Evaluation Of Antiulcer Activity Of Ocimum Sanctum And Its Mechanism In Animal Models

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Abstract

The present study was conducted with the aim of evaluating the antiulcer activity of ocimum sanctum and to explore its mechanism of action. In this study, ocimum sanctum leaf extract was investigated for its activity against aspirin induced gastric ulcers in albino rats. The drug showed significant activity in the doses of 100 mg/kg and 200 mg/kg. To explore the mechanism, its activity was observed on oxytocin induced contractions in rat uterus. It exhibited a significant potentiation of the height of contraction induced by oxytocin. The maximum potentiation was observed in the dose of 0.5 mg/ml which may be due to enhanced synthesis of prostaglandins since they are mediators of uterine contractions. This indicates a cytoprotective mechanism for the antiulcer activity of ocimum sanctum.

INTRODUCTION

Ulcerations in the gastrointestinal mucosa are excavated defects that results when epithelial cells succumb to the caustic effects of acid and pepsin.

The drugs currently available for peptic ulcer may be associated with adverse effects, patient non-compliance, drug resistance and disease relapse. Some of the drugs may be expensive which may again lead to patient non-compliance and recurrence of peptic ulcer disease 1

Therefore, for this research project, a herbal drug (plant), ocimum sanctum was selected to study its effect on peptic ulceration as well as the mechanism of action in some animal models.

Ocimum sanctum is an erect hairy annual herb, found throughout India upto an altitude of 1800m in the Himalayas 2. It has been claimed to be useful as a diaphoretic, diuretic, expectorant, febrifuge and vermifuge. It is also useful in cardiopathy, asthma, otalgia, hepatopathy, vomiting, genitourinary disorders and skin diseases 3.

Though the antiulcer activity of ocimum sanctum has been evaluated 4,5,6,7 studies in this regard are limited and the mechanism of action has not been explored.

Therefore, it was decided to evaluate the antiulcer activity of ocimum sanctum and to explore its mechanism of action in some animal models.

MATERIALS AND METHODS

ANIMALS

Albino rats: Healthy adult albino rats of either sex, weighing between 120-180 gm were used to induce gastric ulcers with aspirin. Virgin female rats were used to evaluate the effect of test drug on oxytocin induced uterine contractions.

They were caged under standard conditions and were allowed to acclimatise to their surroundings for one week before subjecting them to experimentation. Prior to experimentation permission was taken from institutional ethical committee.

DRUGS AND CHEMICALS

Test drug: Fresh leaves of the plant ocimum sanctum were collected during the month of April from a local area in Sevagram and were shade dried and powdered. A hydro alcoholic (70% V/V) extract of shade dried fresh leaves of ocimum sanctum was prepared. This extract was again shade dried and was used to prepare an aqueous solution in desired concentration just before use every time. The same extract was used for all the experiments.

Acetyl salicylic acid or Aspirin (Salg pharma): A 2% suspension in gum acacia was prepared in distilled water and diluted to get the desired concentration.

Ranitidine (Ranbaxy, India): It was diluted in distilled water to get the desired concentration.
Oxytocin (Novartis, India): It was prepared in distilled water just before use.

Oestradiol valerate (German remedies, India): Solution was prepared in desired concentration before use.

De Jalon’s Physiologic salt solution for rat uterus: NaCl - 9 gm/L, KCl - 0.42 gm/L, CaCl$_2$ - 0.06 gm/L, NaHCO$_3$ - 0.5 gm/L and Glucose - 0.5 gm added to 1 litre of distilled water

METHOD FOR ANTIULCER ACTIVITY

Before experimentation or induction of ulcers, the animals were kept fasting for 24 hours but were allowed free access to water. They were caged in wire-meshed cages having a plate at the base to avoid coprophagy.

The animals were divided randomly into five groups of six animals each. Group I received distilled water (10ml/kg) orally and served as control whereas group II, III and IV were administered the test drug (ocimum sanctum) in doses of 50 mg/kg, 100 mg/kg and 200 mg/kg respectively. The animals in group V were administered ranitidine (10 mg/kg) orally which served as standard for comparison. Gastric ulcers were induced by oral administration of a single dose of aspirin (200 mg/kg), 30 minutes after drug treatment. The rats were sacrificed 5 hours later with an over dosage of ether anaesthesia. The abdomen was dissected, stomach was taken out and examined for ulcers. Ulcer index was calculated.

EFFECT OF OCIMUM SANCTUM EXTRACT ON OXYTOCIN INDUCED CONTRACTIONS IN RAT UTERUS

This experiment was done to study the effect of hydroalcoholic leaf extract of ocimum sanctum on oxytocin induced contractions in rat uterus. Adult female virgin rats were primed with a single dose of oestradiol (100 mg/kg, subcutaneously) 24 hours before experiment to bring about the estrus phase. The animals were randomly divided into five groups with six animals in each group. Group I received only oxytocin (0.01 IU/ml) and served as control for comparison. Group II, III, IV and V received oxytocin (0.01) and ocimum sanctum extract in graded doses of 0.1 mg/ml, 0.2 mg/ml, 0.5 mg/ml and 1 mg/ml respectively.

The preparation was mounted in a 20 ml organ bath perfused with Dejalon’s solution which was aerated with oxygen (95%) and carbon-dioxide (5%). The tissue was equilibrated for 60 minutes till spontaneous contraction were abolished during which the physiological saline solution was replaced every 10 minutes and temperature of 30-32°C was maintained.

The responses of uterine tissue were recorded on a kymograph. The percentage potentiation of original response was calculated indicating potentiation of prostaglandin biosynthesis.

STATISTICAL ANALYSIS

Data were analysed by student’s ‘t’ test and expressed as mean + SEM. P values less than 0.05 were considered significant.

OBSERVATIONS AND RESULTS

EFFECT ON ASPIRIN INDUCED GASTRIC ULCERS: (TABLE-1)

Aspirin (200mg/kg, P.O) produce ulcers in 100% animals in the distilled water (10ml/kg, orally) treated control group. Ocimum sanctum extract was administered orally in graded doses of 50 mg/kg, 100mg/kg and 200 mg/kg respectively. In the dose of 50 mg/kg, it did not offer significant ulcer protection. In the dose of 100 mg/kg, it exhibited protection against ulcers in 33.4% animals as well as a significant (p<0.05) decrease in the number of ulcers and ulcer index. In the dose of 200 mg/kg, it protected 50% animals against ulcers and reduced the number of ulcers and ulcer index significantly (p<0.01 and p<0.05).

Ranitidine (10 mg/kg, orally), as a standard drug for comparison protected 83.34% animals against ulcers. It also decreased the number of ulcers and the ulcer index significantly (p<0.001) in comparison to the control group.
Evaluation Of Antiulcer Activity Of Ocimum Sanctum And Its Mechanism In Animal Models

Figure 1
Table 1: Effect of ocimum sanctum extract (OSE) on aspirin induced gastric ulcers in albino rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Dose</th>
<th>Percent Animals Showing Ulcers</th>
<th>Number of Ulcers (Mean ± SEM)</th>
<th>Ulcer Index (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>D.W.</td>
<td>100%</td>
<td>100</td>
<td>4.16 ± 0.20</td>
<td>2.83 ± 0.40</td>
</tr>
<tr>
<td>II</td>
<td>OSE</td>
<td>50%</td>
<td>100</td>
<td>4.00 ± 0.25</td>
<td>2.50 ± 0.50</td>
</tr>
<tr>
<td>III</td>
<td>OSE</td>
<td>100%</td>
<td>66.66</td>
<td>1.66 ± 0.76*</td>
<td>1.00 ± 0.44*</td>
</tr>
<tr>
<td>IV</td>
<td>OSE</td>
<td>100%</td>
<td>50</td>
<td>1.33 ± 0.61**</td>
<td>0.83 ± 0.47**</td>
</tr>
<tr>
<td>V</td>
<td>RAN</td>
<td>100%</td>
<td>16.66</td>
<td>0.16 ± 0.13***</td>
<td>0.10 ± 0.10***</td>
</tr>
</tbody>
</table>

*p = Number of animals (5 in each group),
P.O = Per orally,
SEM = Standard error of mean
RAN = Ranitidine

Figure 2
Table-2: Effect of ocimum sanctum extract on oxytocin induced contractions in rat uterus

<table>
<thead>
<tr>
<th>Group (mg/ml)</th>
<th>Drug</th>
<th>Concentration (mg/ml)</th>
<th>Height of contractions (cm) (Mean ± SEM)</th>
<th>Percent Potentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Oxytocin</td>
<td>0.01 IU/ml</td>
<td>3.73 ± 0.30</td>
<td>NYA</td>
</tr>
<tr>
<td>II</td>
<td>Ocimum sanctum extract + Oxytocin</td>
<td>0.1 + 0.01 IU/ml</td>
<td>4.68 ± 0.04**</td>
<td>25.46</td>
</tr>
<tr>
<td>III</td>
<td>Ocimum sanctum extract + Oxytocin</td>
<td>0.2 + 0.01 IU/ml</td>
<td>6.2 ± 0.08**</td>
<td>66.31</td>
</tr>
<tr>
<td>IV</td>
<td>Ocimum sanctum extract + Oxytocin</td>
<td>0.5 + 0.01 IU/ml</td>
<td>7.1 ± 0.03***</td>
<td>99.24</td>
</tr>
<tr>
<td>V</td>
<td>Ocimum sanctum extract + Oxytocin</td>
<td>1 + 0.01 IU/ml</td>
<td>7.1 ± 0.02***</td>
<td>99.24</td>
</tr>
</tbody>
</table>

*p = Number of animals (5 in each group),
P.O = Per orally,
SEM = Standard error of mean
RAN = Ranitidine

DISCUSSION
In the present study, ocimum sanctum has been investigated for its antiulcer activity against aspirin induced gastric ulcer model in albino rats. Ocimum sanctum showed significant ulcer protective effect as evident from the decrease in the percent incidence, number and severity of the ulcers in aspirin induced gastric ulcers in albino rats. To explore the mechanism of antiulcer activity of ocimum sanctum, its effect was studied on oxytocin induced contractions in rat uterus. In this study, ocimum sanctum extract increased oxytocin induced contractions in rat uterus which may be due to its increasing local prostaglandin levels.

Prostaglandins play a central role in gastric epithelial defence/repair. The gastric mucosa contains abundant levels of prostaglandins. These regulate the release of mucosal bicarbonate and mucus, inhibit parietal cell secretion, and are important in maintaining mucosal blood flow and epithelial cell restitution. It therefore follows that interruption of prostaglandin synthesis can impair mucosal defence and repair, thus facilitating mucosal injury. Aspirin and many nonsteroidal antiinflammatory drugs (NSAIDs) are weak acids that remain in nonionised lipophilic form when found within the acid environment of stomach. Under these conditions, NSAIDs migrate across lipid membranes of epithelial cells, leading to cell injury once trapped intracellularly in an ionised form. Topical NSAIDs can also alter the surface mucus layer, permitting back diffusion of H+ and pepsin, leading to further epithelial damage. Cyclooxygenase (COX) is a key enzyme that controls the dependent manner. The maximum potentiation of height of contraction by ocimum sanctum extract was observed with 0.5 mg/ml.

EFFECT ON OXYTOCIN INDUCED CONTRACTIONS IN RAT UTERUS: (TABLE 2 & FIG-1)
Ocimum sanctum extract in different doses (0.1, 0.2, 0.5 and 1 mg/ml) caused significant potentiation of the height of contraction induced by oxytocin (0.01 IU/ml) in a dose dependent manner. The maximum potentiation of height of contraction by ocimum sanctum extract was observed with 0.5 mg/ml.

Figure 3
Fig- 1: Effect of ocimum sanctum extract (OSE) on oxytocin induced contractions in rat uterus OXY = Oxytocin; OSE= Ocimum sanctum extract; R= Recovery
rate limiting step in prostaglandin synthesis. It is present in two isoforms (COX-1, COX-2). COX-1 is expressed constitutively and plays an important role in maintaining the integrity of renal function, platelet aggregation and gastrointestinal mucosal integrity. In contrast, COX-2 is inducible by inflammatory stimuli, and is expressed in macrophages, leucocytes, fibroblasts and synovial cells. Gastrointestinal mucosal ulceration is related to inhibition of COX-1 isoform.\(^{12}\)

Aspirin induced gastric ulcers are attributed to inhibition of biosynthesis of cytoprotective prostaglandins (PGE\(_2\) and PGI\(_2\)) by inhibition of cyclooxygenase pathway of arachidonic acid metabolism, resulting in overproduction of leukotrienes and other products of lipoxygenase pathway.\(^{14}\) Hence, the antiulcer activity of ocimum sanctum extract against aspirin induced gastric ulcers in our study could be due to its cytoprotective activity by opposing cyclooxygenase pathway inhibition by aspirin. Since, it is possible that enhanced generation of lipoxygenase products contributes to ulcerogenicity, the ulcer protective action of ocimum sanctum against aspirin induced ulcers could also be due to its lipoxygenase inhibitory effect. It could also be due to enhanced prostaglandin synthesis locally since ocimum sanctum extract increased oxytocin induced contractions in rat uterus in vitro. Prostaglandins are also known to increase oxytocin induced uterine contractions.\(^{13}\) The antiulcer activity of ranitidine against aspirin induced gastric ulcers can be attributed to the fact that H\(_2\) receptor antagonists prevent nonsteroidal antiinflammatory drugs induced ulcers. It is therefore inferred that ocimum sanctum may be a useful antiulcer agent as shown by our experimental work. In view of its significant antiulcer activity against different models of ulcers and different possible mechanisms, further studies as well as clinical trials will be required before establishing it as an antiulcer agent for human use.

### References

2. Satyavati GV, Gupta AK and Tandon N. Ocimum sanctum Linn (Tulsi). In: Medicinal Plants of India. Published by ICMR, New Delhi. 1987 ; 27 : 574-575.
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