Evaluation of ulcer protective effects of *Ocimum sanctum* and *Zingiber officinalis* as herbo-formulation for aspirin induced ulcerations in Albino rats

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ABSTRACT

Peptic ulcer disease is a serious GIT disorder that requires well-targeted strategy. A number of drugs including proton pump inhibitors (PPIs) & histamine H$_2$ receptor antagonists are available for treatment of peptic ulcer. Clinical evaluations of their drugs has shown incidence of side effect. The present study includes evaluation of herbo-formulation consisting of *Ocimum sanctum* and *Zingiber officinalis* as ulcer protective in albino wistar rats. The alcoholic extract of ocimum, ginger (100 mg), combination of both (75mg/kg, 100mg/kg), ranitidine 10mg/kg & CMC 1% per oral were used. After ulcer induction with aspirin (200 mg/kg p.o.) the number, scoring area & finally Ulcer index were accessed. We found that extract decreases the incidence of ulcers. The herb oformulation showed dose-dependent ulcer protective effect (62.00-67.20%) in aspirin induced ulcer. Ocimum and Zingiber extract reduced the ulcer index with significant percent protection (60.00% and 34.40% respectively). The herboformulation at a dose of 100mg/kg found to be effective having percent protection (67.20%) as compared to the dose 75 mg/kg (62.00%) & significantly reduce free, total acidity & pepsin activity.

Key words: peptic ulcer disease, *Ocimum sanctum*, *Zingiber officinalis*, aspirin.

INTRODUCTION

Peptic ulcer disease (PUD) encompassing gastric and duodenal ulcer is the most prevalent gastrointestinal disorder [1]. The pathophysiology of PUD involves an imbalance between offensive (acid, pepsin, and *H. pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors) [2]. An estimated 15,000 deaths occur each year as a consequence of PUD. PUD is common in India, the Indian pharmaceutical industry have 6.2 billion rupees drugs share of antacids and antiulcer drugs and occupy 4.3% of the market share.
Today, there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and the second re-enforcing gastric mucosal protection [1, 2].

Recently, there has been a rapid progress in the understanding of the pathogenesis of peptic ulcer. Most of the studies focus on newer and better drug therapy. These have been made possible largely by the availability of the proton pump inhibitors, histamine receptor blockers, drugs affecting the mucosal barrier and prostaglandin analog [3]. However, the clinical evaluation of these drugs showed development of tolerance, incidences of relapse and side effects that make their efficacy arguable. It is the rationale for the development of new antulcer drugs, which includes herbal drugs. Indian medicinal plants and their derivatives are an invaluable source of therapeutic agents to treat various disorders including PUD [4]. Indigenous drug possessing fewer side effects is the major thrust area of the present day research, aiming for a better and safer approach for the management of PUD.

*Ocimum sanctum* (OS) is a sacred plant that belongs to the family *Labiate*. It contains number of chemical constituents that interact in a complex way to produce their pharmacodynamic responses. OS is highly effective in a wide spectrum of diseases and reported to possess anthelmintic, antiseptic antirheumatic, anticarcinogenic, antistress and antibacterial properties. The antiulcer activity of hydroalcoholic extract [5], hydrodistilled leaves extract [6] and the fixed oil [7] of *Ocimum sanctum* has been reported.

Today, pharmacopoeias of many different countries list ginger extract for various digestive diseases [8]. Aromatic, spasmyloytic, carminative and absorbent properties of ginger are probably responsible for the therapeutic applications in digestive tract ailments [9]. Ginger is a rhizome of *Zingiber officinale* commonly used in the treatment of gastrointestinal (GI) disorders including: dyspepsia, nausea and diarrhea. Several studies have shown that ginger extract, essential oils and glycolipids possess a number of pharmacological actions, which at least in part for some of them anti-ulcerogenic or ulcer preventive efficacy may be suggested [10].

So, this study was designed to evaluate and compare the ulcer protective activity of *Ocimum sanctum* and *Zingiber officinalis* in herbo-formulation.

**MATERIALS AND METHODS**

**Plant material and extraction**

Plant materials were procured from local market of Shirpur and were authenticated from Dr. S.R. Kshirsagar (Department of Botany, Shri. Shivaji Vidya Prasarak Sanstha’s Late Karamveer Dr. P.R. Ghogrey Science College, Dhule M.S. India, Voucher no. 79, 47.

Dried powdered leaves of *Ocimum sanctum* (200g) were copulated in glass percolator with ethanol (1:2 w/v) and were allowed to stand at room temperature for about 16 hr. The percolate was collected. This process of extraction was repeated four times. The percentage yield obtained was 8.23% w/w. Dried and coarsely powdered rhizomes of *Zingiber officinalis* (400g) were macerated with 1000 ml of ethanol: water (7:3 v/v) for 72 hours with continuous shaking. The extract was filtered and evaporated in a rotatory evaporator under reduced pressure until dryness.

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Evaporation & removal of the solvent yielded a semisolid mass. The percentage yield obtained was 14.8 % (w/w). Both the extracts were suspended in 1% CMC prior to dosing.

**Animals**
36 male albino wistar rats (250-280g) were obtained from the Animal House facility, School of Pharmacy and Technology Management, Shirpur. Animals were housed in group of 2 in polypropylene cages containing autoclaved rice husk. Animals were provided with rodent pellet feed (Amrut Laboratory Animal Feed, Pranav Agro Ltd, Pune) and water ad libitum. Animals were maintained in 12 hr. light-dark cycle (Light: - 06:00-18:00. Dark: - 18:00-06:00), temperature 22±2°C. The rats were left for 48 hours for acclimatization prior to the commencement of the experiment, and were randomly assigned to 6 groups (n = 6).

**Experimental procedure**
During the experiment the control group received vehicle (1% CMC), four test groups received OS-Ex (*Ocimum sanctum* extract 100mg/kg P.O.), ZO-Ex (*Zingiber officinalis* extract 100mg/kg P.O.), Co-Ex 100 (Combined extract 1:1 w/w 100mg/kg P.O.), Co-Ex 75 (combined extract 1:1 w/w 075mg/kg P.O.), and Standard (ranitidine 10mg/kg P.O.) for three days. On third day aspirin was administered at a dose of 200 mg/kg P.O. after 30 min of extract and ranitidine treatment [11]. The food was withdrawn 18 hours prior to the aspirin treatment. During experiment, to avoid corpophagy and fighting, the rats were kept singly in wire- bottomed cages. After 5 h, all animals were sacrificed by cervical dislocation under carbon dioxide and oxygen (75% CO₂ + 25% O₂) anesthesia and ulcer scoring was done. The ethical guidelines for animal protection rights were followed.

The number of ulcers was counted and scoring was undertaken according to the reported method [12]. The scores were, 0: no ulcer, 1: superficial ulcer, 2: deep ulcer, and 3: perforation. Ulcer area was assessed by using 3M® scaled surgical transpore tapes, which was fixed on a light and transparent sheet. Each cell on the tape was 1 mm² in area, so the number of cells was counted and the ulcer area was measured for each animal [13]. Ulcer index was measured using formula, $UI = UN + US + UA \times 10^{-1}$, where $UI = ulcer$ index, $UN = ulcer$ number, $US = ulcer$ score, and $UA = ulcer$ surface area for each duodenum [14]. The percentage protection was calculated with the formula $%\ protection = [(C-T) / C] \times 100$, where $C = ulcer$ Index in control group, $T = ulcer$ index in treated group.

**Estimation of gastric pepsin:**
0.2ml of centrifuged gastric juice plus 3ml of albumin 3% for each rat test and blank. Then 10ml of 6% trichloracetic acid added to blank to stop enzyme activity. Both blank and test tubes incubated in water bath with temperature 37°C for 30 minutes .Then 10ml of trichloracetic acid added to test tubes, shaken well and filtered. Proteolytic activity determined spectrometrically by optical density measured at 280 wavelength [15]. A standard curve was constructed from which pepsin content of gastric secretion was determined by extrapolation.

**Statistical analysis**
All values are expressed as Mean ± S.E.M. Obtained data was analyzed by One-way ANOVA followed by Dunett’s multiple comparison tests using Graph Pad prism v5.03
RESULTS

In the control group, both duodenal and gastric lesions were observed mainly in the proximal segments (figure 2). A significant reduction (p<0.001) in different parameters it appears that pre-treatment with ranitidine (10mg/kg) reduced duodenal mucosal damage. The ethanolic extract of Ocimum sanctum at a dose of 100mg/kg, shows significant antiulcer effect, while the ethanol extract of Zingiber officinalis (100mg/kg) shows less ulcer protection. The 1:1 w/w combination of the two extracts has shown the dose dependant synergistic ulcer protective effect meaningful and comparable with ranitidine (table 1).

Table 1: Effect of various drugs on aspirin induced ulcer in rat

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number</th>
<th>Scoring</th>
<th>Area</th>
<th>Ulcer index</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.27±0.51</td>
<td>2.41±0.31</td>
<td>68.29±5.94</td>
<td>14.6±0.57</td>
<td>00.00 %</td>
</tr>
<tr>
<td>Co-Ex (075mg/kg)</td>
<td>3.06±0.43</td>
<td>1.07±0.28</td>
<td>14.67±3.62</td>
<td>5.54±0.43</td>
<td>62.00 %</td>
</tr>
<tr>
<td>Co-Ex (100mg/kg)</td>
<td>3.19±0.60</td>
<td>1.26±0.34</td>
<td>18.97±4.16</td>
<td>4.79±0.41</td>
<td>67.02 %</td>
</tr>
<tr>
<td>OS Ex (100mg/kg)</td>
<td>3.51±0.47</td>
<td>1.77±0.24</td>
<td>14.24±3.97</td>
<td>5.84±0.59</td>
<td>60.00 %</td>
</tr>
<tr>
<td>ZO Ex (100mg/kg)</td>
<td>4.13±0.62</td>
<td>2.03±0.29</td>
<td>31.94±4.75</td>
<td>9.58±0.63</td>
<td>34.40 %</td>
</tr>
<tr>
<td>Standard</td>
<td>1.77±0.48</td>
<td>0.79±0.32</td>
<td>10.51±5.42</td>
<td>3.53±0.41</td>
<td>76.00 %</td>
</tr>
</tbody>
</table>

Data is represented as MEAN ± S.E.M (n=6), analyzed by One-way ANOVA followed by Dunnett’s test. a-p<0.001, b-p<0.01, c-p<0.05, significant difference from control group.

This method of treatment with combination was quite more effective than corresponding single oral dose of extract had comparable efficacy with ranitidine treatment. The comparative

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summary of ulcer index is shown graphically in figure 1 whereas; the representative photographs are shown in figure 2.

The combination treatment in higher dose i.e. 100mg/kg shows significant decrease in gastric acid secretion as compared to individual drugs and combination in lower dose (075mg/kg). The concentration of pepsin was also decreased indicating proteolytic activity. The activity exhibited by the combination at a lower dose i.e. 075mg/kg though insignificant, and increase in pharmacological activity at a higher dose indicates dose dependent activity as exhibited in table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Total acidity (mEq/l)</th>
<th>Free acidity (mEq/g)</th>
<th>Pepsin activity (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>42.19±0.93</td>
<td>36.77±0.58</td>
<td>33.58±1.25</td>
</tr>
<tr>
<td>Co-Ex (075mg/kg)</td>
<td>17.65±0.71</td>
<td>8.50±0.49</td>
<td>11.46±0.93</td>
</tr>
<tr>
<td>Co-Ex (100mg/kg)</td>
<td>14.51±0.48</td>
<td>6.25±0.48</td>
<td>9.78±2.17</td>
</tr>
<tr>
<td>OS Ex (100mg/kg)</td>
<td>21.48±1.21</td>
<td>10.11±0.87</td>
<td>15.32±3.16</td>
</tr>
<tr>
<td>ZO Ex (100mg/kg)</td>
<td>36.03±0.69</td>
<td>27.41±1.17</td>
<td>22.76±2.17</td>
</tr>
<tr>
<td>Standard</td>
<td>9.87±0.37</td>
<td>8.90±1.15</td>
<td>12.16±0.97</td>
</tr>
</tbody>
</table>

Data is represented as MEAN ± S.E.M (n=6), analysed by One-way ANOVA followed by Dunnett’s test. a-p<0.001, b-p<0.01, c-p<0.05, significant difference from control group.

**DISCUSSION**

Ayurveda, the oldest medicinal system in the world, leads to find therapeutically useful compounds from plants. Therefore, ayurvedic knowledge supported by modern science is necessary to isolate, characterize, and standardize the active constituents from herbal source. This combination of traditional and modern knowledge can produce better antulcer drugs with fewer side effects. Herbs are widely available in India and other countries. The wide spectrum makes them attractive candidates for further research [16]. There are several factors that may induce ulcer in human being such as stress, chronic use of anti-inflammatory drugs and continuous alcohol ingestion, among others [17]. Although in most cases the etiology of ulcer is unknown, it is generally accepted that it is the result of an imbalance between aggressive factors and maintenance of the mucosal integrity through the endogenous defense mechanism [18]. The candidate for an effective drug against peptic ulcer should basically act either by reducing the aggressive factors on gastro duodenal mucosa or by increasing mucosal resistance against them. It has become imperative to scrutinize herbal products for evaluating their acclaimed properties, as recently numbers of herbs are being introduced in the market [19].

*Ocimum sanctum* and *Zingiber officinalis* are known to possess some anti-ulcerogenic activity. In our study, we went a step ahead and have studied their combination treatment in aspirin induced ulcer model. This model is based on the inhibition of cyclooxygenase enzyme leading to reduced prostaglandin production and increase in acid secretion [20]. This has been reported that the cytoprotective factors of the gastric mucosa like increase in the mucus secretion, bicarbonate secretion, and prostaglandin levels protect the gastric mucosa against corrosive activities of gastric acid and pepsin. However, NSAIDs pretreatment has been found to reduce these cytoprotective measures [21, 22, 23].
Figure 2: Representative photographs
Key- (a): Control, (b): Standard (ranitidine) (c): ZO-Ex 100mg/kg, (d): OS-Ex 100mg/kg, (e): CO-Ex 075mg/kg, (f): Co-Ex 100mg/kg.
It has been reported that gastric cytoprotective effect is antagonized by the nitric oxide synthase inhibitor [24]. It has been indicated that programmed cell death (apoptosis) is an intrinsic part of organismal development and aging. All NSAIDs including aspirin cause apoptosis. The damage in the stomach activates acid secretion by a stimulation pathway in addition to PGs, NO and Ca^{2+} dependent inhibitory mechanism causing acid stimulation in the damaged stomach is mediated by histamine released from the mucosal mast cell [25, 26, 27, 28]. This favours that aspirin causes changes in gastric acid secretion, ulcer indices, and pepsin activities due to apoptosis activity mediated due to oxidative stress induced by the free radicals.

The antioxidant activities have been reported in reported in Ocimum sanctum and Zingiber officinalis [29, 30]. Therefore oxidative damage to endothelial cells, which results in NO might be inhibited due to possible free radical scavenging activity present.

The herboformulation at a dose of 0.75mg/kg significantly reduced severity of ulcer i.e. decrease in ulcer index and increase in % protection, than the individual extracts at a dose of 100mg/kg. The increase in activity at 100mg/kg indicates dose dependent effect of herboformulation. This healing activity may be due to its cytoprotectivity effect coupled with anti-secretory activity by reduction in free acid and pepsin activity as compared to control.

CONCLUSION

Based on our studies, we conclude that the herboformulation of Ocimum sanctum and Zingiber Officinalis was found to be possessing potent ulcer protective properties as compared to individual extract in Aspirin induced Ulcer model. The activity depends on variety of factors including amount of individual constituents in the extract, interaction between individual constituents, and their pharmacokinetics, which requires further studies.

Acknowledgement

We are sincerely thankful to our head of the department, Dr. Sateesh Belemkar for his valuable guidance. We express gratitude to him.

REFERENCES