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Anti-inflammatory activity and effect on gastric acid secretion of Azelain isolated from *Rhododendron dauricum* Linn.

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ABSTRACT
The effect of Azelain isolated from *Rhododendron dauricum* Linn. (Ericaceae) on gastric acid secretion in rats was investigated at a dose of 40 mg/kg, while its anti- inflammatory effect was investigated in two experimental models: arthritis induced by Freund's adjuvant carrageenan and cotton pellet induced granuloma. Azelain did not inhibit gastric acid secretion, suggesting that its anti ulcerogenic effect can be attributed to its action on the mucosa defense factors. On the other hand, Azelain inhibited both chronic and acute adjuvant carrageenan induced inflammation phases, being most effective in the chronic phase. In the granuloma test, Azelain also inhibited inflammation. It is suggested that the anti inflammatory activity of Azelain may be attributed to interference with multiple targets on the level of transcription factors, such as NF-kB and cytokines.
Keywords: Anti-inflammatory, Azelain, Gastric acid, *Rhododendron dauricum*.

INTRODUCTION

Rhododendron dauricum Linn. is an evergreen shrub, belonging to the family Ericaceae and is widely distributed in India, China, Korea and Japan. It is used as antibacterial, antifungal and anti-inflammatory in Ayurveda. Its decoction is used to cure skin diseases, inflammation and bronchitis in folk medicine (1). Earlier, we observed that the aqueous extract of leaves of *Rhododendron dauricum* Linn. exhibited inhibition of carrageenan-induced rat paw oedema and analgesic activity (2). Further, the possible role of TNF-α has been elucidated in the anti-inflammatory activity of the plant (3). In the present work, we investigate the effect of Azelain on the gastric acid secretion of rats, using the technique of ligated pylorus (4), and evaluate the anti inflammatory activity in two experimental rat models: acute and chronic paw inflammation induced by Freund's complete adjuvant carrageenan- induced (5) and cotton pellet induced granuloma (6).

MATERIALS AND METHOD

Plant Material

The leaves were collected from Dalhousie, District Chamba, Himachal Pradesh, India. The plant samples were identified and authenticated in the Herbarium, by Dr. N. N. Sharma. A voucher specimen RD-11 was deposited in the Department of Pharmacognosy, Sri Sai Institute of Pharmaceutical Education and Research, Badhani, Pathankot. Azelain was isolated from the dried leaves of *Rhododendron dauricum* Linn. and confirmed by various spectroscopic methods.

Animals

Albino rats (Wistar strain),of either sex, weighing 150-200g were used in these experiments Animals were provided with standard food and water *ad libitum* and were maintained at a constant temperature of 22 ± 1°C (12 hr light and dark cycle) with humidity of 55 ± 5%. Procedures involving animals and their care were conducted in conformity with Committee for the Purpose of Control and Supervision of Experiments on Animals (Regd. No.911/ac/95/CPCSEA).

Effect of Azelain on the gastric acid secretion in rats

Technique of Pylorus ligation

Albino rats, weighing 150-160 g were placed in individual cages with bottoms to prevent coprophagy. The animals were kept under standard conditions at 22 ± 1 °C with water ad libitum and deprived of food for 24 h before the experiments. The technique of ligated pylorus was used (4). After anaesthetizing with ether, an incision was made in the abdomen and the ligature was performed 0.5-0.7 mm below the pylorus. Care

was taken not to damage the blood supply. Immediately after, the animals were separated into four groups. After closing the incisions, Group 1 (control) and Group 2 were orally administered with 1ml of the carboxy-methylcellulose vehicle (0.4% CMC) in saline and 1 ml of Azelain 40 mg /Kg, respectively. Group 3 and 4 were each intraduodenally administered with 3 and 30 mg/Kg of ranitidine respectively (dose volume: 5 ml/Kg), plus oral administration of 1 ml of the vehicle. All animals were placed in their cages and deprived of water and food for the rest of the experiment. The animals were sacrificed and 4h later, a ligature was placed at the oesophago-cardiac junction and the stomach was removed. The gastric content was collected and centrifuged, supernatant volumes were measured and the acid concentration was estimated by titration to pH 7.0 with 0.1N sodium hydroxide, using an automatic titrator.

Assessment of anti- inflammatory activity

Activity of Azelain in the adjuvant carrageenan induced inflammation model

Adjuvant carrageenan induced inflammation in rats was used (5). Albino rats of either sexes, weighing 180 -190g with food and water ad libitum, received an injection of 0.1 ml Freund's complete adjuvant (Sigma) intradermally at the base of the tail. Six days after the adjuvant inoculation, the animals were divided into seven groups of six rats each. All groups were intraperitoneally injected and treated as follows:

Group 1(inflammation control) received an injection of the vehicle (carboxy-methylcellulose, 0.4% CMC) in saline solution and Group 2 received (injection) phenylbutazone (PBZ) at 80 mg/kg (reference drug) Group 3-7 received Azelain doses of 2.5, 5.0, 10.0, 20.0 and 40.0 mg/kg respectively. One hour after dosing, all groups were injected with 0.1 ml per rat of carrageenan type IV w/v 2% (Sigma) in saline solution in the sub-plantar region of the left hind paw. Pedal oedema was determined by measuring the paw volume at 3, 5, 24, 48, 72 and 96 h after carrageenan administration using a plethysmograph (7). The drug under study and the reference drug were daily administered until the end of the experiment. The oedema volume was expressed as the difference between the left and right hind paw volume. The inhibition percentage of each group was calculated against the control, considered as 100 % inflammation.

Effect of Azelain in cotton pellet-induced inflammation model

Albino rats of either sex, weighing 160-180 g, were used by cotton pellet granuloma method (5). Granuloma was induced by subcutaneous implant of a sterile cotton pellet (50 mg) in the dorsal area of anaesthetized rats. Twenty four hours after the implant , animals were divided into four groups and inoculated as follows : Group 1 (control) received subcutaneously (s.c) 0.4% CMC in saline solution Group 2 (reference) was injected (s.c) with dexamethasone (7mg/kg) and Group 3 and 4 received Azelain (40mg/kg) suspended with 0.4% CMC in saline solution by subcutaneous and oral routes, respectively. At the end of experiments, the animals were anaesthetized and the granulomas were removed and weighed. The anti inflammatory effect was assessed by degree of inhibition of the granuloma weight in the groups under study as compared with the control. The granuloma inhibition percentage for each group was calculated by comparison with the control group considered as 100 % of inflammatory activity.

Statistical Analysis

The experimental data obtained were expressed as the mean ± S.E.M. The significance of the values in treated groups compared with the control were determined using Student's t-test and Dunnet's test (10,11). A probability of $P<0.05$ was considered significant.

RESULTS

Effect of Azelain on Gastric acid secretion

The results obtained in the pylorus ligated assay (Table 1) showed that Azelain did not inhibit the gastric secretion in rats. The volume of gastric content was significantly increased. On the other hand 30mg/kg dose of ranitidine significantly inhibited the acid concentration and the volume of gastric content.

Evaluation of the anti- inflammatory activity

Adjuvant carrageenan- induced inflammation in the acute phase: the maximum effect of oedema inhibition was obtained after 3 and 5 h of treatment (Table 2) at a dose of Azelain of 40 mg/kg (22 and 34 % respectively), while PBZ inhibited 21 and17 % respectively . Doses of 2.5 and 5.0 mg/kg led to non-significant inhibition of 4 and 6% after 3 and 5h respectively. In the chronic phase (Table 3), all the Azelain doses significantly decreased the oedema. The maximum effect of inhibition was obtained after 72 and 96 h of Azelain treatment with a dose of 40 mg/kg (53 and 41% respectively) while PBZ values were 46 and 50 % respectively. In the model of cotton pellet induced granuloma (Table 4) Azelain significantly inhibited the granuloma development: the subcutaneous route was more effective (35.6 % inhibition) than the oral route (19.5 % inhibition).

Table 1. Effect of Azelain on the gastric acid secretion in rat

Treatment	Dose(mg/Kg)	No. of rats	Volume (ml)	pH	Titrable acid conc. (µEq/ml)	Total acid output (µEq/ml/4h)
Control	-	10	3.93±0.73	1.90±0.58	54.50±3.60	226.10±39.6
Azelain	40	7	5.0±0.80*	2.44±0.50	46.0±3.90	240.20±37.0
Ranitidine	3	6	3.47±1.16	2.18±0.40	54.0±5.35	157.6±53.38
Ranitidine	30	6	2.23±0.55**	5.79±0.5**	8.83±1.20**	19.8±4.47**

*Values are means S.E.M. Asterisks indicate significant difference against control at * P < 0.05 and ** P<0.001 (Student's t-test) respectively.*

Table 2. Effect of Azelain on adjuvant carrageenan induced inflammation in rat, acute phase[a]

Drug	Dose(mg/kg)	3h	5h
Group 1 (control)	-	0.94± 0.10	1.20 ± 0.12
Group 2 (PBZ)	80	0.74 ± 0.017*(21)	1.08 ± 0.12*(17)
Group 3 (Azelain)	2.5	0.90 ± 0.13(4)	1.12 ± 0.12(6)
Group 4 (Azelain)	5.0	0.90 ± 0.17(4)	1.10 ± 0.11(6)
Group 5 (Azelain)	10	0.85 ± 0.05* (9.5)	0.87 ± 0.10*(27)
Group 6 (Azelain)	20	0.85 ± 0.07*(9.5)	0.85 ± 0.10*(32)
Group 7 (Azelain)	40	0.73 ± 0.10*(22)	0.75 ± 0.09*(34)

*[a]values indicate mean ± S.E.M. (Dunnet's t-test) with significant variation against control at *p <0.05. Values in parentheses indicate percent reduction in paw volume compared with the group treated only with carrageenan*

Table 3. Effect of Azelain on adjuvant carrageenan induced inflammation in rat, chronic phase[a]

Drug	Dose (mg/kg)	24h	48h	72h	96h
Group 1(control)	-	1.20±0.12	1.54±0.11	1.52±0.10	0.86±0.16
Group 2(PBZ)	80	0.84±0.06*(30)	1.00±0.09*(35)	0.82±0.09*(46)	0.43±0.16*(58)
Group3(Azelain)	2.5	0.99±0.07*(17.5)	1.13±0.11*(29)	0.98±0.10*(35)	0.76±0.06*(12)
Group4(Azelain)	5.0	0.77±0.07*(36)	1.06±0.10(35)	1±0.08*(35)	0.62±0.11*(28)
Group5(Azelain)	10	0.77±0.10*(36)	0.94±0.11*(39)	0.86±0.09*(44)	0.60±0.11*(30)
Group6(Azelain)	20	0.80±0.08*(34)	0.82±0.09*(47)	0.73±0.09*(52)	0.59±0.06*(32)
Group7(Azelain)	40	0.77±0.99*(36)	0.80±0.08*(48)	0.72±0.12*(53)	0.51±0.09*(41)

**values indicate mean ± S.E.M. (Dunnet's test)with significant variation against control at * P<0.05. The values in the parentheses indicate percent reduction in paw volume compared with the control group treated only with the carrageenan .*

Table 4. Effect of Azelain on rat granuloma test[a]

Group	Granuloma weight(g)	Inhibition (%)
Saline sol.(control)	0.855±0.16	-
Dexamethasone (reference)	0.374±0.07*	55.3
Azelain (s.c.)	0.551±0.09*	35.6
Azelain (p.o.)	0.689±0.09*	19.5

*[a]value indicate mean ± S.E.M. (Dunnet's t-test) with significant variation against control at *p<0.001 and ** p <0.05.The percent inhibition for each group was calculated by comparisons with the control group considered as 100% of inflammatory activity.*

DISCUSSION AND CONCLUSIONS

In the ligated pylorus technique, it was observed that Azelain did not inhibit gastric acid secretion at 40 mg/kg so azelain might favours one of the defense factors of the rat gastric mucosa by increasing gastric glycoproteins. This suggests that the anti ulcerogenic effect of Azelain against different necrotizing agents may be due to a cytoprotective activity. Histamine (H2) receptor antagonists and proton pump (H^+, K^+) ATPase inhibitors suppress gastric acid secretion and secondarily include hypergastrinemia. Sustained hypergastrinemia has atrophic effect on the fundic mucosa, resulting in enterochromaffin like ECL cell hypertrophy and hyperplasia (8) .Therefore, it is of interest that Azelain exerts an effective anti-ulcerogenic action without modifying gastric acid secretion.

As regard the anti inflammatory activity of Azelain, the experimental model of inflammation has an acute and a chronic phase and has been proposed as a suitable and simple model system for evaluating potential anti arthritic agents. Azelain was effective in suppressing pedal oedema in the acute as well as in the chronic stage (2). In the granuloma test, Azelain led to a significant inhibition, with the highest effect being obtained by subcutaneous route and the activity may be attributed to interference with multiple targets on the level of transcriptions factors such as NF-kB and cytokines (9).

In conclusion based on the results of our study, at the dose effective as anti- ulcerogenic in rats, Azelain does not inhibit the secretion of gastric acid, but shows an anti-inflammatory effect in the acute and chronic phases, being most effective in the latter. The anti inflammatory effect shown here for Azelain, the active principle of *Rhododendron dauricum* Linn., validates the use of this plant in folk medicine and suggests its potential as a source of an anti- inflammatory agent with gastric cytoprotective properties.

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