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Anti-inflammatory activity of *Rhodiola rosea* – "a second-generation adaptogen"

Pooja, A. S. Bawa and Farhath Khanum*
Defence Food Research Laboratory, Sidddarthanagar, Mysore-570011, Karnataka, India

***Rhodiola rosea* (golden root), a unique phytoadaptogen grown in high-altitude regions has gained attention for its various therapeutic properties. In India, this plant is found in the Himalayan belt and has not been completely explored for its beneficial health effects. The present study was undertaken to evaluate the anti-inflammatory efficacy of the tincture extract of *Rhodiola rosea* roots (RTE). The anti-inflammatory activity was determined through carrageenan-induced paw oedema, formaldehyde-induced arthritis and nystatin-induced paw oedema in rat model. The tincture extract exhibited inhibitory effect against acute and subacute inflammation at a dose of 250 mg/kg body weight. Inhibition of nystatin-induced oedema was also observed in a dose-dependent manner. The *in vitro* inhibitory effects of the tincture extract from *R. rosea* roots was evaluated against the enzymes relating to inflammation. The enzymes include cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2) and Phospholipase A_2 (PLA_2). The extract showed varying inhibitory activities against these enzymes depending on the concentrations. A potent inhibition was observed against Cox-2 and PLA_2. Inhibition of nystatin induced oedema and phospholipase A_2 suggested that membrane stabilization could be the most probable mechanism of action of RTE in anti-inflammation. The findings in this study may provide the use of *R. rosea* root extract in the treatment of inflammatory conditions.**

Keywords: Rhodiola rosea; anti-inflammatory activity; cyclooxygenases-1 and 2; phospholipase A_2: membrane stabilization.

INTRODUCTION

Phytoadaptogens constitute a new class of metabolic regulators that enables an organism to resist adverse stressors of any kind (physical, chemical, biological, etc.). Panax ginseng, American ginseng and Siberian ginseng are well-known 'first-generation' adaptogens. *Rhodiola rosea*, belonging to the family Crassulaceae, can now be classified as a 'second-generation' adaptogen for its unique adaptogenic properties. This plant indigenous to the high-altitude Arctic regions has been used in Russian and Chinese folk medicine (Rege *et al.*, 1999). Many biological activities have been reported on this versatile phytoadaptogen: anti-oxidant (Anilakumar *et al.*, 2006); anti-arrhythmic (Maslov *et al.*, 1998); antistress (Darbiyan *et al.*, 2000; Spasov *et al.*, 2000); anticancer and antibacterial properties (Udintsev and Shakov, 1991; Ming *et al.,* 2005); besides its cardio protective effect (Maslov *et al.*, 1997). Our recent work also established a significant antioxidant activity associated with this plant (Anilakumar *et al.*, 2006). The pharmacological preparations of *Rhodiola rosea* are mainly used as brain tonic and to alleviate fatigue. Most probably these biological attributes are mediated through the active components of the plant-like flavonoids, phenyl propanoids, organic acids and phenyl ethanol derivatives.

In many of the pathological disorders, Inflammation is one of the important processes. Inflammatory cells produce a complex mixture of growth and differentiation cytokines as well as physiologically active arachidonate metabolites. In addition, they possess the ability to generate reactive oxygen species (ROS) that can damage cellular biomolecules which in turn augment the state of inflammation (Cochrane, 1991). Compounds that possess radical scavenging ability may therefore, be expected to have therapeutic potentials for inflammatory diseases (Trenam *et al.*, 1992). In spite of these therapeutic effects of *Rhodiola rosea* extract, little is known about the anti-inflammatory activity. Thus, the aim of the present study was to investigate the anti-inflammatory activity of *Rhodiola rosea* extract and determine its possible mechanism of action.

MATERIALS AND METHODS

Materials. *Rhodiola rosea* roots were obtained from Field Research Laboratory, Leh (India) and the voucher specimen of the same is available in FRL. Cyclooxygenase-1 (sheep) and Cyclooxygenase-2 (human), Phospholipase A_2 (Naja mossambica mossambica), Hematin, N, N, N, N-tetramethyl-p-phenylenediamine, Arachidonic acid, Phosphotidylcholine were purchased from Sigma Aldrich Co., (St Louis, MO, USA) 4-dimethyl aminocinnamaldehyde from Lancaster (England), carrageenan and nystatin from SRL Chemical Co. (Mumbai, India) and while the other chemicals used were of analytical grade.

Animals. Male (Wistar) rats (150–200 g) were used for the study. Animals were housed under standard conditions of temperature (26 ± 2 °C), relative humidity (55 ± 10%), and 12/12 h light and dark cycle and fed on

* Correspondence to: Dr. Farhath Khanum, Biochemistry and Nutrition Discipline, Defence Food Research Laboratory, Biochemistry and Nutrition, Sidddarthanagar, Mysore-570011, Karnataka, India. E-mail: poojs7@sify.com

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the standard pellet diet (Petcare, Bangalore, India) with both food and water *ad libitum*.

Plant extract. The plant material was dried at 60 °C; 5 g of ground dry raw material was dipped in 50 ml of 40% ethanol. Extraction was carried out in flasks on a rotary shaker at room temperature, in darkness for 12 h. The extract was filtered, and the filtrate was evaporated at 35 °C, at reduced pressure (20 mbar). 0.65 g of the dry *Rhodiola rosea* tincture extract/residue (RTE) (containing 4.27 ± 0.5 mg/g root of phenolic compounds) was dissolved in 5 ml of distilled water and used for biological studies following appropriate dilutions.

In vivo screening of anti-inflammatory activity

Formaldehyde-induced arthritis assay. 0.02 ml of formaldehyde solution (2% v/v) was injected on the first and third day to the left hind paw of the rats just beneath the plantar aponeurosis to induce arthritis. RTE (50 mg/kg) was administered intraperitoneally once a day, for 7 days. Dexamethazone (50 µg/kg body weight) was used as the reference standard. Serum aspartate aminotransferase (AST) and alanine amino transferase (ALT) activities were measured in blood on seventh day. (Reitman and Frankel, 1957).

Carrageenan-induced paw oedema. Acute inflammation was induced by injecting 0.1 ml of 1% (w/v) carrageenan into the plantar surface of the right hind paw of the rat (Winter *et al.*, 1962). RTE (250 mg/kg body weight) was administered intraperitoneally 30 min prior to carrageenan injection. Control animals received equal volume of saline while dexamethazone (1 mg/kg body weight) was used as a standard drug. The paw volume was measured at 0, 1 h and 3 h after the carrageenan injection using a micrometer (Mitutoyo, Japan). Anti-inflammatory activity was calculated according to the following equation.

$$\text{Anti-inflammatory activity (\%)} = [(m - m')/m] \times 100,$$

where m and m′ are the differences in thickness between the first and second measurements of the hind paws in control and test groups respectively.

Dose-dependent response on Nystatin induced oedema. Rats were injected with 0.1 ml nystatin into the subcutaneous tissue of the right hind paw as an 8.5% suspension in sterile saline. 50 mg, 125 mg and 250 mg/kg body weight of RTE was administered intraperitoneally, 30 min before inducing oedema. The paw volume was measured at 0 h (before injection of nystatin) as well as after 1, 3, 6, 18, 24, 72, 96, 120, 144 and 168 h using a micrometer (Mitutoyo, Japan). The effect of treatment was expressed as % oedema of inflammation. Dexamethazone (1 mg/kg body weight) was used as a positive control (Arrigoni-Martelli *et al.*, 1971).

In vitro screening of anti-inflammatory activity.

Effect on phospholipase activity. Phospholipase activity was determined using egg phosphatidyl choline (PC) as substrate. The enzyme was incubated in a reaction mixture (1 ml) containing 1 µ mole PC in 0.05 M Tris-HCl buffer pH 7.5, 0.2 ml diethyl ether, 40 µ moles of Ca^{2+} and 0.125 mg, 0.25 mg, and 0.5 mg of the extract were separately incubated at 37 °C for 60 min. The free fatty acids released were extracted as cobalt soap and the cobalt was complexed with α-nitroso-β-naphthol and estimated colorimetrically at 530 nm (Bhat and Gowda, 1989).

Effect on cyclooxygenase-1 and cyclooxygenase-2 activity. *In vitro* enzymatic activity of COX 1 & 2 was measured using chromogenic assay based on the oxidation of N, N, N, N-tetramethyl p-phenylene diamine (TMPD) during the reduction of prostaglandin G_2 to PGH_2 (Copeland *et al.*, 1994). The assay mixture in 1 ml contained 3 µl of 40 nM of TMPD (final concentration 120 µM), 5 µl of 20 mM arachidonic acid (final concentration 100 µM), 5-10 µM of enzyme (50-100 µg of protein) and 987 µl of buffer (100 mM Tris pH 8.0; 10 µM Hematin and 3 µM EDTA). The enzymatic activity was measured by estimation of the initial velocity of TMPD oxidation in the reaction as increase in absorbance at 603 nm. The COX activity was expressed in units/ml enzyme or mg protein. One unit of enzyme is defined as the amount of enzyme required to oxidize one nanomole of TMPD per min at 25 °C. Aspirin was used as the positive control.

Statistics. All the values were expressed as mean ± SD. Statistically significant ($p < 0.05$) differences between the experimental groups were assessed by ANOVA.

RESULTS AND DISCUSSION

Pharmacological and clinical research on *R. rosea* root extract has provided strong evidence that this unique adaptogen possesses beneficial biological activity with no detectable levels of toxicity (Yoshikawa *et al.*, 1996; German and Ramazanov, 1999; Darbiyan *et al.*, 2000). The phenyl propanoids are thought to be critical for the adaptogenic properties. In this study, 40% ethanol (tincture) was used for extraction since it could extract most of the active constituents (Petkov *et al.*, 1986; Linh *et al.*, 2000).

The formaldehyde-induced arthritis in experimental rats was used as a model for subacute condition of inflammation. A measure of AST and ALT enzyme levels provide a simple tool to assess the anti-inflammatory activity of the test samples (Reitman and Frankel, 1957). An expected elevation of serum transaminases as an inflammatory response to formaldehyde induced arthritis was significantly inhibited by RTE administration (Fig. 1). Further, experiments were conducted to assess the anti-inflammatory potential of RTE in acute model of inflammation for which carrageenan-induced oedema was studied. Development of oedema induced by carragenan is commonly correlated with the early exudative stage of inflammation process (Ozaki, 1990). Carrageenan-induced oedema is a multimediated phenomenon with liberation of a diversity of mediators. In the early phase, there is bradykinin, histamine and serotonin liberation by local cells. After a couple of hours there is liberation of prostaglandins. RTE (250 mg/kg) significantly inhibited the oedema formation induced by carrageenan by 62% in comparison to

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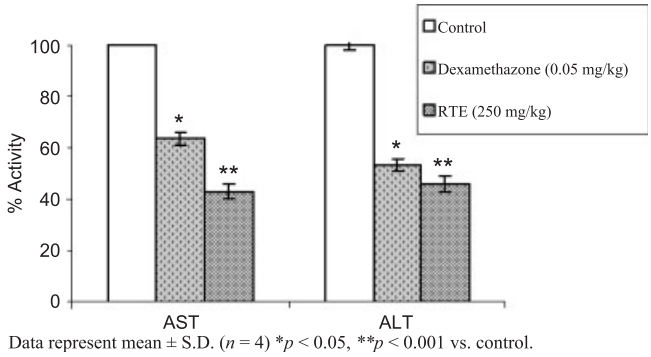
Table 1. Effect of RTE tincture extract on carrageenan-induced oedema

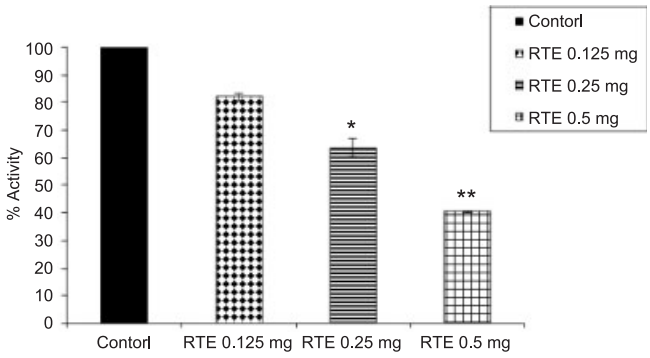
Treatment	Percent inhibition (After 1 h)	Percent inhibition (After 3 h)
Dexamethazone (1 mg/kg body weight)	34.2 ± 0.2	65 ± 12
RTE (250 mg/kg bodyweight)	28.4 ± 2	62 ± 6.75

Values represent mean ± S.D. (n = 6).



Data represent mean ± S.D. (n = 4) *p < 0.05, **p < 0.001 vs. control.

Figure 1. Effect of RTE on formaldehyde induced arthritis.



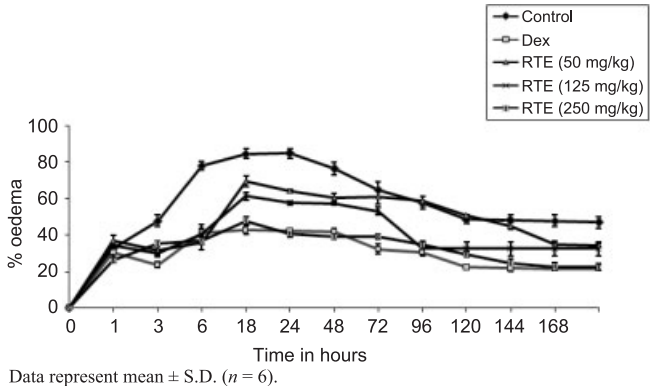
Data represent mean ± S.D. (n = 4) *p < 0.05, **p < 0.001 vs. control.

Figure 3. Effect of RTE on phospholipase A_2 activity.

that of dexamethazone (1 mg/Kg), a known anti-inflammatory drug that reduced the oedema volume by 65% (Table 1). The inhibition of prostaglandin formation through cyclooxygenase pathway is an established mechanism in carrageenan-induced oedema. To reconfirm the involvement of cyclooxygenase inhibition, both *in vitro* and *in vivo* experiments were conducted. Nystatin-induced oedema was chosen as a model for *in vivo* experiment. Nystatin, a polyene antibiotic, was used to induce inflammation that results in oedema by its membrane labilizing action, thereby releasing hydrolytic enzymes which play an important role in promoting the inflammation. It has been demonstrated that prostaglandins are also involved in this oedema. RTE inhibited the nystatin-induced oedema in a dose-dependent manner. A significant inhibition was observed at a concentration of 250 mg/kg body weight as shown in Fig. 2 while a mild inhibition was observed at a concentration of 50 mg/kg body weight. Inhibition of nystatin-induced oedema suggests a more likely action of RTE on membrane stabilization on lysosomal membranes (Shinde *et al.*, 1999).

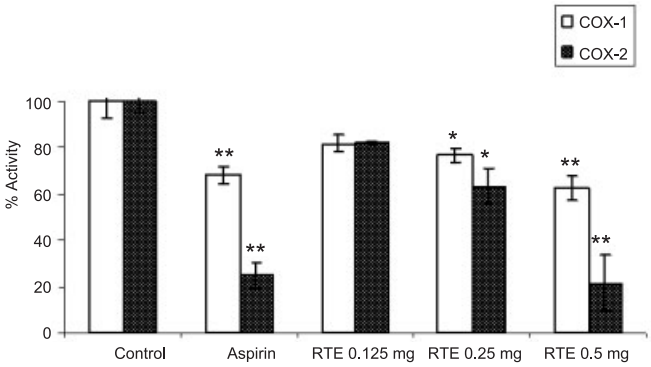
Phospholipase A_2 (PLA_2) catalyses the liberation of arachidonic acid from cell membranes and the subsequent metabolites of arachidonic acid are catalyzed by cyclooxygenases that generate inflammatory mediators like prostaglandins. Inhibition of the biosynthesis of inflammatory mediators by inhibiting the activities of PL A_2 and COX enzymes in *in vitro* experiments would be an additional support to confirm the mechanism of action of RTE. Results in Fig. 3 show that RTE caused significant (p < 0.001) inhibition of PLA_2 in a dose-dependent manner. This may be correlated to the membrane stabilization action of RTE thereby preventing the release of arachidonate from the membrane.

Cyclooxygenase or prostaglandin endoperoxide synthetase (COX) is the enzyme that catalyses the conversion of arachidonate to prostaglandins. COX enzyme performs its biological role in two isoforms namely COX-1 and COX-2. In *in vitro* experiment, RTE could provide a significant inhibition of COX-2 enzyme while a moderate inhibition of COX-1 enzyme in a dose-dependent manner (Fig. 4). COX-1, which is constitutively expressed in most tissues, regulates many physiological functions



Data represent mean ± S.D. (n = 6).

Figure 2. Dose dependent response of RTE on nystatin induced oedema.



Data represent mean ± S.D. (n = 4) *p < 0.05, **p < 0.001 vs. control.

Figure 4. Effect of RTE on Cyclooxygenase-1 and Cyclooxygenase-2 activity.

and hence is generally regarded as the housekeeping enzyme. On the contrary, COX-2 is induced at the site of inflammation and contributes to the inflammation process (Pairet and Enelhardt, 1996). It has been suggested that the selective inhibition of COX-2 isoform could be a relevant target for an anti-inflammatory drug because COX-1 maintains normal gastric mucosa and influences kidney functions (Vane and Botting, 1998). With the significant inhibition of COX-2, a desirable anti-inflammatory effect was clearly evident with RTE, though there was also moderate inhibition of COX-1. Most of the anti-inflammatory drugs result in drastic inhibition of COX-1 thereby resulting in undesirable side effects (Sautebin, 2000). RTE, in comparison to this, might over score because of relatively less-marked inhibition of COX-1.

Our previous study (Anilakumar *et al.*, 2006) demonstrated that *R. rosea* roots contain a significant amount of polyphenols including flavonoids and tannins. The involvement of flavonoid, tannins and phenylpropanoids in anti-inflammatory activity is well documented (Garcia *et al.*, 1996; Xu, 1996; Siani *et al.*, 1999). Hence these phytochemicals present in RTE might be involved in anti-inflammatory activity. It would be interesting to compare the anti-inflammatory potential of RTE with other plants possessing similar biological activity. Application of phytoadaptogens for the use as anti-inflammatory agents through oral route/topical formulations would be much more desirable. We have developed certain functional foods with RTE and with further possible utility as an anti-inflammatory formulation having an added benefit of being a source of natural antioxidants.

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