1. Introduction

Gastric hyperacidity and gastroduodenal ulcer is a very common global problem today. It is now generally agreed that gastric lesions develop when the delicate balance between some gastroprotective and aggressive factors are lost. Major aggressive factors are acid, pepsin, Helicobacter pylori and bile salts. Defensive factors mainly involve mucus bicarbonate secretion and prostaglandins. Hypersecretion of gastric acid is a pathological condition, which occurs due to uncontrolled secretion of hydrochloric acid from the parietal cells of the gastric mucosa through the proton pumping H⁺K⁺ATPase. Even the normal rate of acid secretion may cause ulceration in the breached mucosa when some gastroprotective factors are lost. The modern approach to control gastric ulceration is to inhibit gastric acid secretion, to promote gastroprotection, block apoptosis and stimulate epithelial cell proliferation for effective healing. Most of the antisecretory drugs such as proton pump inhibitors (omeprazole, lansoprazole, etc.) and histamine H2–receptor blocker (ranitidine, famotidine, etc.) are extensively used to control increased acid secretion and acid related disorders caused by stress, NSAIDs and H. pylori; but there are reports of adverse effects and relapse in the long run. On the contrary most of the herbal drugs reduces the offensive factors and are proved to be safe clinically effective, having better patient tolerance, relatively less expensive and globally competitive[1–2].

Chandanasava, one of the commonly used Ayurvedic formulations, is prescribed to treat painful micturatio, spermattorrhoea, heart diseases, general weakness, & dyspepsia (Ayurvedic yog chandanasava). The three different formulations of Chandanasava have been taken to study the antiulcer activity. The first two formulations have been procured from the market belonging to the company Dabur & Baidhyanath respectively. The third formulation was procured from the Ayurvedic College. Medicinal plants have attracted the attention of not only professionals from various systems of medicine, but also the scientific communities belonging to different disciplines, plants are promising source of herbal formulation[3].

There is no scientific report on the effect of Chandanasava on the ulcer. The present investigation was undertaken to evaluate the effect of Chandanasava on experimentally
induced ulcer in rats.

2. Materials and Methods

2.1. Drugs and Chemicals

Aspirin (Cipla Pharmaceuticals limited, Pithampur Indore, India), Omeprezole (Torrent Pharmaceutical, Ahmedabad, India), Ranitidine (Abhirami Pharmachem, India), Catechin (Sigma–Aldrich, MO, USA) and the chemicals used for phytochemical analysis were of analytical grade and procured from local firms.

2.2. Collection of Formulation

The formulations of Chandanasava have been procured from the market area of Rajendra nagar, Indore. The first two formulations have been procured from the market belonging to the company Dabur & Baidyanath respectively. The third formulation was procured from the Ayurvedic College.

2.3. Preparation of Formulation

The formulation made available by the Ayurvedic College was prepared by using the following method. In brief to the required quantity of water, to which jaggery or sugar as prescribed in the formula was added; then it was boiled and cooled. This mixture was poured into a fermentation pot. Fine powder of the drugs (24 ingredients) mentioned in the formula were added. The container was covered with a lid and the edges are sealed with clay–smeared cloth wound in seven consecutive layers; the container was kept in an empty room, in an underground cellar, so as to ensure that for fermentation, as far as possible temperature was maintained. After the specified period, the lid was removed, and the contents examined to ascertain whether the process was complete[44].

2.4. Dosage determination

The dose of the formulation was calculated as per human equivalent dose following the USFDA Guideline[14]. Accordingly 6 ml/kg dose was employed.

2.5. Experimental animals

Albino wistar rats of both sex weighing between 150–250 g were used. The experimental protocol was approved from Institutional Animal Ethics Committee. Animals were housed under standard conditions of temperature (24 ± 2°C) and relative humidity (30–70%) with a 12:12 light: dark cycle. The animals were given standard diet and water ad libitum.

2.6. Antiulcer activity

2.6.1. Cold Restraint Stress Induced Ulcer

Animals of different group were subjected to cold stress after 45 min of the formulation and OMZ treatment. Rats were deprived of food, but not water, for about 18 h before the experiment. Rats were immobilized by strapping the fore and hind limbs in restraint cage and kept for 2 hr, at a temperature of 4°C. After 2 hr, animals were sacrificed, the stomach was incised along the lesser curvature (Govindarajan et al., 2006) and ulcer was scored as: Red coloration (0.5), Spot ulcer (1), hemorrhagic streak (1.5), Ulcers (2), Perforation (3). Mean ulcer score for each, animal was expressed as ulcer index. The percentage of ulcer protection was calculated as mean ulcer index of control—mean ulcer index of test / mean ulcer index of control × 100[5].

2.6.2. Pylorus Ligation Induced Ulcer

After 1 hr of treatment to different groups, the animals were anaesthetized using thiopentone sodium (35 mg/kg, i.p.), the abdomen was opened and pylorus ligation was done without causing any damage in its blood supply. After 4 hr their stomachs were dissected and its contents were collected into tubes for analysis of volume of gastric juice, pH, total and free acidity. The ulcers were scored as described under cold stress induced ulcers. The gastric juice was collected after 4 hr of Pylorus ligation induced ulcers and centrifuged for 5 min at 2000 rpm. The supernatant was collected and the volume of gastric juice was expressed as ml/100 g body weight. Total acidity was determined in the supernatant by titrating against 0.01 N NaOH , using 2–3 drops of topfers reagent as indicator until canary yellow color was observed. Volume of NaOH required was noted and this corresponds to free acidity. Further 2–3 drops of phenolphthalein was added and titrated with 0.01 N NaOH until pink color was restored and this gives total acidity. Free acidity and total acidity is expressed in terms of 0.1 N HCL per 100 g of gastric contents [6].

2.6.3. Ethanol induced ulcer

The animals were divided into five groups as described above except that catechin (200 mg/kg, p.o.) was used as standard. The gastric ulcers were induced in rats by administrating absolute ethanol (99%) (1 ml/200 g) orally, after 45 min of formulations or catechin treatment. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized one hour later with anesthetic ether and stomach was incised along the greater curvature and ulceration was scored. A score for the ulcer was studied similar to cold restraint stress induced ulcer model[7].

2.6.4. Aspirin induced ulcer

The animals were divided into various groups as described in the above sections. After 45 min of formulations (6 ml/kg, p.o.) or ranitidine (50 mg/kg, p.o.) treatment to different groups, the animals were administered with aspirin in dose of 500 mg/kg. The animals were sacrificed after 4 h and the stomach was then excised and cut along the greater curvature, rinsed gently with saline to remove the gastric contents and blood clots. Ulcer index was then calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach[8].

2.7 Statistical Analysis

The data are represented as mean ± S.D., and statistical significance was carried out employing one way analysis of variance (ANOVA) followed by Tukey’s test. P<0.05 was considered statistically significant using Graphpad 5 software.

3. Results

3.1. Phytochemical screening

Preliminary phytochemical screening of different formulations revealed presence of alkaloids, flavonoids, terpenoids, tannins,
carbohydrates, glycosides, and proteins.

3.2. Antiulcer activity

3.2.1. Cold Restraint Stress Induced Ulcer

Pretreatment of rats with three formulations of Chandanasava produced significant protection \( P < 0.001 \) from cold restraint stress induced ulceration as compared to control animals. Omeprazole (20 mg/kg) produced significant gastric ulcer protection \( P < 0.001 \) as compared to control group. The formulations of Chandanasava at dose (6 ml/kg) showed protective effect of 78.57 %, 76.28 %, and 73.85 % inhibition of Baidyanath, Dabur & Prepared respectively against cold restraint stress induced ulcerogenesis which is represented in Fig. 1C.

Table 1

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Vol. of gastric juice (ml)</th>
<th>pH</th>
<th>Free acidity (mEq/L/100g)</th>
<th>Total acidity (mEq/L/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.47 ± 0.40</td>
<td>2.3 ± 0.31</td>
<td>30.75 ± 3.09</td>
<td>47.50 ± 3.10</td>
</tr>
<tr>
<td>OMZ</td>
<td>2.32 ± 0.45**</td>
<td>4.05 ± 0.44***</td>
<td>11.50 ± 1.29***</td>
<td>26.0 ± 3.91***</td>
</tr>
<tr>
<td>F1</td>
<td>1.55 ± 0.34***</td>
<td>3.82 ± 0.17***</td>
<td>12.75 ± 1.70***</td>
<td>26.0 ± 2.16***</td>
</tr>
<tr>
<td>F2</td>
<td>2.02 ± 0.34***</td>
<td>3.72 ± 0.27***</td>
<td>15.0 ± 1.82***</td>
<td>27.5 ± 2.87***</td>
</tr>
<tr>
<td>F3</td>
<td>1.62 ± 0.38***</td>
<td>3.65 ± 0.34***</td>
<td>16.75 ± 2.21***</td>
<td>30.75 ± 2.98***</td>
</tr>
</tbody>
</table>

Each point represents mean ± S.D. of 3 rats. OMZ: omeprazole (20 mg/kg, p.o.); F1 (Baidyanath formulation); F2 (Dabur formulation); F3 (test formulation). **P < 0.01; ***P < 0.001 vs. respective control group (One-way ANOVA followed by Tukey’s test)

3.2.2. Pylorus Ligation Induced Ulcer

The rat pretreated with three formulations of chandanasava produced significant \( P < 0.001 \) decrease in ulcer index, gastric volume, free acidity and total acidity whereas pH was significantly \( P < 0.001 \) increased when compared with control group. The formulations showed 86.85%, 82.95% and 81.40%.

3.3. Ethanol induced ulcers

The results are represented in Fig. 1A. One-way ANOVA revealed significant influence of all treatments on the ethanol induced ulcers in rats. It was observed that ethanol treated rats exhibited marked ulcerations, which were significantly \( P < 0.001 \) reduced after treatment with different formulations of chandanasava. Further the effects were found to be comparable to that of standard drug catechin. The percentage ulcer inhibition in different groups was 84.0 % (catechin); 66.5 % (Baidyanath); 74.7 % (Dabur); and 78.5 % (Test formulation).

3.4. Aspirin induced ulcers

The results are represented in Fig. 1B. One-way ANOVA revealed significant influence of all treatments on the ethanol induced ulcers in rats. It was observed that aspirin treated rats exhibited marked ulcerations, which were significantly \( P < 0.001 \) reduced after treatment with different formulations of chandanasava. Further the effects were found to be comparable to that of standard drug ranitidine. The percentage ulcer inhibition in different groups was 89.0 % (ranitidine); 78.3 % (Baidyanath); 72.3 % (Dabur); and 68.7 % (Test formulation).

Figure 1. Effect of various Chandanasava formulations on the Mean Ulcer Indices of rats treated with different ulcerogens. Each bar represents mean ± S.D. of three observations. A: Ethanol induced ulcers; B: Aspirin induced ulcers; C: Cold stress induced ulcers; D: Pylorus ligation induced ulcers] OMZ: omeprazole (20 mg/kg, p.o.); F1 (Baidyanath formulation); F2 (Dabur formulation); F3 (test formulation); Catechin (200 mg/kg, p.o.); Ranitidine (50 mg/kg) *** P < 0.001 vs. respective control group; \( P < 0.05 \) vs. respective standard group (One-way ANOVA followed by Tukey’s test)
Fig. 2 Photographs showing ulcerous lesions in rat stomach treated with various ulcerogens along with/without different formulations of chandanasava and antiulcer drugs. 1. Cold stress induced ulcers; 2. Pylorus ligation induced ulcers; 3. Ethanol induced ulcers; 4. Aspirin induced ulcers; A: Control; B: Standard; C: Baidyanath; D: Dabur; E: Test.

4. Discussion

Peptic ulcer occurs when there is an imbalance between the damaging effects of gastric acid and pepsin, and the defence mechanisms, which protect the gastric and duodenal mucosa from these substances [7]. To regain the balance, different therapeutic agents including the Ayurvedic herbal formulation are used.

Treatments available for ulcer is generally non-specific and is usually aimed at reducing the production of gastric acid and re-enforcing gastric mucosal protection such as regular food, adequate rest and avoidance of ulcerogenic agents such as coffee, alcohol and tobacco. The drugs used in the treatment of ulcer include receptor blockers, proton pump inhibitors, drugs affecting the mucosal barrier and act on the central nervous system. Even though a range of drugs are available for the treatment of ulcer, many of these do not fulfill all the requirements and have side effects [8].

The marketed herbal formulation of Chandanasava is one of the herbal formulations undertaken in the present study to evaluate its antiulcerogenic potential.

Pylorus ligation induced gastric ulcer model is generally used to study the effect of test drugs on gastric secretions. Ulcers caused by pyloric ligation are due to increased accumulation of gastric acid and pepsin leading to auto digestion of gastric mucosa and break down of the gastric mucosal barrier [9].

The agents that decrease gastric acid secretion and increase mucus secretion are effective in ulcers induced by this method. Chandanasava had significantly reduced the volume of gastric acid secretion, indicating their antisecretory effect.

Stress-induced ulcers are probably mediated by histamine release with enhancement in acid secretion, a reduction in mucus production and generation of free radicals etc., mast cell activation, alterations in prostaglandin generation, cytokine liberation and breakdown of normal cytoprotective mechanism. Ulcers due to cold stress are both due to physiological and psychological factors [10]. The gastro protective action of Chandanasava against stress-induced ulceration could be due to its histamine antagonistic, anticholinergic or antisecretory effects.

Chandanasava contains 24 herbal ingredients and it contains alkaloids, flavonoids, terpenoids, tannins, carbohydrates, glycosides, and proteins and many other chemical constituents. Some of these bioactive constituents have been associated with gastro-protective and antiulcer effects in previous studies. The non-specific gastro-protective activities of the extracts may be the result of a combined effect of the different phytoconstituents present. The flavonoidal compounds were proved to have antisecretory and cytoprotective properties due to free radical scavenging activity during lipid peroxidation [11]. Apart from flavonoids, Chandanasava is also rich in tannins and terpenoids which have been shown to exhibit antiulcer properties. Tannins generally have vasoconstrictive and protein precipitating effects, precipitation of protein at ulcer sites forms impervious layer over the lining that hinders gut secretions and protects the underlying mucosa from toxins and other irritants [12]. The action of terpenes includes reduction of mucosal prostaglandin metabolism and gastric vascular permeability [13]. However, further study is required to isolate the active components responsible for the antiulcer activity.

The results of the present study suggest that use of the herbal formulation Chandanasava may be beneficial in healing of ulcers in patients suffering from peptic ulcer disease.

This study established the protective effect of Chandanasava against pylorus ligation, ethanol induced ulcers, aspirin induced ulcers and cold stress-induced ulcers which provides a support for its putative use in ulcer treatment. Further, this effect may be attributed to the presence of flavonoids, terpenes, and tannins in the preparation.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are thankful to Ayurvedic College Indore (M.P.) for providing the prepared formulation of Chandanasava.
References


[14] U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) 2003, Pharmacology and Toxicology, guidance for industry estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers.