Original Article

The inhibition of gastric mucosal injury by Chinnodbhavadi kwath (decoction) - an Indian Ayurvedic formulation in rats

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Abstract

Objective: As per ancient Indian text, one of the Triphala formulations called as Chinnodbhavadi kwath (decoction) is used for chronic hyperacidity and gastric problems. Triphala formulation is categorized as an Ayurvedic rejuvenator. The purpose of the present study is to evaluate the potential role of Chinnodbhavadi kwath in modulating the extent and severity of experimental gastric ulcer in rats to substantiate its traditional claim.

Methods: Gastric ulcers were induced by aspirin (200 mg/kg, orally for three days) plus pyloric ligation in rats. The test drug was studied for its effect on ulcer index, secretory parameters, mucin activity in gastric juice and nucleic acid content in stomach homogenate of rats.

Results: Chinnodbhavadi kwath (8.7 ml/kg) showed significant anti-ulcer activity. This activity depends mainly on significant decrease in intensity of gastric ulcers, inhibition of acid secretion and strengthening the mucosal defense system by increasing mucin secretion. The increase in nucleic acid content of gastric wall mucosa in drug treated groups indicate decreased cell shedding and increased life span of cells.

Conclusion: Based on the data generated it is suggested that Chinnodbhavadi kwath provides significant protection in experimental gastric ulcer in rats.

INTRODUCTION

Peptic ulcer was assumed to develop when the summation of aggressive factors (like acid, pepsin and Helicobacter pylori infection) is greater than defensive factors (like secretion of bicarbonate, mucus and prostaglandins) [1]. Considering the several side effects of modern medicines, indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer [2]. In traditional Indian medicine, several plants have been used to treat gastrointestinal disorders, including gastric ulcers [3] and the phytochemical analysis of these plants has yielded as compounds with gastro-protective activity [4].

Triphala formulation is one of the renowned formulations used alone or along with other ingredients in Ayurvedic therapeutics for the treatment of gastro-intestinal problems [5] and categorized as rejuvenator [6]. As per Ancient text, one of the Triphala formulations called as Chinnodbhavadi kwath (decoction) is used for chronic hyperacidity and gastric problems [7]. Pharmacological studies have shown that Triphala extract is reported to display intestinal enteroprotective effect [8], anti-diabetic and free radicals scavenging activities [9] and powder of Triphala is reported to possess anti-inflammatory and anti-arthritic activities [10]. Thus, it was thought useful to undertake gastroprotective effects of on Triphala formulation called as Chinnodbhavadi kwath against aspirin plus pyloric ligation-induced gastric ulcers in rats to determine the potential role of Chinnodbhavadi kwath in modulating the extent and severity of experimental gastric ulcer in rats to substantiate its traditional claim.

Received January 30, 2013
Accepted April 20, 2013
Published Online April 28, 2013
DOI 10.5455/jeim.200413.or.067

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Key Words
Chinnodbhavadi kwath; Mucin activity; Pyloric ligation; Triphala

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MATERIALS AND METHODS

Plant materials, drugs and chemicals

Stem of *Tinospora cordifolia* (Willd.) Miers. (Menispermaceae), stem bark of *Azadirachta indica* A. Juss. (Meliaceae) and leaves of *Trichosanthes dioica* Roxb. (Cucurbitaceae) collected from forest of Barda hills, Jamnagar (Gujarat, India), fruits of *Terminalia chebula* Retz. (Combretaceae), *Terminalia belerica* (Gaertn.) Roxb. (Combretaceae) and *Emblica officinalis* Gaertn. (Euphorbiaceae) collected from forest of Dang and Valsad (Gujarat, India) were used in the study. The plant materials were authenticated and voucher specimens of each submitted to phamacognosy laboratory of IPGT & RA, Gujarat Ayurved University, Jamnagar, India. Chinnodbhavadi kwath (decoction) was prepared by mixing equal proportion of *T. chebula, T. belerica, E. officinalis, T. cordifolia, A. indica* and *T. dioica*. Coarse powder (48 g) of mixture and 768 g water was added; boiled on low to medium heat till the liquid portion was reduced to $\frac{1}{16}$ th of the original volume (96 g) and filtered [11]. All chemicals used in the study and for biochemical assay were of analytical grade.

Standardization of formulation and its ingredients

The test formulation and their ingredients were standardized using gallic acid as a marker compound by high performance thin layer chromatography (HPTLC) finger print. The plate was developed in Toluene:Ethyl acetate:Formic acid (5:5:1) solvent system. Gallic acid was observed at 0.52 Rf value, when scanned at 254 nm. Chinnodbhavadi kwath showed almost same peak at 0.49 Rf value when scanned at 254 nm. The concentration of trace heavy metals such as lead, cadmium, arsenic and mercury present in formulation were analyzed by atomic absorption spectrophotometer. The data obtained indicated that trace metals do not seem to be present in significant quantities in Chinnodbhavadi kwath formulation.

Animals

Adult Wistar rats (180 to 220 g) of either sex were used in the experiment. The animals were maintained under standard conditions of temperature, humidity and exposed to 12 h light and dark cycles. All animals were exposed to the same environmental conditions and were maintained on standard diet and water *ad libitum*. The experimental protocol was approved by the institutional animal ethical committee as per guideline of Committee for the Purpose of Control and Supervision on Experiments on Animals, India.

Acute toxicity study

Acute oral toxicity of test drug was carried out in female rats as per the OECD 423 guideline [12]. The result showed that Chinnodbhavadi kwath formulation did not produce any changes in observed parameters and mortality up to dose of 2000 mg/kg. Hence, animal dose of Chinnodbhavadi kwath formulation was fixed on the basis of human therapeutic dose mentioned in literatures [11].

Experimental design

Rats were randomized into three groups, each consisting of six rats. Group I (control), received vehicle as an aqueous suspension of 1% carboxymethyl cellulose (CMC) in dose of 10 ml/kg. Group II was treated with Chinnodbhavadi kwath with a dose of 8.7 ml/kg, p.o. for seven days. Group III was treated with omeprazole 48 h, 24 h and 1 h prior to induction of ulcers at a dose of 2 mg/kg and used as a positive control group.

Gastric ulceration in rats was induced as described earlier [13]. Aspirin suspension in 1% CMC in water was administered one hour after each of drug administration in a dose of 200 mg/kg, orally once daily for three days. The aspirin administration started from 5th day of drug administration. On 7th day, 1 h after aspirin administration pylorus of overnight fasted rats were ligated [14]. The animals were sacrificed at the end of 6 h after pyloric ligation. Abdominal cavity was re-opened carefully and the stomach was excised after ligating the terminal portion of esophagus. Gastric contents were drained into tubes and centrifuged at 3000 rpm for 15 min for estimations of gastric secretion. Stomach tissues were taken for assessment of stomach ulcer and tissue biochemical studies.

Assessment of stomach damage

The stomach was excised, cleaned and opened along its greater curvature, then the inner surface was gently washed with cold saline solution and examined for ulceration with a magnifying lens. Severity and total number of ulcers in each rat were recorded for calculating ulcer index. Ulcer index was determined by following the scoring method of Suzuki *et al* [15].

Gastric secretion

The volume of the gastric juice supernatant was expressed as ml per 100 g body weight of rats. Free acidity and total acidity were determined and expressed for concentration as mEq/100 ml. Peptic activity was determined using hemoglobin as substrate, as described earlier [16] and has been expressed as µmol of tyrosine/ml of gastric juice. Dissolved mucosubstances were estimated in 95% alcoholic precipitates of the gastric juice. This precipitates were employed for estimation of total protein [17], total hexose [18], hexosamine [19], fucose [20], and sialic acid [21]. The result has been expressed as µg/ml of gastric juice. The ratio of total carbohydrates (TC) (sum of total hexose, hexosamine, fucose and sialic acid) to protein (TP) has been taken as index of mucin activity [16].
Nucleic acid estimation

The weighed amount of stomach mucosa was homogenized in trichloroacetic acid and extracted with alcohol diethyl ether reagent. Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in the extracted mucosa of stomach tissue was estimated via a method described previously [22].

Statistical analysis

The data are expressed as mean ± standard error of mean for six rats per experimental group. One way analysis of variance (ANOVA) was used to compare the mean values of quantitative variables among the groups followed by Dunnet’s multiple t-test for unpaired data to determine significant difference between groups at P < 0.05.

RESULTS

Macroscopic results

Aspirin at the dose of 200 mg/kg for three days plus pyloric ligation for 6 h caused gastric mucosal ulcers in rats (Table 1). Pretreatment with the Chinnodbhavadi kwath (P < 0.01) and omeprazole (P < 0.001) showed significant gastric ulcer protective effects, which is evident from the decreased intensity of gastric mucosal ulceration in comparison to control group.

Table 1. Effect of Chinnodbhavadi kwath on ulcer index and gastric secretion in stomach of aspirin plus pylor-ligated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer Index</th>
<th>Volume of gastric juice (ml/100 g)</th>
<th>Free acidity (mEq HCl/100 ml)</th>
<th>Total acidity (mEq HCl/100 ml)</th>
<th>Pepsin activity (µmol of tyrosine/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9 ± 0.73</td>
<td>2 ± 0.09</td>
<td>13.66 ± 1.58</td>
<td>108 ± 4.90</td>
<td>462.82 ± 14.28</td>
</tr>
<tr>
<td>Chinnodbhavadi kwath (8.7 ml/kg)</td>
<td>5.16 ± 0.94b</td>
<td>1.84 ± 0.05</td>
<td>6.33 ± 0.61c</td>
<td>87.33 ± 2.66c</td>
<td>423.31 ± 22.71</td>
</tr>
<tr>
<td>Omeprazole (20 mg/kg)</td>
<td>1.66 ± 0.33c</td>
<td>1.69 ± 0.04a</td>
<td>4.66 ± 0.66c</td>
<td>83.16 ± 0.83c</td>
<td>372.51 ± 24.73a</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SEM of six rats per group; *P < 0.05, **P < 0.01, and ***P < 0.001 compared with control group.

Table 2. Effect of Chinnodbhavadi kwath on the level of total protein, total hexose, total fucose, hexosamine, sialic acid, total carbohydrate and mucin activity (TC:TP ratio) in stomach gastric juice of aspirin plus pylor-ligated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total protein (µg/ml)</th>
<th>Total hexose (µg/ml)</th>
<th>Total fucose (µg/ml)</th>
<th>Hexosamine (µg/ml)</th>
<th>Sialic acid (µg/ml)</th>
<th>Total carbohydrate (µg/ml)</th>
<th>TC:TP ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>679.72 ± 33.31</td>
<td>365.87 ± 7.31</td>
<td>92.59 ± 6.32</td>
<td>209.87 ± 26.47</td>
<td>28.88 ± 4</td>
<td>697.29 ± 31.71</td>
<td>1.027 ± 0.022</td>
</tr>
<tr>
<td>Chinnodbhavadi kwath (8.7 ml/kg)</td>
<td>666.65 ± 18.88</td>
<td>534.23 ± 32.18b</td>
<td>142.66 ± 3.98 b</td>
<td>253.08 ± 26.04</td>
<td>36.66 ± 5.71</td>
<td>966.64 ± 43.31b</td>
<td>1.45 ± 0.05b</td>
</tr>
<tr>
<td>Omeprazole (20 mg/kg)</td>
<td>671.88 ± 34.94</td>
<td>493.82 ± 13.31b</td>
<td>102.04 ± 3.87</td>
<td>320.98 ± 31.23 *</td>
<td>41.1 ± 3.61</td>
<td>957.95 ± 39.88b</td>
<td>1.435 ± 0.06b</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SEM of six rats per group; *P < 0.05, and **P < 0.01 compared with control group.

Effects on gastric secretion

Chinnodbhavadi kwath and omeprazole significantly reduced the free acidity and total acidity in comparison to control group (Table 1). The data related to the concentration of individual carbohydrate constituents and mucin activity is presented in Table 2. The level of total hexose and fucose were significantly increased in Chinnodbhavadi kwath and omeprazole treated groups (P < 0.001). Total carbohydrate values in gastric juice of the rats were significantly increased in Chinnodbhavadi kwath (P < 0.001) treated group. The significant increase in total carbohydrate was due to increase in mucopolysaccharides like hexose, fucose and hexosamine leading to significant increase in mucin activity.

Effects on nucleic acid

The data related to the effect of Chinnodbhavadi kwath on DNA and RNA contents of the gastric wall mucosa in aspirin plus pyloric ligation-induced gastric ulceration in rats are shown in Table 3. The rats pretreated with test drug showed increase in the values of DNA and RNA, indicating the enhancement of life span of mucosal cells. However, only the increase in DNA content was found statistically significant (P < 0.05) in drug-treated groups.
Table 3. Effect of Chinnodbhavadi kwath on nucleic acid in stomach tissue homogenate of aspirin plus pylor-ligated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>DNA (μg/g tissue)</th>
<th>RNA (μg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>510.86 ± 26.17</td>
<td>868.41 ± 78.46</td>
</tr>
<tr>
<td>Chinnod. kwath (8.7 ml/kg)</td>
<td>663.04 ± 42.65*</td>
<td>1054.51 ± 69.81</td>
</tr>
<tr>
<td>Omeprazole (20 mg/kg)</td>
<td>652.17 ± 44.55*</td>
<td>1054.51 ± 83.22</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SEM of six rats per group; *P < 0.05 compared with control group.

DISCUSSION

Triphala is considered as one of the most important formulations in Ayurvedic therapeutics for its multiple organ-protective effects including gastro-protection. Hence, the present study was undertaken to elucidate the probable mechanism underlying anti-ulcer action observed with Triphala formulation called as Chinnodbhavadi kwath. In the pylorus ligation model, it has been proposed that the digestive effect of accumulated gastric juice and interference with gastric blood circulation are responsible for the induction of gastric ulcer. Aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of H⁺ ions. Aspirin was administered to rats with pyloric ligation; in this procedure it is reported that aspirin further aggravates the acidity and attenuates the resistance of the gastric mucosa by causing extensive damage to the glandular region of the stomach [23].

The ability of Chinnodbhavadi kwath to reduce the acidity may be due to the direct action on the acid producing parietal cells or through inhibition of the proton pumping H⁺-K⁺ATPase. An earlier study shows that ellagic acid significantly inhibits the gastric H⁺-K⁺ATPase and acid secretion [24]. Glycosides are also known to inhibit chloride transport in the stomach [25]. Ellagic acid is the major chemical constituent present in the ingredients of Triphala formulations [26]. Glycosides are the major constituents of T.cordifolia [27] and T.belerica [26]; hence the observed antacid effect may be due to the presence of glycosides and ellagic acid in Chinnodbhavadi kwath. Previous reports also suggested that E.officinalis, T.cordifolia and A.indica produced significant anti-ulcer activity [28-30].

Chinnodbhavadi kwath significantly increased the TC:TP ratio, which reflects the functional integrity of mucosal barrier and could be taken as a reliable index for mucin secretion [16]. The increase in mucin activity was due to significant increase in the total carbohydrate content. Hence, the augmentation of the mucosal barrier by Chinnodbhavadi kwath was due to increase in the secretion of dissolved mucus in the gastric secretion.

Increase or decrease in life span of mucosal cells can be expressed as amount of DNA and RNA in the gastric wall mucosa. The increase in DNA content of gastric wall mucosa in the drug-treated groups indicate decreased cell shedding and increased life span of cells [31]. A recent study suggested that phenolic compounds, ascorbic acid and flavonoids in Triphala formulation are responsible for the protection of DNA [26].

From the present study, it is concluded that Chinnodbhavadi kwath provides significant protection against gastric ulceration. This activity depends mainly on inhibition of acid secretion, increase in mucin secretion which enhances the stability of gastric mucosal barrier and gastric cytoprotection against aspirin plus pyloric ligation-induced gastric ulceration in rats.

ACKNOWLEDGEMENT

The authors wish to thank Prof. M.S. Baghel, Director, Institute of Postgraduate Teaching and Research in Ayurveda for their constant support.

COMPETING INTERESTS

The authors declare that they have no conflict of interest.
REFERENCES


