Antiulcer activity of Amlapitta Mishran suspension in rats: A pilot study

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ABSTRACT

Context: Amlapitta Mishran suspension is a poly herbal ayurvedic formulation, which has been traditionally used for acidity and gastric ulcers.

Aim: The aim of this study is to evaluate the antiulcer activity of Amlapitta Mishran on non-steroidal anti-inflammatory drugs (NSAID's) -induced ulcers in the rat model.

Subjects and Methods: The antiulcer activity of Amlapitta Mishran was investigated on indomethacin (100 mg/kg) NSAID's induced ulcers in rats. Effect of two different doses of Amlapitta Mishran was studied by calculating the total number of ulcers, ulcer index and percentage inhibition.

Statistical Analysis Used: Data was analyzed by the Student's *t*-test ($P < 0.05$).

Results: Amlapitta Mishran treated rats have shown significant ($P < 0.0001$) decrease in the total number of ulcers and ulcer index and significant increase in % inhibition of ulcers as compared with positive control group.

Conclusion: The results indicate that Amlapitta Mishran has showed a dose dependent antiulcer activity in experimental animals and confirms ayurvedic use of Amlapitta Mishran in gastric ulcers.

KEY WORDS: Antiulcer activity, cyclooxygenase, gastric ulcers, indomethacin

INTRODUCTION

Gastric and duodenal ulcers are mainly characterized by an imbalance between aggressive (acid, pepsin) and protective factor (secretion and action of mucus and bicarbonate). Both these types of peptic ulcers affect thousands of people all over the world and it is considered as a global health problem.[1] If not treated, it may become malignant.

The traditional Indian system of medicine, Ayurveda, has a long history, of providing plant materials as medicine to treat a number of diseases and disorders. However, sufficient scientific data with respect to safety and efficacy of ayurvedic preparations is lacking due to a large number of constituents in their formulations and mechanism of action being unclear. Drug discovery with a single compound may not be useful in all diseases and conditions. And hence, rationally designed *ayurvedic formulation* could also be considered as a viable alternative.[2] Amlapitta Mishran is a herbo-mineral preparation in suspension form, manufactured by Shree Dhootapapeshwar Limited, Mumbai (India). It contains *Adhatoda vasica* Nees (Vāsa; Fam. *Acanthaceae*),[3,4] *Tinospora cordifolia* (*Willd*) *Miers* (*Guḍūchi*),[5] *Azadirachta indica A.* Juss. (*Nimba*; stem bark; Fam. *Meliaceae*),[6,7] *Swertia chirata Buch Ham.* (Kirātatikta; whole plant; Fam. *Gentianaceae*),[8] *Eclipta alba Hassk.* (Bhṛṅgarājaa; whole plant; Fam. *Asteraceae*),[9] *Trichosanthes dioica Roxb.* (Pointed gourd)[10] and *Glycyrrhiza glabra Linn* (Yaṣṭti; stolon and root; Fam. *Leguminosae*)[11] and (Āmalaki; fruits; Fam. *Euphorbiaceae*),[12] *Terminalia chebula Retz.* (Harītaki; fruits; Fam. *Combretaceae*),[13] *Terminalia belerica Roxb.* (Bibhītaka; dried ripe fruits; Fam. *Combretaceae*)[14] and *Shouktik* (*Muktāshukti*) *bhasma*[15] as active ingredients, which have been investigated for antiulcer activity in various animal models. *Fumaria indica* (*Shahtra; Fumaria parviflora var. indica)* have been studied for antioxidant activity, which would be helpful to prevent the ulcer induce reactive oxygen species (ROS).[16]

Current therapy for ulcers is H_2-receptor blockers, proton pump inhibitors, antacids, anticholinergics and antibiotics. Currently, prevention or treatment of the ulcers is one of the challenging problems because currently available therapy for u limited efficacy and severe side-effects.[17] Health professionals are looking toward compounds with no or minimal side-effects to treat ulcers. In this context, ayurvedic system has the potential to treat a number of diseases with many herbal plants

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and products.[2] In the present study, effect of Amlapitta Mishran suspension was studied on non-steroidal anti-inflammatory drugs (NSAID's) induced ulcers in Wistar rats.

SUBJECTS AND METHODS

Animals and reagents

A total of 30 healthy Wistar rats (100-120 g, 6-8 weeks) were procured from In-House Animal Facility of Shree Dhootapapeshwar Ayurvedic Research Foundation (SDARF). Animals were provided with standard diet and water *ad libitum*. Animals were housed in plastic cages under standard conditions, temperature 20 ± 2°C and humidity 50-60%, with 12 h dark/light cycle. The Animals were acclimatized for a minimum period of 1 week prior to the start of the study. The experimental protocol was approved by Institutional Animal Ethics Committee of SDARF. The experiment was conducted according to the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Amlapitta Mishran suspension was procured from Shree Dhootapapeshwar Limited (Mumbai). All other drugs and analytical grade chemicals were purchased locally.

Experimental design

All the animals were randomly divided into five groups i.e.,

Group I (normal control): 0.1% carboxy methyl cellulose (CMC) solution
Group II (positive control): 0.1% CMC solution + indomethacin (100 mg/kg)
Group III: Ranitidine (100 mg/kg) + indomethacin (100 mg/kg)
Group IV: Amlapitta Mishran suspension (1.35 ml/kg) + indomethacin (100 mg/kg)
Group V: Amlapitta Mishran suspension (2.7 ml/kg) + indomethacin (100 mg/kg).

Amlapitta Mishran dose in rats was selected from the human therapeutic dose (HTD) (15 ml/day) by using the formula, rat dose (200 g) = HTD × 0.018.

NSAID'S-induced ulcers

After 12 h fasting, Group I and II were administered with 0.1% CMC solution and Group III with ranitidine (100 mg/kg), Group IV and V were administered with Amlapitta Mishran suspension at 1.35 ml/kg and 2.7 ml/kg, respectively before 1 h of indomethacin in 0.1% CMC solution (100 mg/kg, p.o). All the test compounds were administered orally. Four hours after indomethacin administration, the animals were sacrificed by using CO_2 chamber. The stomach was removed and opened along the greater curvature. The stomachs were gently rinsed with water to remove the gastric contents and blood clots. The inner surface of free stomach was examined for gastric lesions.[18] The number of ulcers was counted. Ulcer scoring was carried out according to the method by as given below.[19] The scores were: 0 = no ulcer, 1 = superficial ulcer, 2 = deep ulcer, 3 = perforation.

Ulcer score

Ulcer index was measured by using the following formula

$$UI = U_N + U_S + U_P \times 10^{-1}.$$

UI is the ulcer index; U_N is the average number of ulcers as per animal; U_S is the average number of severity score and U_P is the percentage of animals with ulcers.

Percentage inhibition of ulceration

Percentage inhibition of ulceration was calculated as follows:

$$\text{Percentage inhibition of ulceration} = \frac{(\text{UI of control} - \text{UI of test}) \times 100}{\text{UI of control}}$$

Statistical analysis

Data expressed as mean ± SD ($n = 6$) and analyzed by one way analysis of variance was used to compare multiple groups in the study and Kruskal-Wallis test was used for ulcer index. P value less than 0.05 was considered significant.

RESULTS

In the present study, antiulcer activity of Amlapitta Mishran was studied in NSAID's induced ulcers in the stomach in Wistar rats. The rats treated with indomethacin (100 mg/kg, p.o) alone significantly ($P < 0.0001$) produced ulcers in the stomach compared with normal control group [Figures 1a and b]. Amlapitta Mishran showed dose dependent activity on ulcers in indomethacin treated rats. Treatment with Amlapitta Mishran suspension at a dose of 1.35 ml/kg and 2.7 ml/kg and ranitidine (100 mg/kg) showed significant ($P < 0.0001$) reduction in the total number of ulcers compared with positive control group as shown in Figures 1c-e. Treatment with Amlapitta Mishran suspension (1.35 ml/kg and 2.7 ml/kg) and ranitidine in rats showed significant ($P < 0.0001$) decrease in ulcer index

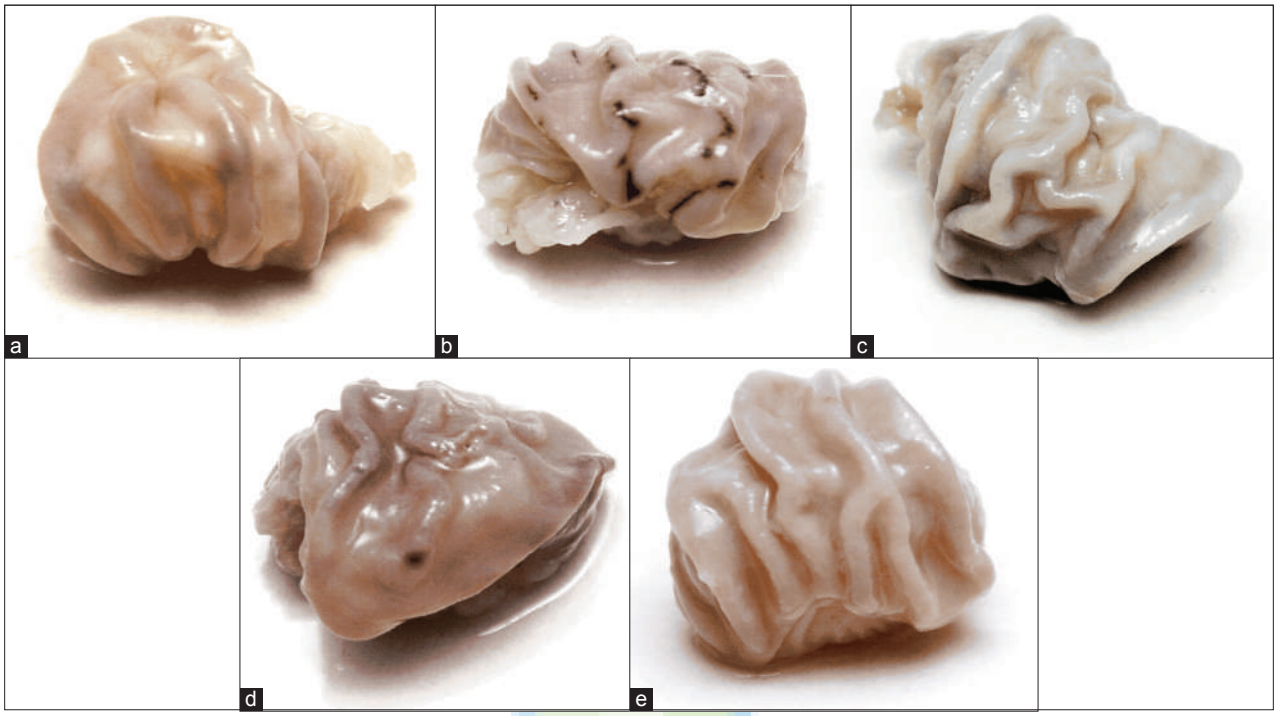

Figure 1: Effect of Amlapitta Mishran on non-steroidal anti-inflammatory drugs induced ulcers in rats. (a) normal control (b) indomethacin (c) ranitidine (d) Amlapitta Mishran (Therapeutic Dose) (e) Amlapitta Mishran (2TD)

Table 1: Effect of Amlapitta Mishran on total no of ulcers, ulcer index and % inhibition of ulcer

Group	Total no. of ulcers (mean ± SD) (n = 6)	Ulcer index (mean ± SD) (n = 6)	% inhibition (mean ± SD) (n = 6)
Group I	0	0	100.00
Group II	11.17±3.19	19.08±10.67	0.00
Group III	0.17±0.41***	0.18±0.43***	99.21±1.94***
Group IV	2.83±1.33***	3.40±2.29***	83.33±11.95***
Group V	0.83±0.75***	0.87±0.76***	96.03±3.58***

***P<0.0001 versus positive control analyzed by one way ANOVA and statistical comparisons of the data for groups were carried out by Kruskal-Wallis test for ulcer index. SD: Standard deviations, ANOVA: Analysis of variance

compared with positive control group and also significant (P < 0.0001) increase in percentage (%) inhibition was observed in two doses of Amlapitta Mishran and ranitidine treated rats compared with positive control rats [Table 1]. Antiulcer activity of Amlapitta Mishran was high at 2.7 ml/kg.

DISCUSSION

Imbalance between aggressive and protective factors may leads to the ulcers in the stomach.[18] Gastric ulcers are caused by several causative factors such as stress,[20] smoking,[21] alcohol[22] and NSAID's,[23] nutritional deficiencies, hereditary predisposition and infection by *Helicobacter pylori*.[18] In the stomach, prostaglandins play a pivotal role in maintenance of mucosal integrity of surface epithelial cells by production of mucus or inhibition of gastric acid secretion and also by stimulating the bicarbonate secretion. Chronic use of NSAID's damages the gastric mucosa by inhibiting the prostaglandin synthesis through inhibiting the enzyme cyclooxygenase,[18] increased expression of interleukin-1, generation of ROS and induction of apoptosis.[24] It was observed that Amlapitta Mishran showed significant reduction in the total number of ulcers in the indomethacin-induced ulcers in rats. These results suggest that gastroprotective effect of Amlapitta Mishran might be due involvement of prostaglandins through mucus secretion in the stomach.

In conclusion, Amlapitta Mishran produces significant antiulcer activity in NSIAD's induced ulcers in rats. According to the present findings, gatroprotective effect of Amlapitta Mishran in prevention of ulcers might be due to production of prostaglandins in the stomach. The results suggest that Amlapitta Mishran having a significant gastroprotective effect in a dose dependent manner.

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