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Antiasthmatic activity of *Ricinus communis* L. roots

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ABSTRACT

Objective: To evaluate the antiasthmatic activity of *Ricinus communis* (*R. communis*) Linn (Euphorbiaceae) to validate its traditional use. **Methods:** The antiasthmatic activity of ethanol extract of *R. communis* (ERCR) root was evaluated on milk induced leucocytosis and eosinophilia in mice, mast cell degranulations in mice and passive cutaneous anaphylaxis in rats at (100-150 mg/kg). **Results:** The ERCR significantly decreases milk induced leucocytosis and eosinophilia and protect degranulations of mast cells in mice. At the same dose ERCR inhibited passive cutaneous anaphylaxis in rats. Phytochemical study revealed the presence of steroids, saponin, alkaloids, flavonoids, and glycosides. **Conclusions:** The flavonoids and saponins are reported to possess mast cell stabilizing and antianaphylactic activity. Hence ERCR shows antiasthmatic activity may be due to presence of flavonoids and/or saponins.

1. Introduction

Asthma is a chronic inflammatory disorder of airway. Asthma affect about 300 million people worldwide and it has been estimated that a further 100 million will be affected by 2025[1]. Asthma is associated with change in the levels of eosinophils, mast cells, lymphocytes, cytokines and other inflammatory cell products. *Ricinus communis* (*R. communis*) Linn (Euphorbiaceae) is a small tree widespread throughout the world. The roots of *R. communis* have been traditionally reported to be used in the treatment of inflammation, fever, asthma, bronchitis's and leprosy[2]. The root possesses antidiabetic activity[3]. Leaves and stem shows antibacterial and anti-inflammatory activity[4]. Monoterpenoids (1, 8-cineole, camphor and α-pinene) and a sesquiterpenoid (β-caryophyllene) isolated from the leaves possesses antitumor activity[5], it is also reported that gallic acid, quercetin, gentisic acid, rutin, epicatechin and ellagic acid from the leaves shows antioxidant activity[6]. Bioassay guided fractions ergost-5-en-3-ol, stigmasterol, γ-sitosterol, fucosterol; and one probucol isolated from ether extract of seeds shows significant antifertility[7]. Seed of the plant is also reported to possesses antibacterial and antitumor activities[8,9]. Hence to validate the traditional use of this plant, the present study was undertaken to evaluate antiasthmatic activity of the ethanol extract of *R. communis* root (ERCR) on milk induced leucocytosis and eosinophilia in mice, egg albumin induced mast cell degranulations in mice and passive cutaneous anaphylaxis in rats.

2. Materials and methods

2.1. Plant material

Roots of *R. communis* were collected in December 2008, from Baramati localities, (Maharastra, India), and authenticated by Prof. RB Deshmukh, Department of Botany, Shardabai Pawar Mahila Mahavidyalaya, Baramati, where voucher specimen (PASR-115) was deposited in herbarium.

2.2. Preparation of extract

Dried and coarsely powdered root of *R. communis* (500 g) was extracted successively with 95% ethanol using Soxhlet extractor. The extract was concentrated to dryness in rotary

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evaporator under reduced pressure to yield ethanol extract of *R. communis* roots 9.46 % w/w.

2.3. Animals

Albino mice (25-30 g) and Wistar rats (150-170 g) of either sex were housed under standard laboratory conditions. The animals had free access to food and water. The animal ethical committee of the institute approved all the protocols of the study (Registration No.1214/ac/08/CPCSEA).

2.4. Drugs and chemicals

Egg albumin, aluminium hydroxide and evan blue were purchased from (Himedia, India), disodium chromoglycate and Dexamethasone.

2.5. Preliminary phytochemical screening

To determine the chemical constituents, qualitative phytochemical screening of ethanol extract of *R. communis* root was carried out for alkaloids, flavonoids, saponins, steroids and glycoside following standard procedure[10,11].

2.6. Acute toxicity studies

Albino mice of either sex were divided into seven groups each containing six animals. Mice were fasted for 18 h with water *ad libitum*. Control group received 1% Tween-80 solution (5 mL/kg) intraperitoneally. Test groups received ERCR at six different doses of 800, 900, 1 000, 1 100, 1 200 and 1 300 mg/kg. All the animals were observed for 72 h, and the LD_{50} was calculated[12].

2.7. Milk induced leucocytosis and eosinophilia

Mice were divided into five groups (n=6) in each group. Blood was collected from retro-orbital plexus. Control group intraperitoneally treated with 1% Tween-80 solution (5 mL/kg); test groups received ERCR at doses of 100-150 mg/kg. Standard group received dexamethasone (50 mg/kg). Boiled and cooled milk, (4 mL/kg, s.c.) was injected to all the groups 30 minute (s) after treatments. Total leukocyte and eosinophile count was done in each group before treatment and 24 h after milk injection. Difference in total leucocytes and eosinophile count before and after 24 h the treatment was calculated[13].

2.8. Passive cutaneous anaphylaxis test

Albino rats were sensitized by subcutaneous injection of 100 mg egg albumin and 12 mg aluminium hydroxide, as adjuvant, on day 1, 3 and 5. On day 10, animals were bled and antiserum was collected. The separated antiserum was stored at -20 ℃. The rats were divided into five groups (n=5). The rat homologous antiserum (100 μL) was injected into the shaved back skin of rats. After 24 h, control group received 1% Tween-80 (5 mL/kg) intraperitoneally, test groups received ERCR at doses (100-150 mg/kg *i.p.*) and standard group received sodium chromoglycate (50 mg/kg *i.p.*). The entire group injected 0.5 mL of mixed solution of 0.5% evan blue, and 1% egg albumin (1:1) through tail vein after 30 min of treatment. Area of leakage of the blue dye was expressed as the longest and shortest diameter of blue spots in mm^2[14].

2.9. Mast cell degranulations

Mice were divided into five groups (n=6) animals in each group. A three day drug treatment schedule was followed. Control group treated with 1% Tween-80 (5 mL/kg), intraperitoneally; test groups were treated with ERCR at doses of 100-150 mg/kg, and standard group received sodium chromoglycate at dose of 50 mg/kg. On the fourth day, entire mice were injected with, 10 mL/kg, 0.9% saline solution, into peritoneal cavity, by gentle massage, the peritoneal fluid was collected after five minute and transferred into test tube containing 7-10 mL RPMI-1640 (Roswell Park Memorrial Institute) buffer medium (pH 7.2-7.4) composed with L- Glutamine and 25 mM Hepes buffer, without sodium bicarbonate. This solution was then centrifuged at 400-500 rpm. Pellets of mast cell were washed with same buffer medium twice by centrifugation, discarding supernatant. The cell suspension from treated and control group of rats were challenged with egg albumin (100 μg/mL) and incubated at 37 ℃ for 10 min. The cell suspension was stained with 1% toludine blue and observed under microscope. Degranulated mast cells observed are like burst instead of intact. Total 100 cells were counted from different visual areas and percent protection against degranulations was calculated[14,15].

2.10. Statistical analysis

The results were reported as mean ± SEM and analyzed for statistical significance using One way ANOVA followed by Student Newman-Keuls test. P<0.05 was considered significant.

3. Results

3.1. Preliminary phytochemical screening

Preliminary phytochemical study of ERCR revealed the presence of steroids, saponin, alkaloids, flavonoids, and glycosides.

3.2. Acute toxicity studies

The LD_{50} value of ERCR when given intraperitoneally and tested in albino mice was found to be more than 1 300 mg/

kg body weight. At higher doses some behavioral signs and writhing were observed immediately after administration of extract but no any death of mice occurred.

3.3. Milk induced leucocytosis and eosinophilia

The maximum increase in difference of leucocytes (4483.34 ± 297.96) and eosinophile (166.67 ± 24.21) count was observed in control group 24 h after administration of milk (4 mL/kg, *s.c.*). ERCR at doses of 100–150 mg/kg significantly inhibit milk induced leucocytosis and eosinophilia in dose dependent manner. The ERCR (150 mg/kg) shows significant inhibition comparable to dexamethasone (50 mg/kg) as shown (Figures 1 and 2).

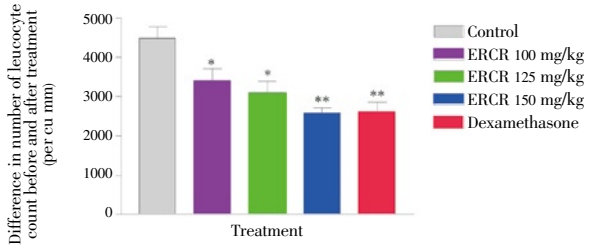

Figure 1. Effect of ERCR on milk induced leucocytosis in mice. values are Mean ± SEM. (*n*=6); **P*<0.01, ***P*< 0.001 when compared with control.

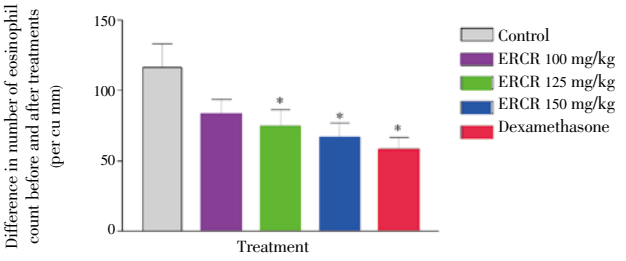

Figure 2. Effect of ERCR on milk induced eosinophilia in mice. Values are Mean ± SEM. (*n*=6); **P*< 0.05 when compared with control.

3.4. Passive cutaneous anaphylaxis test

ERCR at doses of 100–150 mg/kg *i.p.* significantly (*P*<0.001) shows the reduction in the area of dye leakage in dose dependently when compared with control group as shown in Figure 3.

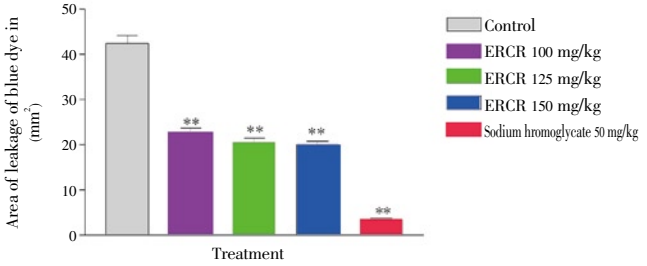

Figure 3. Effect of ERCR on passive cutaneous anaphylaxis in rats Values are Mean ± SEM. (*n*=5); ***P*< 0.001 when compare with control.

3.5. Mast cell degranulations

The control group showed (74.0±1.932) degranulation of mast cells while groups pre-treated with ERCR (100–150 mg/kg, *i.p.*) and disodium chromoglycate significantly (*P*<0.001) protect degranulation of mast cells. ERCR at dose (150 mg/kg) showed (25.83±2.06) and disodium chromoglycate (22.5± 1.118) protection against degranulation as shown in Figure 4.

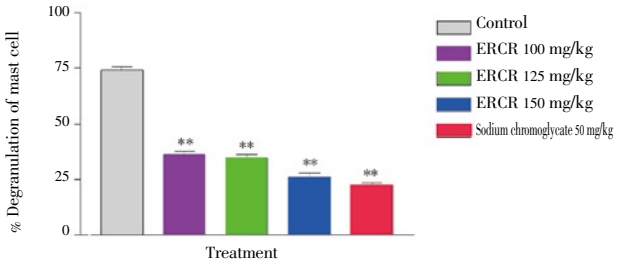

Figure 4. Effect of ERCR on egg albumin induced degranulations of mast cell in rats.
Values are Mean ± SEM. (n=6); ***P*< 0.001 when compare with control.

4. Discussion

In present investigation ERCR at doses of 100–150 mg/kg was evaluated for antiasthmatic activity using different animal models as asthma involve various types of mediator in pathology. It was reported that subcutaneous administration of milk produces a marked increase in the leukocytes and eosinophils count after 24 h[16]. Leucocytes during asthmatic inflammation release the inflammatory mediators like cytokines, histamine, and major basic protein, which promote the ongoing inflammation. An abnormal increase in peripheral eosinophil to more than 4% of total leucocytes count is termed as eosinophilia. In asthmatic patient there is increase in eosinophile count[17–20]. The control group (1% Tween-80) treated mice shows increase in difference of leucocytes and eosinophile while mice treated with different doses of ERCR decreases the difference in leucocytes and eosinophile count. Decrease in leucocytes and eosinophile is mediated by adaptogenic and type I hypersensitivity hence ERCR may be useful in allergic condition.

The degranulation of mast cell occurs in response to the immunological stimuli in which antigen antibody reactions are predominant. ERCR at doses of 100–150 mg/kg significantly protect egg albumin induced degranulation of mast cell in dose dependent. ERCR at 150 mg/kg protect mast cell comparable to disodium chromoglycate. This indicates that ERCR are effective in type I hypersensitivity reactions and effective in stabilizing mast cell. The anaphylactic allergic reaction is a life-threatening reaction inducing release of mediators such as histamine and pro-inflammatory cytokines and can be elicited by various stimuli. ERCR at doses of 100–150 mg/kg significantly inhibit egg abumin induced leakage of dye from dorsal skin of rats

when compared to control group. Control group showed (42.4 ±1.806) mm^2 area of dye leakage while area of dye leakage observed in rats treated with ERCR at dose 150 mg/kg (20.0 ±0.71) mm^2. Hence ERCR shows antihistaminic and anti-inflammatory mechanism by inhibiting egg albumin induced leakage of dye.

Phytochemical screening of ERCR revealed the presence of steroids, saponin, alkaloids, flavonoids, and glycosides. Saponin are reported to possesses mast cell stabilizing. Several flavonoids have been shown to possess smooth muscle relaxant and bronchodilator activity[21]; the flavonoids including apigenin and luteolin were known to inhibit basophil histamine release and neutrophil beta glucuronidase release, and thereby possess *in-vivo* antiallergic activity. These flavonoids also inhibited the histamine release induced by 48/80. Hence antiasthmatic activity of ERCR may be due to presence of flavonoids or saponins.

Present study revealed that ERCR decreases milk induced leucocytosis and eosinophilia. ERCR stabilize antigen induced mast cell and inhibit release of histamine in anaphylactic reaction and possesses antiallergic action. In conclusion ERCR are effective in treatment of asthma as it shows antiallegic and mast cell stabilizing potential.

Conflict of interest statement

We declare that we have no conflict of interest.

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