Indian Journal of Pharmacology 2003; 35: 384-387 **SHORT COMMUNICATION**

ANTIINFLAMMATORY AND ANTIARTHRITIC ACTIVITIES OF LUPEOL AND 19 a -H LUPEOL ISOLATED FROM *STROBILANTHUS CALLOSUS* AND *STROBILANTHUS IXIOCEPHALA* ROOTS

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ABSTRACT

Objective: To study the antiinflammatory and antiarthritic activities of lupeol and 19 a -H- lupeol isolated from the roots of *Strobilanthus callosus* and *Strobilanthus ixiocephala* respectively.

Methods: The antiinflammatory activity was evaluated using carrageenan induced rat paw oedema model for acute inflammation and cotton pellet granuloma model for chronic inflammation. Antiarthritic activity was carried out using Freund's adjuvant induced arthritis model. Prednisolone was used as a standard drug.

Results: The lupeol in the doses of 200, 400 and 800 mg/kg produced a dose dependent inhibition *i.e.* 24%, 40% and 72% where as 19 a -H- lupeol showed 21%, 47% and 62% inhibition after 24 h in acute model of inflammation. In chronic model of granuloma pouch in rats, lupeol exhibited 33% and 19 a -H- lupeol, 38% reduction in granuloma weight. In arthritis model, lupeol exhibited 29% and 19 a -H- lupeol 33% inhibition after 21 days respectively.

Conclusion: Both lupeol and 19 a -H- lupeol isolated from *Strobilanthus callosus* and *Strobilanthus ixiocephala* exhibit significant antiinflammatory and antiarthritic activities respectively.

KEY WORDS Acanthaceae arthritis inflammation

INTRODUCTION

Strobilanthus callosus nees and *Strobilanthus ixiocephala Benth* (Family: Acanthaceae) commonly known in Marathi as Karvi and Waiti respectively are small straggling herbs growing abundantly in the hills of Khandala in Maharashtra state. Both the plants flower once in seven years[1,2]. Traditionally over the ages, the tribals have used the roots of these plants for the treatment of inflammatory disorders. The roots of Karvi in the form of Lepa is reported to reduce the inflammation[3]. *Strobilanthus heyneanus,* an aromatic herb found in South India has also been extensively studied for its antiinflammatory activity. The oil prepared from this whole plant is reported to be effective in various inflammatory conditions[4]. Due to a strong chemotaxonomical relationship among the species of *Genus Strobilanthus*, the above two species *i.e. Strobilanthus callosus* and *Strobilanthus ixiocephala* were selected for the present study. The major constituent of petroleum ether extracts of above plants were isolated and identified as lupeol and 19 a -H lupeol respectively and extensively screened for antiinflammatory and antiarthritic activities.

MATERIALS AND METHODS

Plant material: *Strobilanthus callosus* roots were collected in month of August 1997 from Khandala valley, Maharashtra State and *Strobilanthus ixiocephala* roots in the month of December 1997 from Brahmagiri hills of Trimbakeshwar, Nashik, Pune. Both the plants were authenticated by the Botanical Survey of India, Pune with voucher specimen field no. 165206 and 153115 and Accession no.

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107812 and 103082 respectively and voucher specimens deposited in their office.

Lupeol and 19 a-H lupeol isolation: The dried and powdered roots of *S. callosus* and *S. ixiocephala* (1 kg each) were soxhlated with petroleum ether (60-80) yielding 0.72 and 0.82 % *i.e.* 7.2 g and 8.2 g of extract respectively. Column chromatographic fractionation of petroleum ether extract with petroleum ether and benzene (65:35) as eluent afforded lupeol and 19 a-H lupeol respectively from *S. callosus* and *S. ixiocephala* as major constituents, both to the extent of 50% in total extracts. Lupeol and 19 a-H lupeol were identified by mixed melting points, Co-TLC with authentic samples and by using IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data. The spectral data was found in accordance with the literature data of lupeol[5,6] and 19 a-H lupeol[7]. The main distinguishing observation was seen in melting point of two compounds, lupeol (mp 213-215°C) and 19 a-H lupeol (mp 204-206°C). Another characteristic difference was found on TLC where lupeol showed violet spot while a brick red spot was observed for 19 a-H lupeol at same R_f value 0.37 in solvent system, benzene : ethyl acetate (97.5:2.5) when sprayed with 1% vanillin-sulphuric acid reagent and heated at 110°C for 5 min.

Animals: Experiments were performed on albino rats of either sex (Wistar strain) weighing about 120-160 g, divided into groups of 6 each. Test drug was freshly prepared as a fine homogenized suspension in tween-80 (2% *w/v*). Prednisolone (5 mg/kg) was used as standard drug. All the animal experiments were approved by the ethics committee of the institute.

Carrageenan-induced paw oedema in rats[8]: Oedema was induced by injecting 0.1 ml of carrageenan (1% *w/v*) in distilled water into the subplantar region of the left hind paw after 1 h (oral) of drug administration. The dosage details are given in Table 1. One group served as control and was given only vehicle (2% *w/v*) Tween-80. The volume of the paw was measured with a volume differential meter (Model 7140 UGO Basile) after 3 and 24 h of carrageenan injection. Results were determined as the percent inhibition of oedema compared to the control.

Cotton pellet granuloma in rats[9]: Autoclaved cotton pellets 50±1 mg were implanted subcutaneously by incision on the back under ether anaesthesia. Drugs were administered daily orally for 7 days. Animals were killed on day 7 and the granuloma was dissected out, dried in an oven at 60°C and weighed to determine the percent inhibition of granuloma.

Adjuvant induced arthritis in rats[10]: Arthritis was induced in rats in groups of six animals by injecting 0.05 ml of a 0.5 % (*w/v*) suspension of killed *Mycobacterium tuberculosis* (Difco) in paraffin oil by intradermal injection into the left hind paw. Paw volume was measured till the 12th day by using UGO Basile Plethismometer (Model 7140). Drug treatment was started on day 13 and terminated on day 21. The difference in paw volume on day 3 and day 21 were considered as oedema volume. And the percent inhibition of oedema was determined. The details of drug dosage for the granuloma and arthritis experiments are given in Table 2.

Data analysis: Data are expressed as mean±SEM. Statistical analyses was performed by one-way ANOVA followed by Dunnett's test. P values <0.05 were considered significant.

RESULTS

Lupeol produced a dose dependant inhibition of carrageenan induced paw oedema. Oral administration of 200, 400 and 800 mg/kg of lupeol produced 24%, 40% and 72% inhibition respectively after 24 h as compared to prednisolone (5 mg/kg) which showed 54% inhibition after 24 h. However, after 3 h lupeol did not show any significant antiinflammatory activity (Table 1).

19 a-H lupeol in doses of 200, 400 and 800 mg/kg showed 21%, 47% and 62% inhibition respectively after 24 h as compared to prednisolone (5 mg/kg) which produced 54% inhibition after 24 h. However, 19 a-H lupeol at 400 and 800 mg/kg doses, also showed 27% and 53% inhibition after 3 h respectively which was found to be quite significant. Prednisolone (5 mg/kg) showed 31% inhibition after 3 h (Table 1).

The results of the cotton pellet granuloma model indicated that lupeol and 19 a-H lupeol when administered orally in dose of 600 mg/kg showed 33% and 38% inhibition of granuloma as compared to standard prednisolone (5 mg/kg) which produced 53% inhibition of granuloma (Table 2).

Table 1. Effect of lupeol and 19 a -H lupeol on carrageenan induced rat paw oedema.

Treatment (*p.o.*)	Edema volume (ml)		Percent inhibition		Treatment (*p.o.*)	Edema volume (ml)		Percent inhibition	
	3 h	24 h	3 h	24 h		3 h	24 h	3 h	24 h
Control Tween-80 (2%)	0.37±0.01	0.32±0.01	-	-	Control Tween-80 (2%)	0.41±0.01	0.40±0.03	-	-
Prednisolone (5 mg/kg)	0.25±0.01*	0.15±0.01*	33	54	Prednisolone (5 mg/kg)	0.29±0.01*	0.19±0.02*	31	54
Lupeol					19 a -H lupeoll				
(200 mg/kg)	0.34±0.01	0.24±0.01*	8	24	(200 mg/kg)	0.41±0.01	0.32±0.01*	1	21
(400 mg/kg)	0.33±0.01	0.19±0.01*	11	40	(400 mg/kg)	0.30±0.01*	0.21±0.01*	27	47
(800 mg/kg)	0.27±0.01*	0.09±0.01*	27	72	(800 mg/kg)	0.20±0.03*	0.16±0.03*	53	62
F	364.64	3163.57				296.37	161.37		
P	<0.001	<0.001				<0.001	<0.001		

n = 6 per group. Values are mean±SEM. *P<0.001 (as compared to control); df=4,25.

Table 2. Effect of lupeol and 19 a -H lupeol on cotton pellet granuloma and adjuvant induced arthritis.

Treatment (*p.o.*)	Cotton pellet granuloma		Adjuvant induced arthritis		
	Weight of granuloma (mg)	Percent inhibition	Edema volume (ml)		Percent inhibition
			After 3 days	After 21 days	After 21 days
Control Tween-80 (2%)	188.67± 1.21	–	0.31±0.02	0.23±0.03	–
Prednisolone (5 mg/kg)	88.83± 1.72*	53	0.31±0.02	0.09±0.01*	58
Lupeol (600 mg/kg)	126.50± 2.26*	33	0.31±0.03	0.16±0.01*	29
19 a -H lupeol (600 mg/kg)	116.67± 1.21*	38	0.30±0.01	0.15±0.01*	33
F	3874.29			77.00	
P	<0.001			<0.001	

n = 6 per group. Values are mean±SEM. * P < 0.001 (as compared to control); df=3,20

Oral administration of 600 mg/kg lupeol and 19 a -H lupeol inhibited Freund's adjuvant induced rat paw oedema by 29% and 33% after 21 days respectively where as prednisolone (5 mg/kg) inhibited rat paw oedema by 58% after 21 days (Table 2).

DISCUSSION

The results of carrageenan induced rat paw oedema model indicated the dose dependant antiinflammatory activity for both lupeol and 19 a -H lupeol which were

found to be statistically significant. An earlier report indicates lupeol to be a competitive inhibitor of both trypsin and chymotrypsin whereas its palmitic and linoleic esters are non-competitive inhibitors of trypsin[11]. Lupeol has also been reported to possess dose-dependent suppression of PGE_2 without any effect on leukotriene C_4 release. Above findings indicate that lupeol possessed antiinflammatory activity possibly due to its ability to prevent the production of some pro-inflammatory mediators[12]. Topical antiinflammatory effect of lupeol and its esters have been reported to be due to its effect on keratinocyte proliferation especially by its hemisuccinylation[13]. Triterpenoids as such are reported to be devoid of any ulcerogenic actions[14]. In arthritis, lupeol and its esters are reported to reduce the alterations induced in arthritic animals like significant increase in the levels of lipid peroxide, superoxide dismutase, glutathione peroxidase and catalase[15]. Lupeol is reported as an antiinflammatory agent, however present study is the first report of antiinflammatory and anti-arthritic activities of a rare triterpenic alcohol, 19 a-H lupeol, a stereoisomer of its most abundantly available triterpenic alcohol i.e. lupeol. These isolated compounds have also shown remarkable activity in the chronic cotton pellet granuloma and Freund's adjuvant arthritis model. Results of the present study indicates 19 a-H lupeol to have same action as that of lupeol and therefore its mechanism of action may be possibly similar to that of lupeol. Thus the major compounds, lupeol and 19 a-H lupeol isolated from the petroleum ether extract of *Strobilanthus callosus* and *S. ixiocephala* respectively, possess antiinflammatory activity and may be the reason for the effectiveness of the plant roots in tribal medicine.

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