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## Antiulcerogenic Activity of *Indigofera longiracemosa*

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*Indigofera longiracemosa* root has been used in tribal medicine as an antidote for all snake poisons. Sequential petroleum ether, benzene, chloroform, ethyl acetate and ethanol extracts of *Indigofera longiracemosa* roots when administered either by i.p. (45 min before) or oral (45 min before or for 4 days) against restraint-stress or pylorus-ligated gastric ulcers in rats, the maximum protection was being found to be afforded by the petroleum ether and ethanol extracts. The mechanism of antiulcer effect could be due to decrease in acid-pepsin secretion or augmentation of mucin secretion as observed with the ethanol and petroleum ether extracts.

*Indigofera longiracemosa* Boiv. ex Baill. (Fabaceae), a slender shrub with woody branches and leaves with very thin leaflets<sup>1</sup> commonly known as karunelli in Tamil, grows only in Southern Western Ghats region of Tamil Nadu in India preferentially on sloping soils near the streams<sup>2</sup>. The plant is reported to be useful in tribal medicine as an antidote for all snake poisons<sup>2-3</sup>. In spite of its use in tribal medicine, no systematic studies on phytochemical and pharmacological activities of this plant have been reported. The preliminary successive solvent extraction and chemical tests revealed the presence of phytoconstituents in all the extracts<sup>2</sup>. Hence an effort has been made to establish the pharmacological activity of these extracts in general and antiulcerogenic activity in particular.

### MATERIALS AND METHODS

#### Plant material and extract:

*Indigofera longiracemosa* roots were collected from the vicinity of Courtalam in Tirunelveli district of Tamil Nadu, India. The plant materials were first dried in shade

and then in an oven at 40-50° for 5 h. The dried plant material was then subjected to size reduction to a coarse powder using a grinding mill. The coarse powder (900 g) is used for sequential extraction with petroleum ether, benzene, chloroform, ethyl acetate and ethanol. The extracts were concentrated under steam bath. These concentrated extracts were used for antiulcerogenic studies.

Male wistar rats weighing between 100-150 g were kept in the departmental animal house for one week before the experiment for acclimatization and thereafter the experiment. The rats were then fed with standard Hindustan lever pellets at room temperature 25±2° and 45-55% relative humidity with 10:14 h light and dark cycles. The animals before test had 18 h fast but water was given *ad libitum*. Extracts suspended in 1% gum acacia in double distilled water were used. The chemicals used for the study were petroleum ether, benzene, chloroform, ethyl acetate, ethanol (Sigma Chemicals Co., USA), 5% w/v gum acacia as a vehicle (Ambrosia Remedies Pvt. Ltd., Vadodara) of analytical grade.

#### Assessment of antiulcerogenic activity; Stress - induced gastric ulcers:

The different extracts were administered i.p. at a uniform dose of 50 mg/kg, 45 min before stress on the day

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of experiment in 24 h fasted rats for initial antiulcer study. Rats were fully stretched and strapped on a wooden plank with leucoplast after securing each limb to the wooden plank individually<sup>4</sup>. The rats were then kept at 18° for 4 h and after that animal were killed by decapitation and stomach removed and examined for ulceration<sup>5</sup>. As 50 mg/kg, i.p. dose was showing the tendency of ulcer index in many extracts and a oral route was taken as an intended route of administration to study the antiulcer activity. So a dose of 100 mg/kg was arbitrarily selected for p.o. administration to see antiulcerogenic effect. Extracts were given for one day (45 min before) and for four days In four days study, the extracts were given p.o. once daily for 3 days and on the fourth day 45 min before animals were subjected to stress. The ulcer index was calculated on the basis of number of ulcers per stomach plus severity of ulcers per stomach after histological conformation<sup>6</sup>. The severity of ulceration was calculated according to the following scale: 0 = normal gray colored stomach, 0.5 = pink to red coloration of stomach, 1 = spot ulcer, 1.5 = hemorrhagic streak, 2 = ulcer <5, 3 = ulcer > 5, 4 = ulcers with bleeding. Mean ulcer scores for each treatment group was calculated and expressed as ulcer index (UI)<sup>7</sup>.

#### Pylorus-ligated gastric ulcers:

Pyloric ligation-induced gastric ulcers were produced by 4 h pyloric ligation<sup>8</sup> under ether anesthesia. Different extracts of *Indigofera longiracemosa* roots in the dose of 100 mg/kg p.o. once daily was administered for four consecutive days, the last dose being administered 45 min before subjecting rats to pyloric ligation. The stom-

ach was removed after 4 h pylorus ligation and the gastric juice was collected and filtered through glass wool and centrifuged at 3000 rpm for 5 min. The supernatant was collected and its volume was expressed as ml/100 g of body weight. Total acid was determined by titrating the gastric juice with N/100 NaOH using phenolphthalein as indicator and expressed for concentration as  $\mu\text{Eq/ml}$  and for output as  $\mu\text{Eq/4 h}$ . The peptic activity was determined using hemoglobin as the substrate<sup>9</sup> and expressed for concentration as  $\mu\text{mol}$  of tyrosine/ml and for output as  $\mu\text{ml}$  of tyrosine/4 h. Mucin was estimated in the gastric juice using 95% ethanolic precipitate of the mucosubstances. The precipitate thus obtained was either dissolved in 0.1 N NaOH or 0.1 N  $\text{H}_2\text{SO}_4$ <sup>10</sup>. The former was used for the estimation of total hexoses, hexosamine, fucose<sup>11</sup> and proteins<sup>12</sup> and the latter was used for the estimation of sialic acid<sup>13</sup>. Results were expressed as  $\mu\text{g/ml}$  of gastric juice. Ratio between the total carbohydrate (sum of total hexoses, hexosamine, fucose and sialic acid) and protein (TC:P) was taken as an index of mucin activity.

#### RESULTS

The different extracts of *Indigofera longiracemosa* root, i.p. showed the tendency to reduce the ulcer index (UI) against restraint stress-induced gastric ulcers in rats. The mean ulcer index in the control group was  $23.1 \pm 3.8$ . Though reduction in ulcer index was observed with all the extracts studied (CE 16% NS; EE 18% NS), significant reduction was observed with BE, EAE and PE (33%, 25% and 25%,  $p < 0.05$ ) respectively. However, when the extracts were given p.o. at a higher dose of 100 mg/kg

**TABLE 1 : EFFECT OF INDIGOFERA LONGIRACEMOSA ON 4 h RESTRAINT STRESS INDUCED GASTRIC ULCER IN ALBINO RATS\***

| Treatment (100 mg/kg, p.o.<br>45 min before) | Ulcer Index |           |
|--|-------------|-----------|
|  | One day     | Four days |
| Control                                      | 26.9±4.82   | 45.4±4.61 |
| PE   | 28.4±2.46   | 18.5±2.11 |
| BE   | 16.9±3.12   | 14.9±2.1  |
| CE   | 27.5±12.5   | 21.9±4.92 |
| EAE  | 28.4±6.21   | 31.9±3.64 |
| EE   | 8.6±4.12    | 19.6±4.52 |

\*values are mean ± SE of six animals in each group ( $P \leq 0.05$ )

**TABLE 2 : EFFECT OF *INDIGOFERA LONGERACEMOSA* ON PYLORUS LIGATED GASTRIC ULCERS IN ALBINO RATS\***

| Treatment<br>(100 mg/kg, p.o.) | Ulcer/Stomach(a) | Severity/Stomach (b) | Ulcer Index (a+b) |
|--------------------------------|------------------|----------------------|-------------------|
| Control                        | 6.36±1.12        | 8.4±2.00             | 14.76±3.12        |
| PE                             | 1.43±0.42        | 2.00±0.51            | 3.43±0.93         |
| BE                             | 6.12±1.18        | 8.1±1.18             | 14.22±2.36        |
| CE                             | 6.27±3.92        | 8.3±2.63             | 14.57±6.55        |
| EAE                            | 6.19±2.79        | 8.1±2.21             | 14.29±5.00        |
| EE                             | 2.40±0.42        | 2.64±0.46            | 5.04±0.08         |

\*values are mean± SE of six animals in each group ( $P \leq 0.05$ )

45 min before, significant reduction in ulcer index was observed with EE. When treatment was continued for 4 days (3 days; 100 mg/kg p.o. x once daily + 4th dose 45 min before) significant reduction was observed with PE, BE and EE extracts while CE and EAE tend to decrease the ulcer index Table 1. The results of pylorus-ligated gastric ulcer methods are summarized in Table 2. The data suggests that PE and EE extracts significantly reduced the number of ulcers per stomach as well as severity of ulcers. PE was found to have no effects on gastric volume, acid, pepsin or mucin secretion while EE was found to decrease acid and pepsin concentration significantly Table 3. Further, only ethanol extract treatment was found to increase TC:P ratio which is a reliable index of mucin secretion Table 4.

### DISCUSSION

The extracts of the roots of this plant showed significant antiulcerogenic activity which was found with BE and EE extracts. The effects on offensive acid-pepsin secretion and defensive mucin-secretion were studied in 4 h pylorus-ligated rats. Results showed that PE extract had no or little effect on offensive acid-pepsin and defensive mucin secretion when expressed in terms of TC:P ratio. It is to be noted that gastroduodenal ulcers occur due to an imbalance between offensive acid-pepsin secretion and mucosal resistance. The antiulcer effect of PE extract in the present study could not be explained on the basis of its effect on acid-pepsin or mucin secretion. It is to be noted that stress plays an important role

**TABLE 3 : EFFECT OF *INDIGOFERA LONGERACEMOSA* ON GASTRIC SECRETION IN 4 h PYLORUS LIGATED ALBINO RATS\***

| Gastric Juice                                  | Control       | PE            | BE            | CE            | EAE           | EE            |
|--|---------------|---------------|---------------|---------------|---------------|---------------|
| Volume<br>(ml/100g body wt)                    | 1.39 ± 0.28   | 1.56±0.12     | 1.39±0.46     | 1.42±0.21     | 1.40±0.45     | 1.59±0.18     |
| Acid Conc.<br>( $\mu$ Eq/ml)                   | 136.50±7.20   | 108.60±9.20   | 135.60±6.40   | 135.80±7.60   | 134.10±5.60   | 95.20±9.10    |
| Acid output<br>( $\mu$ Eq/4hr)                 | 183±43.50     | 162.30±26.90  | 183.00±4.60   | 181.70±30.80  | 183.10±26.30  | 152.70±31.40  |
| Peptic Conc<br>( $\mu$ mol/ml)                 | 538.80±90.60  | 462.10±71.30  | 520.70±72.12  | 526.10±6.70   | 523.90±75.10  | 263.80±31.90  |
| Peptic output<br>( $\mu$ mol of tyrosine/4 hr) | 742.80±151.10 | 698.10±154.50 | 741.60±132.60 | 738.10±127.60 | 740.90±126.30 | 409.10±107.30 |

\*values are mean± SE of six animals in each group ( $P \leq 0.05$ )

**TABLE 4 : EFFECT OF *INDIGOFERA LONGERACEMOSA* ON MUCIN ACTIVITY AS  $\mu\text{G}/\text{ML}$  GASTRIC JUICE IN 4 h PYLORUS LIGATED ALBINO RATS\***

| Mucin activity as $\mu\text{g}/\text{ml}$ Gastric Juice | Control            | PE                 | BE                 | CE                 | EAE                 | EE                 |
|---|--------------------|--------------------|--------------------|--------------------|---------------------|--------------------|
| Total hexoses   | 246.20 $\pm$ 21.20 | 263.20 $\pm$ 29.80 | 247.10 $\pm$ 20.80 | 246.70 $\pm$ 21.8  | 246.90 $\pm$ 21.70  | 306.20 $\pm$ 31.40 |
| Hexosamine  | 159.60 $\pm$ 10.30 | 154.20 $\pm$ 12.50 | 159.20 $\pm$ 9.80  | 158.10 $\pm$ 9.9   | 158.30 $\pm$ 9.20   | 149.10 $\pm$ 29.30 |
| Sialic acid   | 56.20 $\pm$ 11.60  | 42.80 $\pm$ 12.80  | 56.00 $\pm$ 11.00  | 54.60 $\pm$ 7.80   | 54.80 $\pm$ 7.90    | 41.80 $\pm$ 4.70   |
| Fucose  | 123.60 $\pm$ 17.80 | 106.20 $\pm$ 21.30 | 123.40 $\pm$ 17.30 | 122.70 $\pm$ 16.30 | 122.10 $\pm$ 16.90  | 116.80 $\pm$ 21.20 |
| Total Carbohydrate (TC)                                 | 576.70 $\pm$ 41.20 | 568.30 $\pm$ 49.20 | 579.60 $\pm$ 32.10 | 573.40 $\pm$ 23.70 | 569.60 $\pm$ 43.50  | 605.60 $\pm$ 26.30 |
| Protein (P)   | 558.90 $\pm$ 52.50 | 512.00 $\pm$ 31.20 | 556.10 $\pm$ 48.70 | 556.70 $\pm$ 48.10 | 558.10 $\pm$ 51.180 | 478.00 $\pm$ 30.10 |
| T.C:P   | 1.06 $\pm$ 0.080   | 1.12 $\pm$ 0.60    | 1.06 $\pm$ 0.01    | 1.07 $\pm$ 0.06    | 1.06 $\pm$ 0.01     | 1.28 $\pm$ 0.04    |

\* values are mean  $\pm$  SE of six animals in each group ( $P \leq 0.05$ )

in the production of gastroduodenal ulceration and anti-stress drugs were found to be effective in stress-induced ulcers in rats<sup>14,15</sup>. Further, it is also possible that the antiulcer effect of the PE and EE may be due to their effects on various other mucosal defensive factors like prostaglandin accumulation, bicarbonate, mucosal glycoproteins, phospholipid layers, tight junctions, cell restitution, cell proliferation and mucosal blood flow. Of these different factors the role of endogenous prostaglandins are well known<sup>16</sup>. It also further necessitates further chemical and pharmacological studies in future.

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