Antiinflammatory activity of leaf and leaf callus of *Silybum marianum* (L.) Gaertn. in albino rats

*Silybum marianum* (L.) Gaertn. is an important medicinal plant of family *Compositae*, commonly known as Milk-thistle, or St. Mary’s thistle. The plant and its extracts are reported to possess hepatoprotective, antioxidant,[3] anticancer,[1] antiinflammatory,[3] and antidiabetic[8] properties. It contains flavonolignan Silymarin, which is an important bioactive principle having anticancer, antiinflammatory, antioxidant, and immunomodulatory effects.[3] However, till date, no antiinflammatory activity has been carried out on tissue cultures developed from *S. marianum*. Therefore, it was thought worthwhile, to determine the antiinflammatory activity of plant cultures developed in vitro. In the present investigation, the leaf callus of plant has been successfully developed and maintained for six months. The methanolic extract of dried leaf callus was examined for antiinflammatory activity, using carrageenan and formalin-induced rat paw oedema models, which was also compared with that of leaf extract. Leaves were collected from the plants grown in the herbal garden of Hamdard University, New Delhi, identified by Department of Botany, and the voucher specimen was kept in the herbarium of the University.

The immature leaves were washed with water and detergent, followed by rinsing with double distilled water to remove the detergent. The cleaned leaves were then transferred aseptically to Mercuric chloride solution (0.5% w/v) and stirred for five minutes. Then these were removed and washed six times with double distilled water to complete removal of chemical sterilent. The sterilized leaves were then transferred in culture tubes (Borosil glass works Ltd.) containing Murashige and Skoog (MS) medium[6] supplemented with several hormones like indole acetic acid (IAA), Indole butyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D), naphthalene acetic acid (NAA), 6-benzyl adenine (6-BA), and kinetin (Sigma chemicals) in different concentrations, and kept in a BOD incubator (25±2°C temperature, 16 and 8 h light/dark cycle). Out of several hormonal combinations tried, MS medium supplemented with IAA + IBA + 2, 4-D and kinetin (1 ppm each), showed best results for initiation of creamy soft and friable leaf callus, within 12-16 days. The leaf calli initiated on the above medium, were further developed and maintained for six months on the same medium.

Dried and powdered leaves (35 g) and callus (20 g), were separately extracted with methanol in soxhlet apparatus (Borosil glass works Ltd.), for four hrs. The methanolic extracts were evaporated to dryness under vacuum evaporator (Scientific system, New Delhi), and the residue obtained was triturated with gum acacia in distilled water (1:1), and administered to adult female Wistar albino rats by oral route (100 mg/kg, body wt).

Forty-eight female Wistar albino rats weighing 150-200 g were used. The rats were housed in colony cages in an animal house, at an ambient temperature of 25±2°C, with 12 h light/dark cycle. The rats were allowed standard laboratory feed, and water ad libitum.

Preliminary phytochemical screening of methanol extracts of leaf and leaf callus were carried out for the detection of phytoconstituents, using standard chemical tests. Alkaloids, amino acids, flavonoids, carbohydrates, phenolics, steroids,

### Table 1

<table>
<thead>
<tr>
<th>Drug dose/route</th>
<th>Carageenan-induced paw oedema</th>
<th>Formalin-induced paw oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oedema volume (ml) % Inhibition</td>
<td>Oedema volume (ml) % Inhibition</td>
</tr>
<tr>
<td>Control</td>
<td>1.22±0.20</td>
<td>1.18±0.11</td>
</tr>
<tr>
<td>Aspirin (150 mg/kg, oral)</td>
<td>0.26±0.13**</td>
<td>0.16±0.04**</td>
</tr>
<tr>
<td>Leaf extract (100 mg/kg, oral)</td>
<td>0.32±0.08**</td>
<td>0.17±0.04**</td>
</tr>
<tr>
<td>Leaf callus extract (100 mg/kg, oral)</td>
<td>0.07±0.02**</td>
<td>0.10±0.05**</td>
</tr>
<tr>
<td>One-way</td>
<td>F</td>
<td>7.91</td>
</tr>
<tr>
<td>ANOVA</td>
<td>df</td>
<td>3, 20</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n = 6 animals in each group. **P < 0.01 as compared to control (Dunnett’s test).
and tannins, were detected in both the extracts. HPTLC fingerprints of methanolic extracts were established using CAMAG HPTLC and chloroform: acetone: formic acid (9:2:1) as solvent system, which showed presence of 10 spots (RF-value: 0.03, 0.07, 0.10, 0.19, 0.42, 0.51, 0.59, 0.66, 0.74 and 0.79) and 8 spots (RF-value: 0.03, 0.07, 0.19, 0.42, 0.52, 0.59, 0.67 and 0.75) respectively at 254 nm wavelength.

In carrageenan-induced paw edema model, groups of rats were orally administered with the leaf extract (100 mg/kg, bw), leaf callus extract (100 mg/kg, b.w.), Aspirin (150 mg/kg) or saline. 1 h before administration of an intradermal injection of carrageenan (0.1 ml of a 1% in 0.9% saline), into the plantar surface of the right hind paw. The doses of extracts were chosen, based on those used in an earlier study. The paw volume was measured by recording the volume displacement by digital plethysmometer (UGO-BASILE-7140 Barcelona), just before, and three hours after the injection of carrageenan. The average percent increase in paw volume of each group was calculated, and compared with that of the control (saline) and aspirin groups.

In formalin-induced paw edema model, the same procedure was carried out, except that 0.05 ml of 1% formalin was injected, instead of carrageenan.

The data were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett’s test. P <0.01 was considered as statistically significant. The data are expressed as mean ± SEM. The results are shown in Table 1.

The leaf and leaf callus of Silybum marianum (L.) Gaertn. inhibited the formation of paw oedema to significant levels in rats treated either with carrageenan or formalin. [Table 1] At a dose of 100 mg/kg orally, the leaf extract produced 74% inhibition, while leaf callus produced 93.9% inhibition in case of the carrageenan-induced oedema (P<0.01), and there was 85.61% inhibition in leaf extract, and 91.27% inhibition in leaf callus extract, in formalin-induced oedema (P<0.01). The % inhibition showed by leaf callus extracts (100 mg/kg) was found to be more than that of reference standard i.e., aspirin (93.9% vs 78.79% inhibition in carrageenan-induced rat paw oedema , and 91.27% vs 86.86% inhibition in formalin-induced rat paw oedema).

The in vitro culture-generated callus extract showed maximum inhibition in rat paw oedema, which is due to presence of higher amount of secondary metabolites, as compared to natural plant leaf. Our results strongly suggest that the methanolic extract of leaf and leaf callus of Silybum marianum possesses a potent antiinflammatory activity, that could inhibit the acute inflammation in rat paw, induced either by carrageenan or formalin.

S. Balian, S. Ahmad, R. Zafar
Plant Tissue Culture Laboratory,
Department of Pharmacognosy and Phytochemistry,
Faculty of Pharmacy, Jamia Hamdard,
New Delhi - 110 062, India
E-mail: swatimadan30@rediffmail.com

References