Antiulcer Activity of an Isolated Compound (SP–1) from the Seeds of Nirmali (Strychnor potatorum Linn.)

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An active compound (SP-1) was isolated from the seeds of Nirmali (Strychnor potatorum Linn.) and its antiulcer activity was studied against ethanol, hydrochloric acid, indomethacin, stress and pyloric ligation induced gastric ulceration in albino rats. A significant antiulcer activity of SP-1 was observed in all the models. SP-1 thus provides a scientific rationale for the use as antiulcer drug.

Keyword: Antiulcer activity, Ethanol, Stress, Indomethacin, Pyloric ligation, Nirmali (Strychnor potatorum Linn.)

1. Introduction

Sanyal et al. 1961 found that vegetable banana is efficacious not only for experimentally induced gastric ulcers in albino rats, mice, guinea pigs etc. but also for humans suffering from gastric ulcers11. Akah et al. 1999 demonstrated anti gastric ulcer activity of the herb Cassampelos mucronata12, Shetty et al. 2000, Sairam et al. 2001, Maity et al. 1995, 2003 and Dharmani and Palit, 2006 confirmed anti gastric ulcer activities of Ginkgo biloba, Convulvulus pluricaulis Chois, tea root extract and Vernonia lasiopus respectively13-17. We also reported anti gastric ulcer activities of few medicinal plants of this part of India in different experimental ulcer models (Mitra, 2001; Mitra & Mitra 2005, 2008; Mitra et al. 2008, 2010)18-121. During the work we have noted that seeds of Nirmali (Strychnor potatorum Linn.) could protect albino rats from ethanol and restraint induced gastric ulcers19,12. It was thought worthwhile to isolate the active compound(s) from the seeds of Nirmali (Strychnor potatorum Linn.) and to find its anti ulcerogenic effect in certain other experimental ulcer models.

2. Materials and Methods

Seeds of Nirmali (Strychnor potatorum Linn.) were collected from the local market and were identified by the experts of the department of Botany of the University of North Bengal. Seeds were kept in the department with proper voucher number for future reference.

2.1 Isolation of active principle (SP-1) from the seeds of Nirmali (Strychnor potatorum Linn.)

Seeds of Nirmali were shade dried at room
temperature, ground into fine powder and then extracted (amount 100g) with 600 ml chloroform–water mixture(10:1, v/v) for 30 minutes using soxhlet apparatus at room temperature. The extract was concentrated under reduced pressure by a rotary evaporator to a volume of about 10 ml. This was then subjected to column chromatography using alumina as adsorbent. Elution was done by 50% ethanol-chloroform mixture. Eluted material was evaporated to dryness and extracted with 10 ml ethyl acetate. The ethyl acetate extract was further subjected to column chromatography using silica gel mesh (200-400 size) as adsorbent. The fraction obtained after elution with 50% methanol-chloroform mixture was subjected to repeated crystallization when a compound was crystallized. The compound was given a trivial name SP-1. The compound was preserved for acute toxicity study as well as for anti-gastric ulcer activity.

2.2 Experimental Animals
Wistar strain albino rats of both sex were used for the study. The animals were housed in colony cages (4 rats/cage) and were kept for at least a week in the experimental wing of the animal house (room temperature 25–28 degree centigrade and humidity 60–65% with 12 h light and dark cycle) before experimentation. Animals were fed on laboratory diet with water ad libitum. For each set of experiment 10 animals were used. The animal experiment had approval of the institutional ethics committee.

2.3 Chemicals
Indomethacin (Torrent Research Centre, Gandhinagar), ethanol (Baroda Chemical industries Ltd., Dabhoi), HCl LR (Thomas baker, Mumbai), omeprazole (Kopran Pharma Ltd. Mumbai).

2.4 Test drug
Isolated compound (SP-1) was used as the test drug.

2.5 Production of gastric ulcers
Ethanol induced gastric ulcer (Sairam et al. 2001)[4]
Rats were fasted for 18 h when no food but water was supplied *ad libitum*. Gastric ulcers were induced by administering ethanol (95%, 1 mL/200 g body weight) orally through a feeding tube. 1h after administration of ethanol, animals were sacrificed by cervical dislocation and the stomach was taken out and incised along the greater curvature. Stomach was then examined for the presence of ulcers.

2.6 HCl induced gastric ulcer (Parmar and Desai, 1993)[13]
0.6M HCl (1 mL/200 g body weight) was orally administered to all rats. Rest part is same to that of ethanol induced gastric ulcer group.

2.7 Indomethacin induced gastric ulcer (Parmar and Desai, 1993)[13]
Indomethacin (10 mg/kg) was given orally to rats in two doses at an interval of 15 hour. Rest part is same to that of ethanol induced gastric ulcer group.

2.8 Stress induced gastric ulcer (Alder, 1984)[14]
Rats were fasted for 24h when no food but water was supplied *ad libitum*. Stress ulcer was induced by forced swimming in the glass cylinder (height 45 cm, diameter 25 cm) containing water to the height of 35 cm maintained at 25degree centigrade for 3h. Rats were then sacrificed. Rest part was same to that of ethanol induced gastric ulcer group.

2.9 Induction of gastric ulcer by pyloric ligation method (Parmar and Desai, 1993)[13]
Rats were fasted for 24h when no food but water was supplied *ad libitum*. Under light ether anesthesia, abdomen was opened and the pylorus was ligated. The abdomen was then sutured. After 4h the rats were sacrificed with excess of anesthetic ether and the stomach was dissected out. Rest part was same to that of ethanol induced gastric ulcer group.
2.10 Acute oral toxicity study (Ghosh, 2005)[15]
Acute toxicity studies were carried out on Swiss albino mice. Isolated compound (SP-1) from the seeds of Nirmali (Strychnor potatorum Linn.) was given orally at doses of 100, 200, 500, 1000 and 3000 mg/kg to five groups of mice, each group containing six animals. After administration of the compound, the animals were observed for the first three hours for any toxic symptoms followed by observation at regular intervals for 24 hours up to seven days. At the end of the study, the animals were also observed for general organ toxicity, morphological behavior and mortality.

2.11 Anti gastric ulcer study
Rats were divided into 5 groups;
Group 1: Control
Group 2: Ulcerogenic drug or Method (Ethanol / HCl / Indomethacin / Stress / Pyloric ligation)
Group 3: Ulcerogenic drug or method + SP-1 (5 mg/kg)
Group 4: Ulcerogenic drug or method + SP-1 (10 mg/kg) (SP-1 was given orally 30 minutes prior to administration of ulcerogenic drug or method)
Group 5: Ulcerogenic drug or method + Omeprazole (8 mg/kg orally 30 minutes prior to administration of ulcerogenic drug or method). Omeprazole was used as per the method of Malairajan et al., 2008[16].

2.12 Evaluation of ulcer index (Szelenyi and Thiemer, 1978)[17]
Gastric lesions were counted and the mean ulcerative index was calculated as follows:
   i. Presence of edema, hyperemia and single sub mucosal punctiform hemorrhage.
   ii. Presence of sub mucosal hemorrhagic lesions with small erosions.
   iii. Presence of deep ulcer with erosions and invasive lesions.

Ulcer index = (number of lesion I) x1 + (number of lesion II) x2 + (number of lesion III) x 3.

2.13 Statistical analysis
The values were expressed as mean ± SEM and were analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) 20th versions. Differences between means were tested employing Duncan’s multiple comparison test and significance was set at p<0.05.

3. Results and Discussion
3.1 Acute toxicity studies
Acute toxicity studies revealed that SP-1 did not produce any toxic symptoms when administered orally to mice in doses of 100, 200, 500, 1000 and 3000 mg/kg. Animals were healthy, cheerful and behaved normal throughout the experimental period. No death of animal was recorded during seven days of experiment.

3.2 Effect of SP-1 on ethanol induced gastric ulcer
Result is given in Table-1
Ethanol produced massive gastric ulcers in all albino rats. Ulcers were mostly superficial. Bleeding of the stomach was followed by adhesion and dilatation. Ulcer index came 30.5 ±1.88. Pretreatment of rats with SP-1 produced a dose dependent protection (13.11% and 43.60% for the doses of 5 mg/kg and 10 mg/kg of SP-1 respectively) from ulcer production induced by ethanol. However, the protection was statistically significant (p<0.001) only at 10 mg/kg dose of SP-1. Omeprazole gave significant protection (64.26%) to the rats from ethanol induced gastric ulcers. Anti-ulcer effect of SP-1 in the dose of 10 mg/kg was comparable to that of omeprazole.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ulcer index (mean ± SEM)</th>
<th>% Ulcer protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Nil</td>
<td>--</td>
</tr>
<tr>
<td>Ethanol</td>
<td>30.5 ± 1.88</td>
<td>--</td>
</tr>
<tr>
<td>Ethanol+ SP-1 (5 mg/kg)</td>
<td>26.5 ± 1.31</td>
<td>13.11</td>
</tr>
<tr>
<td>Ethanol+ SP-1 (10 mg/kg)</td>
<td>17.2 ± 1.22**</td>
<td>43.60</td>
</tr>
<tr>
<td>Ethanol + Omeprazole (8mg/kg)</td>
<td>10.9 ± 1.35**</td>
<td>64.26</td>
</tr>
</tbody>
</table>

Results were in mean ± SEM, Each group had ten rats, ** p<0.001
Table-2. Effect of SP-1 on hydrochloric acid induced gastric ulcer

<table>
<thead>
<tr>
<th>Group</th>
<th>Ulcer index (mean ± SEM)</th>
<th>% Ulcer protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Nil</td>
<td>--</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>29.6 ± 1.61</td>
<td>--</td>
</tr>
<tr>
<td>Hydrochloric acid + SP-1 (5 mg/kg)</td>
<td>20.9 ± 1.52*</td>
<td>29.39</td>
</tr>
<tr>
<td>Hydrochloric acid + SP-1 (10 mg/kg)</td>
<td>17.5 ± 1.46**</td>
<td>40.87</td>
</tr>
<tr>
<td>Hydrochloric acid + Omeprazole (8mg/kg)</td>
<td>11.3 ± 1.22**</td>
<td>61.82</td>
</tr>
</tbody>
</table>

Results were in mean ± SEM, Each group had ten rats, *P<0.01, ** p<0.001

Table-3. Effect of SP-1 on indomethacin induced gastric ulcer

<table>
<thead>
<tr>
<th>Group</th>
<th>Ulcer index (mean ± SEM)</th>
<th>% Ulcer protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Nil</td>
<td>--</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>29.5 ± 1.62</td>
<td>--</td>
</tr>
<tr>
<td>Indomethacin+SP-1 (5 mg/kg)</td>
<td>22.5 ± 1.22*</td>
<td>23.72</td>
</tr>
<tr>
<td>Indomethacin + SP-1 (10 mg/kg)</td>
<td>15.7 ± 1.31**</td>
<td>46.77</td>
</tr>
<tr>
<td>Indomethacin + Omeprazole (8mg/kg)</td>
<td>10.1 ± 1.18**</td>
<td>65.76</td>
</tr>
</tbody>
</table>

Results were in mean ± SEM, Each group had ten rats, *P<0.01, ** p<0.001

Table-4. Effect of SP-1 on swimming stress induced gastric ulcer

<table>
<thead>
<tr>
<th>Group</th>
<th>Ulcer index (mean ± SEM)</th>
<th>% Ulcer protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Nil</td>
<td>--</td>
</tr>
<tr>
<td>Swimming stress</td>
<td>30.8 ± 1.72</td>
<td>--</td>
</tr>
<tr>
<td>Swimming stress + SP-1 (5 mg/kg)</td>
<td>22.9 ± 1.43*</td>
<td>25.64</td>
</tr>
<tr>
<td>Swimming stress + SP-1 (10 mg/kg)</td>
<td>15.1 ± 1.34**</td>
<td>50.97</td>
</tr>
<tr>
<td>Swimming stress + Omeprazole (8mg/kg)</td>
<td>11.4 ± 1.29**</td>
<td>62.98</td>
</tr>
</tbody>
</table>

Results were in mean ± SEM, Each group had ten rats, *P<0.01, ** p<0.001

Table-5. Effect of SP-1 on pyloric ligation induced gastric ulcer

<table>
<thead>
<tr>
<th>Group</th>
<th>Ulcer index (mean ± SEM)</th>
<th>% Ulcer protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Nil</td>
<td>--</td>
</tr>
<tr>
<td>Pyloric ligation</td>
<td>27.2 ± 1.71</td>
<td>--</td>
</tr>
<tr>
<td>Pyloric ligation + SP-1 (5 mg/kg)</td>
<td>23.2 ± 1.55</td>
<td>14.70</td>
</tr>
<tr>
<td>Pyloric ligation + SP-1 (10 mg/kg)</td>
<td>11.8 ± 1.31**</td>
<td>56.61</td>
</tr>
<tr>
<td>Pyloric ligation + Omeprazole (8mg/kg)</td>
<td>10.5 ± 1.1**</td>
<td>61.39</td>
</tr>
</tbody>
</table>

Results were in mean ± SEM, Each group had ten rats, ** p<0.001

3.3 Effect of SP-1 on hydrochloric acid induced gastric ulcer

0.6M HCl when administered to rats orally produced massive ulcers in stomach of all rats. Adhesion and dilatation of the stomach were seen. Ulcer index was 29.6 ± 1.61. Pretreatment with SP-1 with the doses of 5 mg/kg and 10 mg/kg gave ulcer index 20.9 ± 1.52 and 17.5 ± 1.46 respectively. Thus, there was protection with SP-1 from the production of HCl induced gastric ulceration in rats. Protection was statistically significant (p<0.001) and comparable with omeprazole group where ulcer index came 11.3 ± 1.22 and protection was 61.82% (Table -2).

3.4 Effect of SP-1 on indomethacin induced gastric ulcer

Result is given in Table-3
Indomethacin produced gastric ulcers in all albino rats. Ulcers were superficial in nature. There were adhesion, dilatation and bleeding in the stomach. Ulcer index came 29.5 ± 1.62. Pretreatment of rats with SP-1 produced dose dependent protection (23.72% and 46.77% for the doses of 5 mg/kg and 10 mg/kg of SP-1 respectively) from indomethacin induced ulceration. Protections were statistically significant (p<0.001) by both the two doses of SP-1. Omeprazole produced more protection (65.76%) in course of production of gastric ulcer by indomethacin.

3.5 Effect of SP-1 on stress induced gastric ulcer
Swimming stress produced massive ulcers in stomach of all rats. Adhesion and dilatation of the stomach were seen. Ulcer index came 30.8 ± 1.72. Pretreatment with SP-1 gave dose dependent protection (25.64% and 50.97% for the doses of 5 mg/kg and 10 mg/kg respectively). Protection was statistically significant (p<0.001) and comparable to that of omeprazole group. In omeprazole group ulcer index came 11.4 ± 1.29. Protection was 62.98%. Results were shown in Table -4.

3.6 Effect of SP-1 on pyloric ligation induced gastric ulcer
Result is given in Table-5. Pyloric ligation produced gastric ulcers in all albino rats. Ulcers were superficial in nature. There were adhesion, dilatation and bleeding in the stomach. Ulcer index came 27.2 ± 1.71. Pretreatment of rats with SP-1 produced dose dependent protection. For the doses of 5 mg/kg and 10 mg/kg of SP-1 ulcer index came 23.2 ± 1.55 (14.70% protection) and 11.8 ± 1.31 (56.61% protection) respectively. Protection, however, was statistically significant only at 10 mg/kg dose of SP-1. Omeprazole produced significant protection (61.39%) in course of formation of gastric ulcer by pyloric ligation method. Ulcer index in this group came 10.5 ± 1.1.

The term “Peptic ulcer” refers to an ulcer in the lower oesophagus, stomach or duodenum, in the jejumum after surgical anastomosis to the stomach or, rarely in the ileum adjacent to a Meckel’s diverticulum. Ulcer in the stomach (gastric ulcer) may be acute or chronic. Quincke (1963) was probably the first to use the term ‘Peptic ulcer’. Because of its frequency and worldwide distribution, peptic ulcer continues to be a subject of numerous investigations, both experimental and clinico pathological. In this respect peptic ulcer occupies a place secondary to carcinoma in the field of gastroenterology.

There is medicine to treat peptic ulcer (Tierrey et al.1978). In case, the ulcer is due to infection of Helicobacter pylori (H. pylori), the different medications are usually prescribed. This is known as “Triple therapy”. This includes a proton pump inhibitor viz. omeprazole to reduce acid production and two antibiotics to get rid of the organism. Sometimes, instead of one of the antibiotics, bismuth salicylate may be the third medication recommended. This drug, available over the counter, coats and soothes the stomach, protecting it from the damaging effects of acid. Two, rather than three, drug regimens are currently being developed. For non H. pylori ulcers number of drugs are now available for treatment. These drugs are broadly classified into two categories:

1. Those that decrease or counter acid–pepsin secretion viz. ranitidine, famotidine etc. (H2 - blockers), pirenzepine, telenzepine etc. (M1 – blockers), omeprazole, lansaprazole etc. (proton pump inhibitors)

2. Those that affect cytoprotection by virtue of their effects in mucosal defense factors like sucralfate, carbenoxolone etc. (Yeomans et al. 1998).

No doubt the above said drugs have brought about remarkable changes in peptic ulcer therapy, reports on clinical evaluation of these drugs show that there are incidences of relapses and adverse effects and danger of drug interactions during ulcer therapy. Hence, the search for an ideal anti-ulcer drug continues and has also been extended to
medicinal plants / herbs in search for new and novel molecules, which afford better protection and decrease the incidence of relapse.

Strychnos potatorum Linn (Family, Loganiaceae) is a moderate sized tree found in southern and central parts of India, Sri Lanka and Burma. In Hindi and in English the tree is known as ‘Nirmali’ and ‘Clearing Nut Tree’ respectively. It is a common tree of medicinal importance in India popularly used to purify water for drinking. According to Ayurveda fruit, root and seed of Strychnos potatorum Linn. have medicinal property. Fruits are emetic, diaphoretic and alexiteric, also useful in eye diseases, thirst, poisoning and hallucinations. Root cures Leucoderma. Seeds are bitter, astringent to bowels, aphrodisiac, tonic, diuretic, anti ulcerogenic and good for liver, kidney complaints, colic as well as gonorrhea. Sanmuga and Venkataraman observed that seeds of Strychnos potatorum Linn could protect gastric ulcer induced by aspirin and pyloric ligation in albino rats. We also found antiulcerogenic potency of the seeds of Strychnos potatorum Linn against restraint ulcer model in experimental rats. Based on these observations we undertook study to isolate active compound(s) from the seeds of Strychnos potatorum Linn responsible for anti-ulcer effect. By different solvent extraction processes and chromatographic experiments an active compound was isolated from seeds of Nirmali (Strychnor potatorum Linn.). A trivial name of the compound was given as SP-1. Anti-gastric ulcer activity of SP-1 was studied against ethanol, hydrochloric acid, indomethacin, stress and pyloric ligation induced gastric ulceration in albino rats. Two doses of SP-1 (5 mg/kg and 10 mg/kg) were used. Results were compared with omeprazole, a known anti-gastric ulcer drug. Significant anti-gastric ulcer activity of SP-1 was observed in all the models employed. Results showed that pretreatment of rats with SP-1 produced dose dependent protection. The protection was statistically significant (p<0.001) specially in the dose of 10 mg/kg of SP-1 and was comparable to that of omeprazole group.

It is known that peptic ulcer is formed either through offensive mechanism (acid – peptic secretion) or through defensive mechanism (mucus secretion). Anti-gastric ulcer activity of SP-1 may be related with any one of the two mechanisms. Work in this direction is now under progress.

4. Conclusion
An active compound (SP-1) was isolated from the seeds of Nirmali (Strychnor potatorum Linn.). The compound had significant anti-ulcer activity against ethanol; hydrochloric acid, indomethacin, stress and pyloric ligation induced gastric ulceration in albino rats.

5. References