EVALUATION OF ANTIALLERGIC ACTIVITY (TYPE I HYPERSENSITIVITY) OF INULA RACEMOSA IN RATS

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Abstract: Alcoholic extract of root of Inula racemosa, was studied for its antiallergic effect in experimental models of type I hypersensitivity, viz. egg albumin induced passive cutaneous anaphylaxis (PCA) and mast cell degranulation in albino rats. The alcoholic extract was prepared by the process of continuous heat extraction. LD₅₀ of this extract was found to be 2100±60 mg/kg, i.p. Assessment of protection against egg albumin induced passive cutaneous anaphylaxis by different doses of Inula racemosa was done by giving drug intraperitoneally or orally for seven days or once only. Mast cell degranulation studies were done by using compound 48/80 as degranulation agent with same dosage schedule. Inula racemosa (i.p. as well as p.o.) showed significant protection against egg albumin induced PCA. Protection against compound 48/80 induced mast cell degranulation by alcoholic extract of Inula racemosa (single dose) was similar to that of disodium cromoglycate. The seven days drug treatment schedule showed greater protection than disodium cromoglycate intraperitoneally. The results suggest that Inula racemosa possesses potent antiallergic properties in rats.

Key words: inula racemosa passive cutaneous anaphylaxis mast cell degranulation antiallergic

INTRODUCTION

The powder of the roots of Inula racemosa (IR) of compositae family, in Ayurvedic system of medicine is a well known remedy for many ailments. These include allergic skin disorders, cough, dyspnoea, precordial pain and dysmenorrhoea etc. (1). Many bioactive constituents have been isolated from the root powder of plant like alantolactone, isoalantolactone, dihydroisoalantolactone (2), inunolide (3), inunal, isoalloalantolactone (4), epoxypetelekine, epoxysotelekine (5) and isoinunol (6) etc.

The biological activities of IR root powder have been correlated with its active

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constituents. Thus the antifungal and cholinergic activity of alcoholic extract of IR roots are due to alantolactone and isolantone (7). Antidermatophytic potential has been found to be due to hexane soluble fraction of alcoholic extract of IR (8). Clinically, root powder of IR has been found to be effective in patients of ischaemic heart disease (9-12), diabetes mellitus (13,14) and bronchial asthma (15). It possesses hypolipidemic (16), antimicrobial (17) and anthelmintic (18) properties. The antihistaminic (19) and antifungal (20) activity of IR root powder has also been studied experimentally. However, although it has significant anti-allergic properties clinically, the various aspects of this property pushkar mool have not been explored in detail. Therefore, in the present study, IR was evaluated for its antiallergic (Type I hypersensitivity) properties by different experimental methods in rats.

METHODS

Experimental animals

Albino rats of 100-150 gms of either sex were used. They kept in standard laboratory conditions in natural day-night cycle and given pellet diet and water ad-libitum.

Preparation of Alcoholic Extract

Roots of IR were collected from Kashmir region in winter months (Sept.-Nov.). After authentication, this was dried in shed and powdered in an electric grinder. Alcoholic extract was prepared by the method of Devis 1961 (21). Briefly, 5 kg of powdered material was extracted with 70% alcohol in Soxhlet apparatus by the process of continuous heat extraction at 55-60°C. After filtration the extract was concentrated on a water bath under reduced pressure. The semisolid material thus obtained was dried again in a vacuum dessicator over anhydrous calcium chloride. The total yield was 15.5% of root powder. The solution of required strengths were freshly prepared by mixing extract with normal saline and gum acacia (5% w/v) before experiments.

Determination of LD₅₀

Rats were taken in group of 5 each. First and second group were given alcoholic extract of IR in the doses of 1500 mg/kg and 3000 mg/kg i.p., observed for 24 hrs. and mortality noted. On the next day, two groups of rats were given 2000 mg/kg and 2500 mg/kg of alcoholic extract and mortality noted in next 24 hrs. Same experiment was repeated thrice and results were analysed by the method of Fisher 1954 (22).

Passive cutaneous anaphylaxis (PCA)

(A) Single dose experiments

The experiments were conducted by method of Ovary 1952 (23).

Three doses of 10 mg egg albumin (Sigma Chemical, U.S.A.) with 1 ml Freund adjuvant (60 mg aluminium hydroxide per ml, John-Wyeth Chemicals, U.S.A.) were given subcutaneously to rabbits (weight 1.5-2.0 kg) on 1, 3, 5 days. On 10th day, blood was collected from dorsal vein of pinna and serum containing IgG type of antibodies separated by centrifugation and stored at -20°C, till it was used for PCA testing.

Rats were sensitised with 0.1 ml of rabbit’s antiserum (diluted 1:10 in normal
saline), injected intradermally, on the shaved dorsal surface of each rat on either side of mid-line. Three hours later, alcoholic extract of \textit{IR}, in doses of 60, 120, 240 mg/kg, i.p., was given to the rats of group I, II and III. The fourth group was given disodium cromoglycate (DSCG, Fisons Pharmaceuticals, U.S.A.) 50 mg/kg, i.p. The last group was taken as control and was given 0.2 ml of 2% gum acacia solution intraperitoneally. One hour later 1% egg albumin along with 0.5% Evan’s blue (0.25 ml each) was injected into the tail vein of each group of rats. All rats were sacrificed after 45 minutes and blue coloured area on the inner aspect of skin was measured with the help of calliper and scale.

(B) Seven day study

100 mg egg albumin coated with 12 mg aluminium hydroxide per rat was given subcutaneously on 1, 3 and 5 days to ten rats. On the 10th day, blood was collected by puncturing orbital venous plexus and serum containing IgE type antibodies was separated and stored at \(-20^\circ\text{C}\) for further use.

Rats were divided randomly in five groups of five rats each for the study. Rats of group I, II & III were treated with 60, 120, 240 mg/kg, i.p., alcoholic extract of \textit{IR}, by i.p. seven consecutive days respectively, whereas group IV was given DSCG 50 mg/kg, i.p., for the same period. Group V served as control and animals were given solution of gum acacia (5% w/v) 0.2 ml intraperitoneally.

On day 5, rats of all five groups were sensitised with antiserum as above and on the seventh day, after one hour of giving test compound, 1% egg albumin along with 0.5% Evan’s blue (0.25 ml each) was administered intravenously. Then after a gap of 45 minutes the blue coloured area measured with the help of calliper and scale after sacrificing the animals and results were compared with DSCG and control groups.

(C) PCA by oral route

Both the studies (single dose and seven days) were also done by giving the drugs orally viz. alcoholic extract of \textit{IR} in doses of 280 and 560 mg/kg and DSCG in the dose of 50 mg/kg. The other steps were same as above. Mean values of coloured area of skin were calculated for each group separately and percent protection of PCA for each treated group calculated by the following formula:

\[
\text{Percent protection} = 100 \times \frac{\text{Coloured area of skin in treated group}}{\text{Coloured area of skin in control group}}
\]

Mast cell degranulation study

Five groups of 5 rats each were taken for study. Rats of group I, II and III were given alcoholic extract of \textit{IR} in doses of 60, 120 and 240 mg/kg, intraperitoneally. The group IV rats were given DSCG, 50 mg/kg, i.p. The 5th group served as control and was given 5% w/v gum acacia solution in a dose of 0.2 ml/rat.

After one hour of drug treatment, 10 ml buffered normal saline was injected i.p. and abdomen massaged gently for 90 seconds.
The rats were sacrificed by cervical dislocation and the suspension of peritoneal cells was aspirated by glass dropper after opening abdomen by midline incision and aspirate was suspended in 5-7 ml of RPMI 1640 medium (prepared by mixing 10.4 gm RPMI 1640 (Sigma Chemicals, U.S.A.) and 2.25 g sodium bicarbonate in one litre of distilled water and pH adjusted to 7.2-7.4) in siliconised tubes. Mast cells were washed thrice with RPMI 1640 and each time this was centrifuged at 200 RPM for 10 minutes. Supernatant was discarded and pellets of mast cells were taken into medium. The cell suspensions of all specimens were challenged with one drop of 1 ng/ml of compound 48/80 (Sigma Chemicals, U.S.A.) and incubated on a water bath at 37°C for 10 minutes. The pellets of peritoneal mast cells were taken on slide and stained with 0.1% toluidine blue and counted in four different fields, under a microscope, for intact and degranulated cells.

For seven days study, same procedure was repeated except that the treatment for various groups was for 7 days. On the seventh day mast cell suspension was taken out after 1 hour of drug treatment and counts were done. For both these studies (single dose and seven days) drug administration was by oral route in doses of 280 and 560 mg/kg of alcoholic extract of IR and DSCG in a dose of 50 mg/kg, orally.

Mast cells per ten high power field (HPF) were calculated under microscope as degranulated or intact. Mean values for control as well as for each treated group were calculated. Percent inhibition of mast cell degranulation for each treated group was calculated by formula:

\[
\text{Percent inhibition of MCD} = \left( \frac{100 - \frac{\text{Number of degranulated cells}}{\text{Total number of mast cells}} \times 100}{\text{Total number of mast cells}} \right) \times 100
\]

Statistical analysis

Mean ± SE was calculated for each dose of compound and significance determined by Students 't' test.

RESULTS

LD₅₀

The LD₅₀ of alcoholic extract of IR in rats, by intraperitoneal route was found to be 2100 ± 60 mg/kg.

Passive cutaneous anaphylaxis

The effects of intraperitoneal of IR on PCA test in rats are summarised in Table 1. The mean percent protection values were found to be 49 ± 0.54, 66 ± 0.42 and 70.0 ± 11% with 60, 120 and 240 mg/kg, i.p. of alcoholic extract of IR respectively given once only. While the mean percent protection values were 53 ± 2.17, 77.0 ± 0.36 and 83.0 ± 0.46% with 60, 120 and 240 mg/kg, i.p. doses of alcoholic extract of IR given for a period of 7 days, respectively. The percent protection by 50 mg/kg DSCG single dose was 71.0 ± 2.3%, whereas the percent protection after the seven days i.p. treatment with 50 mg/kg DSCG was 81.5 ± 5.2%. The anti PCA activity after i.p. doses of IR were found to be significant (P<0.01).

The mean percent protection values were 51.0 ± 5.42 and 65 ± 3.19% with 280 and
TABLE I: Effect of alcoholic extract of *Inula racemosa* on passive cutaneous anaphylaxis induced by egg albumin.

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Route</th>
<th>Single day treatment</th>
<th>Seven days treatment</th>
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<tr>
<td></td>
<td></td>
<td>Mean ± S.E. n = 25</td>
<td>Mean ± S.E. n = 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coloured area (cm²)</td>
<td>Percent* protection</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>352.0±0.57</td>
<td></td>
</tr>
<tr>
<td>IR 60</td>
<td>ip</td>
<td>181±0.67</td>
<td>49±0.54</td>
</tr>
<tr>
<td>IR 120</td>
<td>ip</td>
<td>121±0.86</td>
<td>66.0±0.42</td>
</tr>
<tr>
<td>IR 240</td>
<td>ip</td>
<td>106±2.73</td>
<td>70.0±3.11</td>
</tr>
<tr>
<td>DSCG 50</td>
<td>ip</td>
<td>103±2.81</td>
<td>71.0±2.3</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>337.0±11.2</td>
<td></td>
</tr>
<tr>
<td>IR 280</td>
<td>po</td>
<td>166±4.86</td>
<td>51±5.42</td>
</tr>
<tr>
<td>IR 560</td>
<td>po</td>
<td>166±2.36</td>
<td>65±3.19</td>
</tr>
<tr>
<td>DSCG 50</td>
<td>po</td>
<td>335±10.6</td>
<td></td>
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</tbody>
</table>

*P<0.01. All values were found to be significant.

TABLE II: Effect of alcoholic extract of *Inula racemosa* on mast cell degranulation induced by compound 48/80.

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Route</th>
<th>Percent protection Mean ± SE, n=25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Single dose*</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR 60</td>
<td>ip</td>
<td>49±0.83</td>
</tr>
<tr>
<td>IR 120</td>
<td>ip</td>
<td>54±0.92</td>
</tr>
<tr>
<td>IR 240</td>
<td>ip</td>
<td>58±0.57</td>
</tr>
<tr>
<td>DSCG 50</td>
<td>ip</td>
<td>69±0.76</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR 280</td>
<td>po</td>
<td>49±0.69</td>
</tr>
<tr>
<td>IR 560</td>
<td>po</td>
<td>54±0.40</td>
</tr>
<tr>
<td>DSCG 50</td>
<td>po</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*P<0.01. All values were found to be significant.

560 mg/kg of a single dose of alcoholic extract of *IR* orally. Similarly the mean percent protection were 53.0±3.53 and 68±2.14% with 280 and 560 mg/kg of *IR* for 7 days orally. However, DSCG was ineffective orally. The results with PCA test with orally administered *IR* were also significant (P<0.01) (Table I).

Mast cell degranulation

The effect of intraperitoneal administration of *IR* against compound 48/80 induced mast cell degranulation are summarised in Table II. The mean percent protection values were found to be 49±0.83, 54.0±0.92 and 58.0±0.57% with 60,
120 and 240 mg/kg, respectively, of alcoholic extract of IR given once only. Similarly, the mean percent protection after single dose i.p. administration of 50 mg/kg DSCG was 69.0 ± 76%, whereas the percent protection after seven days treatment with same dose of DSCG was 74.0 ± 2.93%.

Animals treated with either single dose or seven daily doses of the alcoholic extract of IR by oral route also showed significant protection against compound 48/80 induced mast cell degranulation. The mean percent protection values were 49.0 ± 0.69 and 54.0 ± 0.40% with 280 and 560 mg/kg of extract of IR. Similarly, the mean protection values were found to be 67 ± 2.69 and 75 ± 0.27 with 280 and 560 mg/kg doses of IR in the seven day treatment schedule.

DISCUSSION

*Inula racemosa* has been advocated for the prophylaxis of acute exacerbation of chronic bronchial asthma and for the treatment of allergic skin disorders in Indian traditional system of medicine. Since the principal target cells of hypersensitivity reactions are mast cells and basophils (24-25), the antiallergic property of IR was evaluated by assessing its capability to protect mast cell degranulation, when induced by some highly antigenic substance like compound 48/80 (p-methoxy-N-methylphenylethylamine) and protection of allergen (egg albumin) induced passive cutaneous anaphylaxis (Type I hypersensitivity).

In the present study, alcoholic extract of IR showed significant protection against egg albumin induced passive cutaneous anaphylaxis both with single dose as well as with seven days administration, confirms its antiallergic property (type I hypersensitivity). The results of our study also indicate that IR is orally effective, an added advantage over DSCG, which is ineffective orally (26).

The second parameter taken for evaluating antiallergic property of IR was protection of mast cell degranulation induced by compound 48/80. The compound 48/80 acts on mast cells principally by mobilizing Ca²⁺ from cellular stores (27,28). On degranulation, mast cells release histamine, leukotrenes and other granular contents (29,30). Thus prevention of this degranulation process can be a very effective criteria to measure prevention of type I allergic disorders.

Our results showed that IR affords significant protection against mast cell degranulation induced compound 48/80, indicating a possible stabilizing effect on the biomembrane of mast cells.

Since the LD₅₀ values were found to be very high (2100 ± 60 mg/kg, i.p.), the compound seems to be very safe for use in clinical situations, though further evaluation for chronic toxicity is required.

However, its use in bronchial asthma and allergic disorders in Ayurvedic system of medicine without any toxicity for centuries supports our observation for its safety as well as its effectivity as an antiallergic compound. Its potential usage in prevention as well as treatment of bronchial asthma and allergic disorders needs to be explored extensively specially when treatment of such disorders remain unsatisfactory with modern system of medicine.
REFERENCES


