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Anti-inflammatory properties of *Rhododendron dauricum Linn*. Inhibition of lipopolysaccharide induced septic shock and vascular permeability

Harwinder Singh Rao*, Balwinder Singh*, Narinder Singh, Ramnik Singh†**

**Department of Pharmacy, Government Institute of Pharmaceutical Sciences and Engineering, Amritsar.,Punjab, India*
***Department of Pharmacology, S.G.R.D Institute of Medical Sciences & Research, Amritsar. Punjab, India*
† Principal, SSS College of Pharmacy, Gurdaspur, Punjab, India
Correspondence : *E-mail: ramnik1144@yahoo.co.in ; Mobile: 09876064547*

ABSTRACT

The anti inflammatory activity of the aqueous extract of leaves of *Rhododendron dauricum* L. was further evaluated in models which are mediated by tumour necrosis factor – alpha (TNFα). The effect of the extract on lipopolysaccharide (LPS) induced septic shock was evaluated by measuring the number of deaths and the level of serum alanine and aspartate aminotransferase following intraperitoneal injection of LPS (1μg/kg) into D-galactosamine primed mice. LPS-induced vascular permeability on the back skin of mice was measured by local accumulation of Evan's blue after subcutaneous injection of LPS. Pretreatment with *Rhododendron dauricum* L.extract (10-80 mg/kg) produced a dose dependent inhibition of the septic shock syndrome in mice, with 80 mg/kg of the extract exhibiting comparable activity as pentoxifylline (100mg/kg). LPS- induced dye leakage in the skin of mice was also suppressed by the extract (10-80 mg/kg). This study suggests that one of the mechanisms of the anti inflammatory effects of *Rhododendron dauricum* L. possibly involves the suppression of TNFα up-regulation.

KEYWORDS: *Rhododendron dauricum,* anti-inflammatory; tumour necrosis, lipopolysaccharide, septic shock, microvascular permeability.

INTRODUCTION

Rhododendron dauricum L. 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. Earlier, we observed that the aqueous extract of leaves of *Rhododendron dauricum L.* exhibited inhibition of carrageenan induced rat paw oedema and analgesic activity (1). In this study, the anti-inflammatory profile of the aqueous extract of leaves of *Rhododendron dauricum* L.was further evaluated in both LPS-induced septic shock and LPS-induced microvascular permeability. The pro-inflammatory cytokine-tumor necrosis factor alpha (TNFα) has been shown to be one of the endogenous chemicals, which mediate the inflammatory response in these models. They were, therefore, employed in this study, to elucidate the possible roles of TNFα in the anti-inflammatory activity of the plant.

MATERIAL AND METHODS

Animals **-** Male swiss mice (20-25g) were used for this study. The animals were bred and housed in the pre-clinical animal house, Department of Pharmacy, Government Institute of Pharmaceutical Sciences & Engineering, Amritsar which was well ventilated. The animals were fed on standard diet and given water *ad libitum*.

Plant Material

Rhododendron dauricum L. leaves were collected in the month of June 2006 from Dalhousie, Distt. Kangra, Himachal Pradesh, India.The plant samples were identified and authenticated in the Herbarium; Botany Department, Guru Nanak Dev University, Amritsar, Punjab, India. A voucher specimen (B-09) of the collected plant samples was also deposited in the Department of Pharmacy, Government Institute of Pharmaceutical Sciences & Engineering, Amritsar. The leaves were air dried at room temperature, powdered and extracted in distilled water for 18 hr. The extract obtained was concentrated to a solid-greenish residue. The yield of the aqueous extract was 16.2 % based on air-dried starting material. The extract obtained was stored in a refrigerator. A stock solution of 100 mg/ml of the extract was prepared in 0.9 % normal saline for pharmacological studies.

Effect of extract on LPS induced septic shock

The experiment was carried out as described by Gantner et al. (2) with slight modifications. The mice were divided into seven treatment groups with six animals in a group (n=6). Group of mice were administered intraperitoneally with 10, 20, 40 and 80 mg/kg of *Rhododendron dauricum* L. extract. The positive control group was administered with saline at a dose of 10 ml/kg. Thirty minutes later, all the animals were injected with D-galactosamine (700 mg/kg, i.p.), followed by LPS (1 µg/kg, i.p.). The negative control group was injected only with D-galactosamine and saline. Eight hours later, blood was collected from all the mice by cardiac puncture following lethal anaesthesia with pentobarbital (150 mg/kg). In case animals died before the termination of the experiment (i.e. before the eight hours), the number of animals that died were recorded and blood was quickly withdrawn by cardiac puncture as soon as the animals lapsed into paralysis, just before death. The blood samples from each mouse were collected into tubes, and the serum level of both alanine and aspartate aminotransferases were determined using the method of Reitman and Frankel (3).

Effect of the extract on plasma leakage in mouse skin

This was done using the method described by Irie et al. (4), with slight modification. The microvascular permeability of the skin was assessed by extravasation of Evan's blue. The dye (100 mg/kg) was injected into the mouse via tail vein and 5 min later, LPS (400 µg/site) was administered, subcutaneously, at the back of the mouse. Two hours later, the mice were killed by cervical dislocation and the stained area of the skin at the site of injection was excised (about 1g) and minced. The skin specimen was dispersed in 6ml of 0.5% sodium sulphate and the dye was extracted by addition of about 14 ml of acetone. After 3.5 hr of extraction period, the dye concentration was determined by measuring absorbance at 590 nm with a spectrophotometer. The extracts (10-80 mg/kg doses) and pentoxifylline (100 mg/kg) were administered 1hr prior to Evan's blue injection.

Statistical Analysis

All data are expressed as mean ± S.E.M. Statistical significance was determined using student's t-test. Values with $p < 0.05$ were considered significant.

RESULTS

Effect of the extract on LPS-induced septic shock

Three parameters were measured in the experiment (Table 1):

a) Number of deaths per group.
b) Serum level of alanine aminotransferase.
c) Serum levels of asparatate aminotransferase.

No deaths were recorded in the group of mice administered with only saline and D-galactosamine. However, in galactosamine primed mice injected with LPS, all the animals died before the completion of the experiment. The number of deaths in the group of animals pretreated with 10, 20, 40 and 80 mg/kg of the *Rhododendron dauricum* L. extract prior to LPS-challenge were three, two, one and none , respectively. No deaths were recorded in the group of mice that received pentoxifylline (100 mg/kg). D-galactosamine injection before saline treatment resulted in serum levels of 270.7±3.2 U/ml and 304.5±6.6 U/ml of alanine and aspartate aminotransferases, respectively. In D-galactosamine primed mice injected with LPS, the levels elevated to 297.8±3.5 U/ml and 335.8±7.8 U/ml for alanine and aspartate aminotransferases, respectively. Pretreatment with *Rhododendron dauricum* L.extract, however, caused a statistically significant ($p<0.05$) reduction in the levels of both enzymes. Pentoxiyfylline pretreatment also caused a significant ($p<0.05$) reduction in the serum enzyme levels.

Effect of the extract on plasma leakage in the mouse skin

The effects of the extracts on LPS-induced dye leakage were evaluated 2 hr after subcutaneous injection of LPS. Pretreatment with *Rhododendron dauricum* L.extract produced a dose related inhibition of dye leakage (Table 2). Dye leakage in mice given only saline before LPS injection was 57.3±4.9 µg/g wet weight. This was significantly ($p < 0.05$) reduced to 38.0±2.8µg/g wet weight by the extract given at 10 mg/kg. The highest dose of the extract used in this study (80 mg/kg) inhibited dye-leakage to a similar degree as pentoxifylline (100 mg/kg).

DISCUSSION

The extract of *Rhododendron dauricum* L. protected mice from septic shock in a dose dependent manner. The serum levels of liver enzymes were also reduced in mice treated with the extract. There were no deaths recorded in mice given only saline and D-galactosamine. This shows that the death of the animals primed with D-galactosamine and injected with LPS was due to the action of the hepatotoxic agent. LPS/ D-galactosamine – induced sepsis has been reported to be mediated by TNFα (5). In addition,

Table 1. Effect of Rhododendron dauricum leaf extract (RDE) on lipopolysaccharide LPS induced septic shock in mice.

Treatment	No. of deaths	Serum enzyme activity (µg/ml) [a]	
		ALT	AST
Saline + D-galactosamine (DG)	0/6	270.7 ± 3.2	304.5 ± 6.6
Saline + DG + LPS	6/6	297.8 ± 3.5	335.8 ± 7.8
RDE(10mg/kg) + DG + LPS	3/6	284.0 ± 5.5[b]	315.5 ± 6.5[b]
RDE(20mg/kg) + DG + LPS	2/6	278.2 ± 4.2[b]	308.7 ± 7.4[b]
RDE(40mg/kg) + DG + LPS	1/6	274.3 ± 1.9[b]	304.0 ± 5.6[b]
RDE(80mg/kg) + DG + LPS	0/6	266.3 ± 2.4[b]	290.5 ± 3.3[b]
pentoxifylline(100 mg/kg) + DG + LPS	0/6	271.0 ± 4.2[b]	309.5 ± 6.7[b]

[a] *each value is the mean ± S.E.M of 6 animals.*
[b] *p<0.05 compared with DG + saline + LPS treatment ; student's t- test*

Table 2. Effect of Rhododendron dauricum leaf aqueous extract (RDE) on lipopolysaccharide (LPS) induced micro vascular permeability in mice.

Treatment	Amount of dye leaked (µg/g of wet weight)[a]
Saline + LPS	57.3 ± 4.9
RDE(10 mg/kg) + LPS	38.0 ± 2.8[b]
RDE(20 mg/kg) + LPS	28.3 ± 1.9[b]
RDE(40 mg/kg) + LPS	19.3 ± 1.1[b]
RDE(80 mg/kg) + LPS	12.7 ± 1.2[b]
Pentoxifylline (100 mg/kg) + LPS	12.8 ± 1.4[b]

[a] *each value is the mean ± SEM of 6 animals*
[b] *p<0.05 compared with saline + LPS treatment ; student's t- test*

TNFα has been demonstrated to mediate LPS-induced systemic inflammatory response syndrome i.e. liver failure, characterized by early apoptosis and subsequent liver cell death (6,7). TNFα activity in the model has been shown by Gantner et al. (2) to be correlated with both elevated levels of transaminase enzymes and septic death. Pentoxifylline, the reference agent in this study, is a class IV phosphodiesterase inhibitor which has been shown to be TNFα antagonist in animal experiments (8,9). Other class IV phosphodiesterase inhibitors like rolipram and CP-77059 were shown by Sekut et al. (10) to exert anti inflammatory activities in different murine models. The effects of *Rhododendron dauricum L.*extract was also tested on LPS induced dye leakage in the mouse skin. The effects of the extract and pentoxifylline were evaluated 2 hr after injection of LPS (11). The extract was found to produce inhibition of dye leakage to the same extent as pentoxifylline. Subcutaneous injection of LPS on the back of mice and rats induces a plasma leakage at the site of injection and thus is used as a model of inflammation (12). The LPS induced increase in vascular permeability has been reported to be mediated in the mouse (by TNFα) (13). This study has, therefore provided an evidence for a possible role of TNFα in the anti-inflammatory effects of the aqueous leaf extract of *Rhododendron dauricum* L. It is yet to be determined if other cytokines like interleukin-I, play any role in this activity of the plant.

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