenan induced paw oedema in Albino rats. Materials and methods: The benzene, chloroform and ethanol extracts of leaf galls of *Piper nigrum* Linn. were obtained by continuous soxhlet extraction. Each extract was assessed for anti-inflammatory activity in carageenan induced Albino rats by measuring the paw volume and ulcerogenic activity was also carried out. Results: All the extracts showed significant ($P<0.05$) anti-inflammatory activity in which ethanol extract showed prominent ($P<0.01$) effect. The extracts were devoid of ulcerogenic action. Conclusion: From the results, it is revealed that, the active ethanol extract of leaf galls of *Piper nigrum* is worthwhile to develop the bioactive principle for inflammatory disorders.

Keywords: *Piper nigrum*, Antiinflammatory, Ulcerogenic.

1. Introduction

Inflammatory diseases, including different types of rheumatic disease are a major cause of morbidity of the working force throughout the world. Many drugs produced a dramatic symptomatic relief in rheumatic disease, but all of them shared the common side effect, gastrointestinal irritation [1]. The body can assimilate the medicinal substance packaged in a plant, since most of the plant species are used as natural food either directly or after prepared suitably. In India many ayurvedic practitioners are using various indigenous plants for the treatment of different types of arthritic conditions [2]. *Piper nigrum* Linn. (Piperaceae) [3], a stout glabrous climber is found in South Western parts of India. Galls are pathologically developed cells, tissues and organs of plants which have risen mostly under the influence of parasitic organism. This plant has been used as a stimulant, carminative, antitubercular [4,5], oxytocic [6], and insecticide [7]. Tribal groups residing in the

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Western region of Kanyakumari district, Tamilnadu state use the leaf galls extract for rheumatic disorders (personal communication). However no scientific study on the anti-inflammatory activity of leaf galls of *Piper nigrum* has been reported. Moreover the presence of anti-inflammatory activity in triterpenes seems interesting and has remarkably less side effects [8]. The literature survey revealed the presence of terpenes [9] in various piper species. Hence the present work was undertaken to evaluate the anti-inflammatory activity of leaf galls of *Piper nigrum*.

2. Materials and methods

Fresh leaf galls of *Piper nigrum* Linn. were collected from the local areas of Kanyakumari district, Tamilnadu state in the month of November and were identified by Prof. Pamela Mohandoss, Dept. of Botany, Lady Doak College, Madurai. A voucher specimen (LPN-01) has been deposited at the Departmental Herbarium, K.M.College of Pharmacy, Madurai.

Healthy, male, adult Albino Wistar rats weighing between 150–200 g, maintained in our animal house facility under standard animal house conditions were used. CPCSEA guidelines were adhered to during the maintenance and experimentation. Experiment protocol was submitted to Institutional Animal Ethics Committee and approval was taken.

2.1 Preparation of extract

The leaf galls (500 g) were shade dried and reduced to coarse powder, subjected to continuous hot extraction with benzene, chloroform and 90% ethanol in a Soxhlet extractor for 72 h. The obtained extracts were filtered and evaporated to dryness at 40° C under reduced pressure in a rota evaporator. The yields of benzene, chloroform and ethanol extracts were found to be 10 g, 14 g and 17 g respectively. All the extracts were kept in a dessicator till experimentation. The extracts were suspended in 0.5 % w/v of sodium lauryl sulphate (SLS) for the pharmacological studies.

2.2 Preliminary Phytochemical studies

The preliminary phytochemical screening of the extracts was performed to identify the presence of sterols, triterpenoids, flavonoids and tannins [10].

2.3 Acute Toxicity studies

Acute toxicity study was carried out on all three extracts following OECD guidelines [11]. Overnight fasted, healthy Wistar Albino rats (n=3) were administered orally the respective extracts in the dose of 2000 mg/kg body weight and observed continuously for 4 h and 24 h for mortality. No visible change was observed in any test animal and all animals survived beyond 24 h.

2.4 Evaluation of anti-inflammatory activity

The extracts were evaluated for their anti-inflammatory activity by carageenan induced rat paw oedema method [12,13]. Male albino Wistar rats (150–200 g) were randomly distributed into 5 groups of 6 animals each. First group served as a control (received 0.5% SLS, 1 ml, po). Second group served as the positive control (received diclofenac sodium 10 mg/kg, po) while the third, fourth and fifth group received benzene, chloroform and ethanol extracts of leaf galls of *Piper nigrum* at a oral dose of 200 mg/kg body weight respectively. After 1 h 0.1ml of 1 % w/v suspension of carageenan was injected into the subplantar region of left hind paw to all the five groups. The paw volumes were measured using plethysmometer at 1, 2 and 3 h after carageenan injection, and mean increases in paw volumes were noted.

2.5 Ulcerogenic activity [14]

Animals of five groups of six rats in each were fasted for 16 h. Control (Sodium Lauryl sulphate 0.5% w/v solution, 1 ml), diclofenac sodium (10 mg/kg), benzene extract (200 mg/kg), chloroform extract (200 mg/kg), ethanolic extract (200 mg/kg) were orally administered. Animals were sacrificed 4 h after the administration of the drugs, the stomach were removed and cut along the lesser curvature, and the gastric mucosa were washed with normal saline and scored according to the scale. The following scale was used: 0 = no lesion, 0.5 = hyperaemia, 1 = one or two lesions, 2 = severe lesions, 3 = very severe lesions, 4 = mucosa full of lesions. The technician who performed the scoring procedure did not know the treatment to which the animals had been submitted. In the second model [15], the above said procedure was followed after administering the respective drugs orally for 7 days.

2.6 Statistical analysis

The difference in the paw volume at different time intervals and ulcer scores were analysed for statistical significance by performing one-way ANOVA followed by Dunnet's multiple comparison test. $P < 0.05$ implies significance.

Table 1: Effect of leaf galls of *Piper nigrum* Linn. in carageenan induced paw oedema in Albino rats.

Drug	Dose	Paw Volume (mean ± SEM)		
		1 hour	2 hour	3 hour
Control (0.5 % w/v SLS)	1 ml	0.504 ± 0.04	0.543 ± 0.04	0.558 ± 0.02
Standard (Diclofenac sodium)	10 mg/kg	0.264 ± 0.04	0.192 ± 0.02**	0.186 ± 0.04**
Benzene extract	200 mg/kg	0.408 ± 0.02	0.394 ± 0.04**	0.376 ± 0.01*
Chloroform extract	200 mg/kg	0.456 ± 0.02	0.434 ± 0.02*	0.40 ± 0.06*
Ethanolic extract	200 mg/kg	0.312 ± 0.02	0.240 ± 0.02**	0.206 ± 0.06**

All the results were expressed in mg/100ml
Values are mean ± SEM,(n=6). * P<0.05, **P<0.01 compared to control group (One-way ANOVA followed by Dunnet's Multiple Comparison test). SLS – Sodium Lauryl Sulphate.

Table 2: Effect of leaf galls of *Piper nigrum* Linn. in ulcerogenic activity in Albino rats.

	Control (0.5 % w/v SLS, 1 ml)	Diclofenac sodium (10 mg/kg)	Benzene extract (200 mg/kg)	Chloroform extract (200 mg/kg)	Ethanol extract (200 mg/kg)
Ulcer score (4 hrs)	0.16±0.10	2.33±0.34**	0 .84±0.24	0.66±0.10	0.58±0.82
Ulcer score (7 days)	0.25±0.12	3.84±0.16**	0.82±0.24	0.74±0.12	0.66±0.10

Ulcer scores: 0 = no lesion, 0.5 = hyperaemia, 1 = one or two lesions, 2 = severe lesions, 3 = very severe lesions, 4 = mucosa full of lesions.
Values are mean ± SEM, (n=6). **P<0.01 compared to control group (One-way ANOVA followed by Dunnet's Multiple Comparison test). SLS – Sodium Lauryl Sulphate

3. Results and Discussion

Neither mortality nor any visible changes were observed during the acute toxicity studies. Hence the extracts in the dose up to 2000 mg/kg body weight were found to be safe in laboratory animals.

The phytochemical study showed the presence of triterpenoids, flavonoids and tannins in all the three extracts All the three extracts showed significant ($P< 0.05$) decrease in paw oedema at 2h and 3h after the injection of carrageenan. However the ethanolic extract showed more prominent effect ($P< 0.01$) at 2 h and 3 h (Table 1).

The groups of animals treated with extracts did not show ulceration in the stomach after 16 h of fasting, whereas the ulcer score was found to be significantly high ($P<0.01$) in rats administered diclofenac sodium (Table 2). Treatment of the extracts for seven days did not show any ulceration whereas the ulcer score was significantly ($P<0.01$) high with diclofenac sodium treated rats (Table 2).

Carrageenan induced paw oedema was taken as a prototype of exudative phase of inflammation. Inflammatory stimuli microbes, chemicals and necrosed cells activate the different mediator systems through a common trigger mechanism. The development of carageenan-induced oedema is believed to be biphasic. The early phase is attributed to the release of vasoactive amines (Histamine and serotonin) [16,17] and the delayed phase is sustained by the acidic lipids, SRS-A (Leucotrienes) and prostaglandins (PG) [18]. Flavonoids and tannins are reported to inhibit PG synthesis [19]. As phytochemical tests showed presence of triterpenoids, tannins and flavonoids in all the three extracts used, they might suppress the formation of PG or antagonize their action and exert the activity. A strong correlation between the potency of NSAIDs as an inhibitor of prostaglandin (PG) synthesis and ulcerogenic activity has been suggested [20]. Most of the NSAIDs have well-balanced anti-inflammatory and ulcerogenic activities, which are considered to be due to PG synthetase inhibitor activity. The ethanolic extract of *Piper nigrum* possesses a marked anti-inflammatory activity and its lack of ulcerogenic activity is suggestive that it does not act mainly by PG synthetase inhibition, (but through some selective mechanism viz. Cox-2). Further, chronic administration of the extracts did not produce ulcer which proves the safety of the extracts. This is a point of distinct advantage when considering the chronic administration.

In conclusion, the leaf galls of *Piper nigrum* Linn. whose spectrum of anti-inflammatory activity appears to be different from classical NSAIDs, with the distinct advantage of its freedom from gastric ulcerogenic effects, is likely to have therapeutic potential. It is worthwhile to isolate the bioactive principle that responsible for the anti-inflammatory activity that is in process. However studies are essential to elucidate the detailed mechanisms of action for anti-inflammatory activity.

4. Acknowledgement

The authors are greatful to K.M.College of Pharmacy, Madurai for necessary facilities provided to carry the research work.

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