Section: Pharmacology

Original Research Article

SCREENING OF ANTI-ULCER ACTIVITY OF EMBLICA OFFICINALIS FRUIT EXTRACT ON SWIMMING STRESS INDUCED PEPTIC ULCER MODEL IN ALBINO RATS

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Abstract

Background: To screen the anti-ulcer activity of Emblica officinalis (EO) aqueous fruit extract and to compare it with omeprazole in Swimming Stress Induced Peptic Ulcer Model of albino rats. **Materials and Methods:** Albino rats were divided into 4 groups and each group received distilled water, omeprazole (standard), test doses 250 mg/kg and 500mg/kg. After 30 minutes rats were forced to swim for 3 hours and stomach was examined for ulceration. Total Severity score (TSS), ulcers index (UI) and percentage inhibition (PI) were calculated. **Result:** The congestion and hemorrhagic lesions were less and showed significant reduction in TSS with the test dose of 500 mg/kg body weight. When the UI and PI of ulceration by omeprazole was compared with EO the difference was not significant and also the TSS of standard and test 1, standard and test 2, and test 1 and test 2, when compared using Mann Whitney U test, the difference is statistically insignificant. EO in its both doses showed to be gastroprotective compared to control (P value < 0.05). **Conclusion:** EO proves to be gastroprotective in the above model, may be due to its known anti-oxidant and anti-secretory property.

INTRODUCTION

Peptic ulcer Disease (PUD) is one of the common health issue affecting humans. Peptic ulcer is the disruption of the mucosal integrity of the stomach and / or duodenum leading to a local defect or excavation due to active inflammation.

Peptic ulcers include gastric and duodenal ulcers. An estimated 15000 deaths occur worldwide every year due to peptic ulcer disease.[1] The pathophysiology of PUD involves an imbalance between offensive factors (acid and pepsin) and defensive factors (mucin, prostaglandins (PGs), nitric oxide (NO), biocarbonate, growth factors and other peptides). Apart from the harmful effects of acid, Reactive Oxygen Species (ROS), especially the hydroxyl radical (OH) is known to play a significant role in causing oxidative damage of mucosa in different types of ulcers.[2]

Most common causes of peptic ulcer are Helicobacter pylori infection, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) related mucosal damage, alcohol, smoking and stress induced mucosal damage. Complications of peptic ulcers include hemorrhage, perforation, pyloric stenosis and the development of malignancy.[3]

Stress ulcers commonly seen in critically ill patients4 with shock, sepsis or severe injury. Stress and chronic illnesses for longer duration leads to gastric mucosal ischaemia and bleeding mainly due to hypersecretion of gastric acid, which is commonly observed in patients with head trauma or neurosurgical procedures. Preventive strategies aim to reduce gastric acidity, strengthen mucosal defensive mechanisms and to normalise gastric mucosal microcirculation.[4,5]

The therapeutic goal of treating peptic ulcer disease is to manage pain, treat the ulcer and prevent the ulcer recurrence.[6] The treatment available for peptic ulcer is aimed at decreasing the production of gastric acid, reinforcing gastric mucosal protection and reduction of stress. Though different anti-ulcer agents like Proton Pump Inhibitors (PPIs), H2 receptor blockers, prostaglandin analogues etc., are available, have their own limitations. The clinical evaluation of these drugs showed development of tolerance, incidence of relapses and adverse effects that make their efficacy arguable.[7] Thus, there is need for search of newer

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synthetic and herbal formulations which have lesser adverse effects with improved efficacy. The use of cytoprotective agents could be a new strategy that can help to modulate the antioxidant defenses in the body and thus prevent mucosal damage and gastric ulceration. As plants are rich sources of active principles and antioxidants, there has been a growing trend to identify and scientifically validate agents that have traditionally been used in ayurveda practice in the treatment of peptic ulcers and related diseases. About 80% of the world's population depends on traditional medicine for primary health care.[8]

Herbs are believed to be well compatible with the human body and to produce lesser side effects than modern medicine. In the Indian system of medicine, Emblica officinalis Gaertn or Phyllantus emblica linn is an important medicinal plant used for prevention and treatment of peptic ulcers. It is also used as stomachic, laxative, anti-oxidant, anti-inflammatory, antipyretic, etc.[9]

Objectives of the Study

- To screen the anti-ulcer activity of Emblica officinalis fruit extract in a swimming stress induced peptic ulcer model of albino rats.
- To compare the anti-ulcer effect of Emblica officinalis fruit extract with the standard drug omeprazole in preventing stress-induced ulcers.

MATERIALS AND METHODS

A) Animals: Albino rats.

B) Drugs and Chemicals: omeprazole, distilled water, and test compound.

C) Tools and Equipments: Feeding tube, scalpel, scissors, forceps, small beakers, stirring rods, thermocol, pins, petri dishes, glass cylinder (height 45cm, diameter 25cm), magnifying lens.

A) Animals: 28 Albino rats of either sex weighing between 150–250 grams were procured from the Central Animal House, J N Medical College, KLE University, Belagavi, which is registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and maintained under standard laboratory conditions. The animals were acclimatized under standard laboratory conditions for one week. They were housed in cages and maintained for 12 hours on a dark/light cycle and were fed with standard rat feed and water ad libitum was provided before starting the experiment.

B) Drugs and Chemicals

Omeprazole is used as the standard drug for peptic ulcers and the results obtained with the test drug were compared with those of omeprazole. For the study, Omeprazole (Reddy's Laboratories Limited) was diluted in distilled water and was administered orally at a dose of 10 mg/kg body weight.[10] Distilled water was used as the vehicle for the test compound, omeprazole - the standard drug.

Ethical clearance for use of the animals was obtained from the Institutional Animal Ethical Committee (IAEC) with the Certificate No BIMS, IEC/PG/47/2014.

Test compound: Aqueous extract of fruits of Emblica officinalis.

Preparation of the extract: Amla fruits [Figure 1] were bought from the local market in Belagavi, Karnataka. The fruits were sundried. For the preparation of the aqueous fruit extract, 100 g of the dried amla fruits were ground in an electrical grinder and soaked in 500 ml distilled water.[11] The mixture was left with a magnetic stirrer at room temperature for 24 hours. Twenty-four hours later, the mixture was strained out using a fine sieve and the crude extract air-evaporated for 3 days.[12] The aqueous fruit extract was then orally administered to the rats in the dose of 250 mg/kg p.o and 500 mg/kg p.o.[13] Doses of the drugs were calculated for each animal based on the body weight and respective volumes were administered using the rat feeding tube.

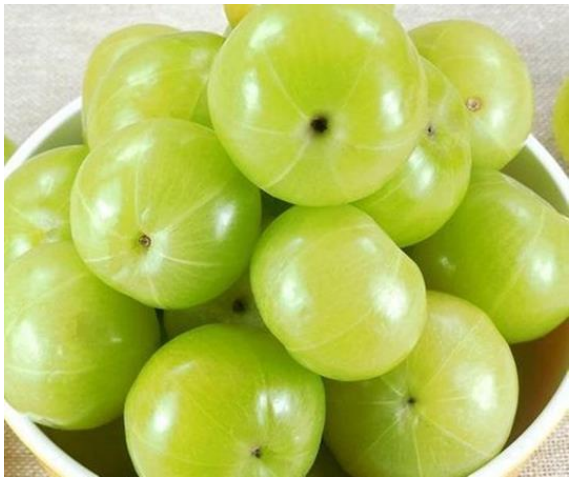

Figure 1: Amla fruit – Emblica officinalis

METHODS

Swimming Stress Induced Peptic Ulcer Model.[14]

Stress ulcers were induced in albino rats by forced swimming for 3 hours in a glass cylinder (height 45cm, diameter 25cm) containing water to the height of 35cm and maintained at 25 degree. Albino rats weighing between 150–250 grams were divided into 4 groups consisting of 7 in each group. The rats were fasted for 24 hours and forced to swim after 30 minutes of administration of standard and test drugs.

Group 1: Control rats received distilled water orally and after 30 minutes, the rats were forced to swim for 3 hours.

Group 2: Standard rats received omeprazole 10 mg/kg orally and after 30 minutes, the rats were forced to swim for 3 hours.

Group 3: Test rats received a test drug at the dose of 250 mg/kg orally and after 30 minutes, the rats were forced to swim for 3 hours.

Group 4: Test rats received a test drug at the dose of 500 mg/kg orally and after 30 minutes, the rats were forced to swim for 3 hours. Then the rats were sacrificed under light ether anesthesia, the stomach was opened along the greater curvature, and the inner

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surface was gently washed with cold normal saline and examined for ulceration with a magnifying lens. Severity score was determined by calculating ulcer index and percentage inhibition.

Calculation of Ulcer Index and Percentage Inhibition of Ulceration

The ulcers were examined and scored according to the method described by Laurence and Bacharach.[15]

Observation	Grading
Normal	0
Scattered hemorrhagic spots	1
Deeper hemorrhagic spots and some ulcers	2
Hemorrhagic spots and ulcers	3
Perforation	4

The ulcer index was calculated for each group by the Sunita and Devdas method.[16]

Ulcer Index = Arithmetic mean of the intensity in a group + [Ulcer positive No x 2] /Total no of rats Percentage Inhibition of Ulceration = Ulcer index control – Ulcer index test x 100 /Ulcer Index control Statistical Analysis The total severity score is expressed as Mean ± Standard Deviation (S. D) (n=7). The severity scores of control groups are compared with the standard and test groups with Mann-Whitney U test using SPSS (Statistical package for Social Sciences software) Statistics version 22. Significance was established with a probability value < 0.05.

RESULTS

Swimming Stress Induced Peptic Ulcer Model.

The rats in the control group were made to swim for 3 hours to induce stress. The examination of the inner surface of the stomach with a magnifying lens, 5 out of 7 rats showed excessive gastric secretions and gastric ulceration mainly in the glandular portions [Figure 2], with scattered hemorrhagic spots, and the remaining 2 rats showed scattered superficial hemorrhagic spots with minimal amount of gastric juice. The total severity score was 1.71±0.49 and Ulcer Index was 3.14. [Table 1]

In the standard group (Group 2), 5 out of 7 rats showed scattered superficial hemorrhagic spots with a lower amount of gastric juice secretion, and the gastric mucosa of the other 2 rats appeared normal. The total severity score, Ulcer index and Percentage Inhibition were 0.71±0.49, 0.71 and 77.39% respectively. When the Total Severity Score of control was compared with the standard group, the P value was < 0.05 and hence it was statistically significant.

Table 1: Effect of Standard drug Omeprazole and E. officinalis on swimming stress induced ulcers.

Groups	Total Score	Mean	Median	IQR	Mean ± SD Total Severity Score	Ulcer Index	Percentage Inhibition
Group 1 (Control)	12	1.71	2.00	1.00	1.71±0.49	3.14	00
Group 2 (Standard)	5	0.71	1.00	1.00	0.71±0.49*	0.71	77.39%
Group 3 (Test 1)	8	1.14	1.00	0.00	1.41±0.38*	1.43	54.46%
Group 4 (Test 2)	6	0.86	1.00	0.00	0.86±0.38*	0.86	72.61%

Test 1 (Group 3): treated with aqueous extract of fruits of E. officinalis 250 mg/kg body weight, only 1 rat showed gastric ulcers and 6 showed only congestion of gastric mucosa with some hemorrhagic spots out of 7 rats. The total severity score, Ulcer index and Percentage Inhibition were 1.41±0.38, 1.43 and 54.46% respectively. When the Total Severity Score of the control group was compared with Test 1, P value was < 0.05 and hence it was statistically significant, and when the standard was compared with test 1, the P value was > 0.05 and was statistically insignificant.

Test 2 (Group 4) treated with aqueous extract of fruits of E. officinalis 500 mg/kg body weight, 6 out of 7 rats showed only scattered hemorrhagic spots and gastric mucosa of one of the rats appeared normal. The total severity score, Ulcer index and Percentage Inhibition were 0.86±0.38, 0.86 and 72.61% respectively. When the Total Severity Score of the control group was compared with Test 2, the P value was < 0.05 and hence it was statistically significant. When standard was compared with test 2 and test 1 with test 2 both showed a P value > 0.05 and were statistically insignificant.

The total severity Score expressed as Mean ± Standard Deviation (n=7). *P value < 0.05 is significant, when control is compared with the standard drug omeprazole (10 mg/kg), Test 1 (EO 250 mg/kg) and Test 2 (EO 500 mg/kg) using Mann Whitney U Test. IQR: Interquartile range.

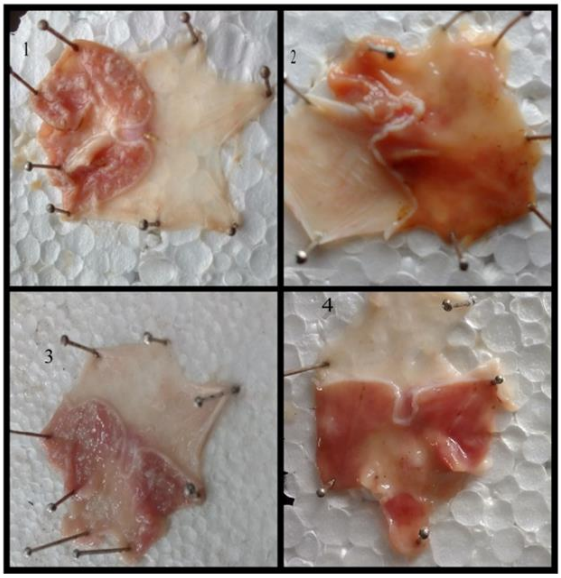

Figure 2: Swimming stress Induced Peptic Ulcer Model: 1. Control, 2. Standard, 3. Test 1, 4. Test 2.

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DISCUSSION

In this study, the gastroprotective effect of Emblica officinalis was screened in a swimming stress induced peptic ulcer model.

Swimming stress was chosen to induce gastric ulcers in rats because stress plays a significant role in the pathogenesis of gastric ulcers in human beings. The incidence of stress-induced acute gastric injury secondary to other serious medical conditions is as high as 1.5% and is usually associated with significantly higher morbidity and mortality.[17] Stress-induced gastric ulceration is a typical example of stress-associated organ injuries.[18] Swimming stress induced model resembles the clinical acute gastric ulcerations caused by trauma, surgery, or sepsis and has been accepted for studying stress ulceration. The ease of production of ulcers in this model is an advantage. The suspected pathophysiological mechanism responsible for acute gastric lesion development involves inflammatory infiltration, endothelial dysfunction, free radical oxygen mediated cell membrane damage in response to mucosal damage.[19] The treatment should be aimed at preventing the above pathophysiology and hence reducing the risk of stress induced gastric mucosal damage.

The results showed that E. officinalis in the doses of 250 mg/kg and 500 mg/kg is gastro protective in swimming stress induced gastric ulcers (P value < 0.05). The congestion and hemorrhagic lesions were comparatively less with the test dose of 500 mg/kg body weight than the test dose of 250 mg/kg body weight. There was significant reduction in total severity score and gastric juice secretion in the test 1 and test 2 groups. When the ulcer index and percentage inhibition of ulceration by the standard drug omeprazole was compared with EO in the dose of 250 mg/kg and 500 mg/kg body weight, the difference was insignificant and also the total severity score of standard and test 1, standard and test 2, and test 1 and test 2, when compared using Mann Whitney U test, the difference is statistically insignificant. The gastro-protective role of EO may be due to its rich composition of tannins, where the fruit alone contains 28% of the total tannins distributed in the whole plant. It contains two hydrolysable tannins, Emblicanin A and B, which have antioxidant properties. It is also a rich source of vitamin C, flavanoids and other phytochemicals known to have antioxidant property. Flavanoids isolated from EO are reported to lower the Lipid peroxidation (LPO) levels in rats.

In a study by Sai Ram et al., methanolic extract of fruits of EO showed significant antioxidant effect in stressed animals and did not have any effect on cell proliferation in terms of DNA or glandular weight.[20] It has been shown that an increase in acid production is mainly associated with the occurrence of stress-induced ulceration.[21] The mechanism of action of H2 Receptor blockers and the PPIs is mainly by decreasing the acid secretion. Hence, in this model, it appears that the efficacy of the test compound may be due to its anti-oxidant and anti-secretory properties. Further biochemical and histological studies may help us to know the exact mechanism of action of EO.

CONCLUSION

The present study was done to screen the anti-ulcer activity of aqueous extract of fruit of Emblica officinalis in albino rats-which revealed its gastroprotective activity in swimming stress-induced peptic ulcer models. When the test groups treated with 2 doses of EO (250 mg/kg and 500 mg/kg body weight) were compared with the standard group pretreated with omeprazole, the difference in results did not show any statistical significance. Hence, we can conclude that the efficacy is comparable to omeprazole (10 mg/kg body weight). The gastroprotective activity may be due to its anti-oxidant and anti-secretory property. Amla is considered as the wonder berry in ayurveda and is used in almost all the diseases of mankind including the PUD. This study proved the gastroprotective action of EO. Further toxicological, histological, biochemical and molecular studies may help us determine the safety and efficacy of the EO.

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