APPENDIX A

PHARMACOLOGICAL METHODS

1. CARRAGEEANAN-INDUCED RAT PAW OEDEMA

Experimental

Carrageenan Suspension:

Suspension of carrageenan sodium salt (Sigma-Aldrich Chemicals Ltd.), 1% was prepared by sprinkling 100 mg of carrageenan powder on 10 ml of saline (0.9%) solution and set a side to soak for 1h. A homogeneous suspension was then obtained by thorough mixing with a magnetic stirrer.

Induction of Paw oedema:

Wistar rats (150-200 gm, purchased from national Institute of Nutrition, Hyderabad, A.P, India) were used. Oedema was induced by injecting, subcutaneously (s.c) into the sub plantar tissue of the left hind paw of each rat, 0.1 ml of 1% carrageenan suspension in saline. The right hind paws of the same rats received 0.1 ml of saline alone in the same manner as control. Before the induction of oedema, the thickness of the both paws of each rat between lower and upper surface was measured using an instrument consisting of a graduated micrometer combined with a constant loaded lever system (Fig. A) to magnify the small changes in paw thickness during the course of the experiment. The measurements were then taken at 1hr intervals after the induction of the oedema for up to 6hr. Oedema was monitored as the percentage increase in paw thickness in the carrageenan injected paw. To
assess the effect of saline on the oedema produced, the percentage increase in paw thickness produced in the saline injected paw was subtracted from that of carrageenan injected left paw (Al-Haboubi and Zeitlin, 1983). The percentage increase in paw thickness was plotted against the time (h) and the maximal oedema response induced during the 6hs was determined. The total oedema response as the area under the time course curves (AUC) was also determined.

Figure A: Zeitlin’s Constant Loaded Lever
(Paw thickness measuring device)

1. Place where the paws use to be kept to measure the thickness
2. Constant loaded lever
3. Graduated scale numbered between 1-10 and divided by 0.5 equal to 20 divisions
4. Thread to pull down the lever with right leg in order to facilitate to keep the paw in between pointer 1a and basement 1b.

Sample calculations for the results
Paw thickness (mm):

<table>
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<tr>
<th>Time (hr)</th>
<th>Rat 1</th>
<th>Rat 2</th>
<th>Rat 3</th>
<th>Rat 4</th>
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<tbody>
<tr>
<td></td>
<td>RT</td>
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<td>LT</td>
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<td>6.5</td>
<td>3.0</td>
<td>5.5</td>
<td>3.0</td>
</tr>
</tbody>
</table>

RT: Right paw; LT: Left paw

**Calculations:**

% Increase in paw thickness = \([Y_t - Y_o]/ Y_o \times 100\)

Where, \(Y_t\) = Paw thickness at time \(t\) hr (after injection)

and \(Y_o\) = Paw thickness at time 0 hr (before injection)

Accordingly, for Rat 4, the oedema in the 4th hr was calculated as:

% increase in RT = 7.5-3.5/ 3.5 \times 100 =114.3%

% increase in LT = 3.5-3.0/ 3.0 \times 100 =16.7%

Therefore, % increase due to carrageenan = (114.3-16.7) % = 97.6%
Assessment of drug effects:

For screening purposes, drugs (extract or compounds) in sodium carboxymethyl cellulose were always pre dosed to rats prior to the induction of carrageenan paw oedema. The actions of drugs were evaluated by comparing the maximal paw oedema response during 6hr (monitored as % increase in paw thickness) in the drug treated groups with that produced in the drug vehicle (control) treated group. The total (area under the time course curve, AUC, calculated using Trapezoid Rule) oedema response as the area under the time course curve, produced in the drug treated groups was also compared with that from the control group.