Effect of standardized extract of *Ocimum sanctum* Linn. on gastric mucosal offensive and defensive factors

R K Goel, K Sairam, M Dorababu, T Prabha & Ch V Rao

Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221 005, India

Received 20 October 2004; revised 9 May 2005

The standardized methanolic extract of leaves of *O. sanctum* (OSE; eugenol content 5%) given in doses of 50-200 mg/kg, orally, twice daily for five days showed dose-dependent ulcer protective effect against cold restraint stress induced gastric ulcers. Optimal effective dose (100 mg/kg) of OSE showed significant ulcer protection against ethanol and pyloric ligation-induced gastric ulcers, but was ineffective against aspirin-induced ulcers. OSE significantly healed ulcers induced by 50% acetic acid after 5 and 10 days treatment. OSE (100mg/kg) significantly inhibited the offensive acid-pepsin secretion and lipid peroxidation and increased the gastric defensive factors like mucin secretion, cellular mucus, and life span of mucosal cells and had antioxidiant effect, but did not induce mucosal cell proliferation. The results indicate that the ulcer protective and healing effects of OSE may be due to its effects both on offensive and defensive mucosal factors.

**Keywords:** Antioxidants, Eugenol, Gastric ulcer protection and healing, Mucosal offensive and defensive factors, *Ocimum sanctum*.

*Ocimum sanctum* Linn. (Family- Labiatae) known as holy basil, is reported to possess several pharmacological and adaptogenic properties. Adaptogens are medicinal substances which cause a state of non-specifically induced resistance. This definition fits into the Ayurvedic concept of rasayanas. Rasayanas are drugs that promote longevity and prevent diseases by providing strength and immunity. Many rasayanas have also been reported to possess significant antiulcer activity, as stress is one of the important factors responsible for ulcerogenesis. Though antiulcerogenic activity of *O. sanctum* (OS) have been reported due to its anti-secretory activity, the role of defensive mucosal factors, whose imbalance with the offensive factors leads to gastric ulcers, needs exploration for better understanding of antiulcerogenic activity of this plant.

Eugenol forms the major active constituent of OS, even though other minor constituents like fixed oils and flavones have also been reported to have pharmacological activities. Eugenol is the major constituent responsible for anti-stress activity and significant anti-oxidant activity. Fixed oils of OS have been reported to possess antiulcer activity and anti-inflammatory activity. Most of the natural drugs possessing anti-stress and anti-oxidant activities have also been reported to have anti-ulcer activity and in accordance, the antiulcerogenic activity of juice of fresh leaves of OS was reported by Sairam *et al.* Recent report on OS also indicated its antiulcerogenic and ulcer healing properties.

In view of these information, the present study has been undertaken to evaluate the gastric ulcer protective and healing effects of methanolic extract of fresh leaves of OS, standardized to eugenol and also to elucidate its possible mechanism of action by studying its effect on various offensive factors like acid-pepsin and lipid peroxidation and defensive factors like mucin secretion, cellular mucus, life span of mucosal cells, cell proliferation and anti-oxidants in albino rats.

**Materials and Methods**

**Animals**—Inbred Charles-Foster (CF) albino rats (130-180 g), of either sex, obtained from the central animal house of the Institute used for the study, were kept in the departmental animal house at 26 ± 2°C, 44-56% RH, 10:14 hr L:D cycle for 1 week before and during the experiments. The animals were provided with standard rodent pellet diet (Hind Lever) and the food was withdrawn 18-24 hr before the experiment though water was allowed *ad libitum*. 'Principles of laboratory animal care'
Drug treatment—Fresh leaves of cultivated variety of OS were collected in the month of December from the Ayurvedic garden of the Institute and were identified with the standard sample preserved in the Department of Dravyaguna, Institute of Medical Sciences, Varanasi. The fresh leaves were size reduced and macerated with methanol for 7 days. The extract was filtered, vacuum dried and stored in a refrigerator until further use. The yield was 6.04%. The methanolic extract of OS (OSE) was quantified for the essential oil, eugenol by HPTLC using a CAMAG assembly (evaluation software © 1990 TLC system; Scanner II. V. 3.14/PC/CTS version), toluene: ethylacetate (93:7) as developing solvent, eugenol (Sigma, U.S.A.) as standard reference compound and quenching at 260 nm. The percentage of eugenol was 5.0%. The doses were fixed based on our earlier studies on the fresh juice of OS.

Treatment protocol—OSE, suspended in 1% carboxy methyl cellulose (CMC) in distilled water in doses of 50, 100 and 200 mg/kg were administered orally twice daily at 1000 hrs and 1600 hrs respectively for five days for dose response study. Further, the optimal effective dose of 100 mg/kg of OSE and standard ulcer protective drug sucralfate (SFT) in the dose of 250 mg/kg, twice daily for 5 days was used for ulcer protective studies, gastric secretion and mucosal studies, and up to 10 days for ulcer healing study. Control group of animals received suspension of 1% CMC in distilled water.

Anti-ulcer study—Antitranucular activity was studied by using cold restraint stress (CRS), aspirin (ASP), ethanol (EtOH) and pyloric ligation (PL) induced gastric ulcer (GU) models while, ulcer healing study was studied against acetic acid (AA)- induced chronic gastric ulcer (GU) models while, ulcer healing study was studied against acetic acid (AA) as well as sodium salicylate (NAS) induced chronic gastric ulcer (GU) models as described earlier. A dose dependant study for the selection of optimal ulcer protective dose was undertaken by using 50, 100, 200 mg/kg of OSE against CRS-induced gastric ulcer. Statistical significance was calculated by using unpaired Student’s t test.

Gastric secretion study—The gastric juice was collected 4 hr after PL and centrifuged for 5 min at 2000 rpm and the volume of the supernatant was expressed as ml/100g body weight. Total acid output was determined by titrating with 0.01 N NaOH, using phenolphthalein as indicator and is expressed as μEq/ml concentration or μEq/4hr as output. Peptic activity was determined using hemoglobin as substrate and has been expressed as μmol of tyrosine/ml as concentration or μmol of tyrosine/4 hr as output. Dissolved mucosubstances were estimated in the 90% alcoholic precipitate of the gastric juice. The precipitate, thus obtained was either dissolved in 1 ml of 0.1 N NaOH or 1 ml of 0.1 N H2SO4. The former was used for the estimation of protein, total hexoses, hexosamine and fucose, while the latter was used for the estimation of sialic acid. The results are expressed in μg/ml. The ratio of total carbohydrate (TC) (sum of total hexoses, hexosamine, fucose and sialic acid) to protein (P) has been taken as the index of mucin activity. DNA content was estimated and expressed as μg/ml gastric juice/100 g weight of rat.

Estimation of mucosal glycoproteins—Samples of gastric mucosal scraping were homogenized in distilled water and treated with 90% ethanol and were subjected for the estimation of carbohydrates and proteins using the methods described above for gastric juice contents.

Statistical analysis of data were done by using unpaired Student’s t test.

Cell proliferation

Estimation of DNA in gastric mucosa—Mucosal scraping was homogenized in 2.5 ml of ice cooled 0.6 N perchloric acid (PCA). DNA and protein were estimated. The concentration of DNA is expressed as μg DNA/mg protein.

Measurement of glandular weights of stomach—The weights of the whole stomach (rumen and glandular portion) and rumen were taken and the weight of the glandular portion was calculated. The weights of the glandular portions are expressed in mg/100 g body weight of animals. Statistical analysis was done by Student’s t test.

Estimation of free radical generation—OSE (100 mg/kg) was given orally, daily for 5 days and on day 6 of experiment, 1 hr prior to subjecting the animals to CRS. The animals were then sacrificed and the ulcer index was calculated as described earlier. The gastric fundic mucosal scrap was homogenized by using a Potter-Elvehjem glass homogenizer for 30 sec either in ice cold 0.9% saline (5%) for estimation of lipid peroxidation and superoxide dismutase or in M/150 phosphate buffer (2%) for estimation of catalase.

(a) Measurement of lipid peroxidation (LPO)
LPO levels were estimated in terms of malondialdehyde (MDA). To 0.4 ml of the homogenate was added 0.2 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of 20% acetic acid solution (adjusted to pH 3.5 with NaOH), and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid (TBA). The mixture was made up to 4 ml with distilled water, and then heated in an oil bath at 95°C for 60 min. After cooling with tap water, 1 ml of distilled water and 0.5 ml mixture of n-butanol and pyridine (15:1, v/v) were added and shaken vigorously. After centrifugation at 4000 rpm for 10 min the organic layer was taken and its absorbance at 532 nm was measured against blank containing 0.4 ml of distilled water in place of sample. 1,1,3,3-tetramethoxypropane was used as external standard and the level of LPO was expressed as nmol MDA/g wet tissue.

(b) Superoxide dismutase (SOD)

SOD was estimated as per Kakkar et al. The inhibition of reduction of nitro blue tetrazolium (NBT) to blue colored formazan in presence of phenazine metha sulpha te (PMS) and NADH was measured at 560 nm using n-butanol as blank. Briefly, to 0.4 ml of the homogenate was added 1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 ml of 186 nM of PMS, 0.3 ml of 300 nM NBT and 0.8 ml of distilled water was added to make up the volume up to 3 ml. The reaction was started by the addition of 0.2 ml of NADH (780 nM). It was incubated at 30°C for 60 sec. The reaction was then stopped by the addition of 1 ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 ml of n-butanol. The mixture was allowed to stand for 10 min, centrifuged and butanol layer was taken out. Colour intensity of the chromogen in the butanol was measured against butanol at 560 nm using Spectronic-20 spectrophotometer. A system devoid of enzyme served as control. One unit of enzyme activity is defined as enzyme concentration required to decrease the rate of reaction by 50% in one min under the assay conditions. The results were expressed as units (U) of CAT activity/g wet tissue.

(c) Catalase (CAT)

Gastric mucosal scrap was homogenized (2%) in M/150 phosphate buffer at 1°C-4°C and centrifuged. Sediment was stirred in cold phosphate buffer and was allowed to stand in the cold with occasional shaking. The extraction was repeated twice and the combined supernatant was used for the assay. The supernatant was diluted ten times with distilled water and 0.04 ml of the diluted supernatant was taken for the assay. Decomposition of H2O2 in presence of catalase was followed at 240 nm. Results were expressed as units (U) of CAT activity/g wet tissue.

Results

OSE (50-200 mg/kg, po, twice daily for 5 days) showed dose-dependant ulcer protective effect against 2 hr cold restraint stress induced gastric ulcers. Further, optimal effective dose of OSE showed significant ulcer protective effect against ethanol and 4 hr pyloric ligation induced gastric ulcers but tended to decrease ulcer index against aspirin-induced ulcers (Table 1). OSE showed significant ulcer healing against 50% acetic acid induced gastric ulcers after 5 and 10 days treatment (Table 2).

OSE in the dose of 100 mg/kg significantly decreased the gastric juice volume, acid and peptic output (Table 3). OSE in the same dose showed a

| Oral treatment (mg/kg, bd for 5 days) | Ulcer index | Protection (%) |
|-------------------------------------|-------------|----------------|                |
| **CRS-induced ulcers**              |             |                |
| Control                             | 17.8 ± 3.2  | 44.9           |
| OSE                                 | 9.8 ± 2.2   | 61.2           |
| SFT                                 | 6.9 ± 2.3*  | 72.3           |
| **EtOH-induced ulcers (mm²/rat)**   |             |                |
| Control                             | 26.9 ± 4.3  |                |
| OSE                                 | 8.9 ± 2.9*  | 67.1           |
| SFT                                 | 7.5 ± 2.5*  |                |
| **ASP-induced ulcers**              |             |                |
| Control                             | 19.3 ± 3.2  |                |
| OSE                                 | 12.8 ± 2.9  | 33.7           |
| SFT                                 | 5.2 ± 1.2*  | 73.3           |
| **PL-induced ulcers**               |             |                |
| Control                             | 13.3 ± 3.7  |                |
| OSE                                 | 7.2 ± 1.9*  | 71.4           |
| SFT                                 | 2.3 ± 1.0   | 76.7           |

P values: *< 0.05; **< 0.01; ***< 0.001. Statistical analysis was done by unpaired Student’s t test.
tendency to increase the total carbohydrates (TC, which is the sum of the individual carbohydrates, like total hexoses, hexosamine, fucose and sialic acid) and significantly decreased the protein (P) content of the gastric juice (Table 4), leading to significant increase in the TC:P ratio in the gastric juice, the marker for mucin secretion. It also significantly decreased cell shedding as evidenced from the decrease in DNA content of the gastric juice, which is indicative of increase in life span of cells (Table 3). Again OSE in the above dose significantly increased the TC:P ratio in the gastric mucosa with significant increase in sialic acid indicating enhancement of mucosal glycoproteins, the source of dissolved mucin in the gastric juice (Table 4). OSE did not show any effect on cell proliferation as shown by no changes in μg DNA/mg protein or weight of glandular portion of the stomach (Table 5). Cold restraint stress significantly caused gastric ulcers with increased lipid peroxidation with concomitant increase in superoxide dismutase (SOD) and decrease in catalase (CAT) levels compared to control unstressed group. OSE treatment (100 mg/kg) decreased the ulcer index significantly with decrease in LPO and SOD and increase in CAT levels near to the control unstressed values (Table 6).

### Table 2—Effect of OSE on 50% acetic acid-induced (healing) gastric ulcers in rats

<table>
<thead>
<tr>
<th>Oral treatment (mg/kg, bd)</th>
<th>5 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ulcer index</td>
<td>Incidence of perforations (%)</td>
</tr>
<tr>
<td>Control</td>
<td>20.2 ± 3.5</td>
<td>80</td>
</tr>
<tr>
<td>OSE 100</td>
<td>7.0 ± 1.3</td>
<td>0.0</td>
</tr>
</tbody>
</table>

P values: *p < 0.05; *p < 0.01. Statistical analysis was done by unpaired Student’s t test.

### Table 3—Effect of OSE and SFT on gastric juice volume, acid, pepsin and DNA content

<table>
<thead>
<tr>
<th>Oral treatment (mg/kg, bd for 5 days)</th>
<th>Volume (ml /100g)</th>
<th>Acid output (μEq/4hr)</th>
<th>Pepsin output (μmol/4 hr)</th>
<th>DNA (μg/ml/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.39 ± 0.35</td>
<td>352.8 ± 47.6</td>
<td>588.5 ± 55.6</td>
<td>17.9 ± 1.3</td>
</tr>
<tr>
<td>OSE 100</td>
<td>1.41 ± 0.16*</td>
<td>147.1 ± 23.1*</td>
<td>337.4 ± 67.1*</td>
<td>13.0 ± 1.0*</td>
</tr>
<tr>
<td>SFT 250</td>
<td>1.93 ± 0.18</td>
<td>297.8 ± 32.4</td>
<td>229.7 ± 11.4*</td>
<td>11.1 ± 1.1*</td>
</tr>
</tbody>
</table>

P values: *p < 0.05; *p < 0.01. Statistical analysis was done by unpaired Student’s t test.

**Discussion**

Extract of *O. sanctum* in the dose of 100 mg/kg showed ulcer protective effect in acute GU induced by ethanol, cold restraint stress and pyloric ligation and healed chronic GU induced by acetic acid though its protective effect against aspirin induced GU was less marked. As ulcers are essentially due to imbalances between offensive and defensive mucosal factors, the activity of OSE in the above experimental models can be explained based on these factors.

In PL-induced ulcers, OSE in the dose of 100 mg/kg significantly decreased the gastric juice volume, acid and pepsin output indicating decrease in offensive acid and pepsin secretion. On the defensive factors, OSE significantly increased the TC: P ratio in the gastric juice, which is taken as a reliable marker for mucin secretion. This was due to the tendency to increase dissolved mucopolysaccharides and decrease in protein content of the gastric juice. Decrease in protein content of the gastric juice indicates increased mucosal resistance as the increase in proteins in the gastric juice during ulceration is mostly from shedded mucosal cells and bleeding. This is further substantiated by decrease in DNA content of the gastric juice, which is a reliable index for cell exfoliation. Decrease in DNA content indicates increase in life span of mucosal cells. OSE also significantly increased the TC:P ratio of the gastric mucosa indicating increase in cellular mucus. This increase was due to increase in individual carbohydrates specifically sialic acid, which was increased significantly. Mucus is an important pre­epithelial defensive factor, which also serves as the first line of defense against ulcerogens. OSE did not show any effect on cell proliferation as seen from the absence of any significant changes in μg DNA/mg protein in the gastric mucosa and glandular weights of the stomach. Hence protection afforded by OSE should involve the process of restitution rather than cell proliferation. PL-induced ulcers are caused
predominantly due to enhanced acid-pepsin secretion leading to breakdown of mucosal barrier\textsuperscript{12}. Hence the decrease in acid pepsin secretion and increase in the mucosal protective factors may be involved in the protection afforded by OSE in PL-induced ulcers.

OSE also offered significant protection against gastric ulcers induced by ethanol and cold restraint stress. The mechanisms of ulcerations induced by ethanol and cold restraint stress are different from one another. Ulcerations by ethanol are caused due to perturbations of superficial mucosal cells, notably the mucosal mast cell leading to release of vasoactive mediators including histamine, thus causing damage to gastric mucosa\textsuperscript{35}. As ethanol induced ulcers are independent of luminal acid\textsuperscript{36}, the increase in mucosal protective factors should then be the major factors responsible for ulcer protective property of OSE rather than the decrease in acid secretion. CRS-induced ulcers are due to both psychological and physiological factors\textsuperscript{37}. The role of acid is questionable and decrease in mucin secretion has been reported during stress-induced ulcers\textsuperscript{38}. Hence, protection afforded by OSE in stress-induced ulcers may principally involve the mucosal defensive factors, which were significantly increased.

However, OSE (100 mg/kg) did not offer significant protection against aspirin-induced ulcers. This may be due to various reasons as many factors, both topical and systemic are reported to be involved in ulcers induced by aspirin. Aspirin is reported to interfere with PG synthesis, increase acid secretion and back diffusion of H\textsuperscript{+} ions and thus leading to breaking up of mucosal barrier\textsuperscript{39,40}. OSE significantly decreased acid secretion and increased mucin secretion, which should be capable of inhibiting back diffusion of H\textsuperscript{+} ions. However a tendency to decrease aspirin-induced GU with OSE was observed. This may be due to the difference in the dose and amount of active principle/s present in the test extracts of leaves of \textit{O. sanctum} used or the amount of aspirin.
used to produce GU. Most of the workers have used oral aspirin either in the dose of 100 mg/kg or 150 mg/kg to induce GU or have studied the ulcer protective effect of aqueous extract, fixed oil or ethanolic extract of leaves of O. sanctum against aspirin-induced GU. Recently, a significant ulcer protective effect of dried juice of leaves of OS has been observed against aspirin-induced GU though at a higher dose of 500 mg/kg (unpublished data). This could be due to biological variation shown by test substances of herbal origin due to change in soil, weather, temperature etc that could lead to change in the amount of active principle/s present in them.

OSE not only protected gastric ulcers due to different ulcerogens but also significantly healed ulcers induced by acetic acid both after 5 and 10 days of treatment. Acetic acid induced ulcers are thought to be mostly due to increase in gastric secretion and volume of acid output and subsequent pyloric obstruction. Hence, the healing effect may be predominantly due to decrease in acid secretion supported by augmented increase in mucosal defensive factors.

Free radicals, a common factor in the aetiopathogenesis of ulcers by different ulcer models are thought to play a decisive role in stress-induced ulcers and as OSE has been reported to have adaptogenic property, the cold restraint stress model was chosen to study the effect of OSE on free radicals. OSE significantly reduced lipid peroxidation in stressed rat gastric mucosa. OS has been reported to possess significant antioxidant properties in rat brain regions. During acute stress LPO and SOD were significantly increased while CAT level was significantly decreased, while CAT level was significantly increased. This could be due to biological variation shown by test substances of herbal origin which increase non-specific resistance, cAMP etc. would provide more insight into the activity of OSE.

Acknowledgement

RKG is thankful to Indian Council of Medical Research for grant-in-aid. The authors are thankful to Indian Herbs Ltd, Saharanpur, India, for technical facilities for chemical standardization of the extract.

References


Okabe S & Pfeiffer C J, Chronicity of acetic acid ulcer in the rat stomach, Dig Dis, 7 (1972) 619.


