

**Evaluation of Antiulcer Activity of Abelmoschus Esculentus in Laboratory Animals**

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Conflicts of Interest: Nil.

Abstract

The objective of present study is to evaluate the anti ulcer activity of methanolic extract of seeds of *Abelmoschus esculentus*. The cause of ulceration in patients is mainly due to hypersecretion of gastric juice and pepsin. In traditional system of medicine a number of herbal preparations have been used for the treatment of peptic ulcers. The anti-ulcer activity of methanolic extract of *Abelmoschus esculentus* seeds was investigated by aspirin plus pylorus ligation induced gastric ulcer in rats, Indomethacin induced ulcer in rats, water immersion stress test induced ulcer in rats. In aspirin plus pylorus ligation model, *Abelmoschus esculentus* at doses of 200 and 400 mg/kg produced significant reduction in gastric volume, free acidity and ulcer index compared to control. In Indomethacin and water immersion stress test induced ulcer models both doses (200mg/kg & 400mg/kg) of *Abelmoschus esculentus* extract significantly reduced severity of ulceration. This present study indicates *Abelmoschus esculentus* seeds extract have potential anti ulcer activity.

Keywords: Antiulcer, *Abelmoschus esculentus*, Indomethacin.

Introduction

Peptic ulcer is an excoriated area of the gastric or duodenal mucosa caused by action of the gastric juice. It is a chronic and recurrent disease, and is the most

predominant of the gastrointestinal diseases¹. It is generally recognized that peptic ulcer is caused by a lack of equilibrium between the gastric aggressive factors and the mucosal defensive factors [1].

Gastric ulcer is among the most serious diseases in the world. The etiology of gastroduodenal ulcers is influenced by various aggressive and defensive factors such as acid-pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents such as prostaglandins and epidermic growth factors [3].

Some other factors, such as inadequate dietary habits, excessive ingestion of non-steroidal anti-inflammatory agents, stress, hereditary predisposition and infection by *Helicobacter pylori*, may be responsible for the development of peptic ulcer [4].

Although a number of antiulcer drugs such as H₂ receptor antagonists, proton pump inhibitors and cytoprotectants are available for ulceration all these drugs have side effects and limitations. Herbal medicine deals with plants and plant extracts in treating diseases. These medicines are considered safer because of the natural ingredients with no side effects [5].

Abelmoschus esculentus synonym of okra, known in many English-speaking countries as lady's fingers or gumbo is a flowering plant in the mallow family [8]. It is valued for its edible green seed pods. It is an important vegetable and

widely distributed from Africa to Asia, Southern Europe and America [9] In Asia, okra is typically prepared as traditional medicine as a dietary meal in the treatment of gastric irritations [10]. The plant has a wide range of medicinal value and has been used to control various diseases and disorders. The fiber in okra helps to stabilize blood sugar by regulating the rate at which sugar is absorbed from the intestinal tract. It is a good vegetable for those feeling weak, exhausted, and suffering from depression and it is also used in ulcers, lung inflammation, sore throat as well as irritable bowel. Okra is good for asthma patients and it also normalizes blood sugar and cholesterol levels [11,12]. Previous studies reported that okra polysaccharide possesses anti complementary and hypoglycemic activity in normal mice [13]. Also, okra polysaccharide lowers cholesterol level in blood and may prevent cancer by its ability to bind bile acids [10,14]. Based on the above scientific data, current literature research revealed that gastric ulcer prevention/healing is scientifically not yet documented. Therefore, the present study was aimed to investigate antiulcer potential of *Abelmoschus esculentus* extract in laboratory rats.

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Evaluation of Antiulcer Activity:

Aspirin plus Pylorus Ligation induced Ulcer in rats:

The rats were divided into 5 groups of 6 each. The ulcer was induced from group II to group V by oral administration of Aspirin (200mg/kg) for 3 days and pylorus was ligated on the fourth day following 36 hour fasting. The group I was served as normal control. All the drug solutions were prepared using 0.1% Tween 80 as emulsifying agent and given 0.2 ml/200g of body weight, 1hour prior to pylorus ligation. The different groups were assigned as described below [11,12].

Group I: Vehicle control (0.1 % Tween 80)

Group II: Ulcer control, Aspirin (200mg/kg)

Group III: Ulcer control + Omeprazole (20mg/kg)

Group IV: Ulcer control + *Abelmoschus esculentus seed extract* (200 mg/kg)

Group V: Ulcer control + *Abelmoschus esculentus seed extract* (400 mg/kg).

4 hours after the pyloric ligation, the animals were sacrificed by decapitation. The stomach was removed, opened along with greater curvature and the ulcer index was determined. The gastric content was titrated against 0.1N NaOH to find out the free acidity and total acidity.

Indomethacin- induced Gastric Mucosal Damage:

Wistar albino rats (180 to 200 g) were randomly divided into 5 groups of 6 each. The ulcer was induced from group II to group V by oral administration of Indomethacin (20 mg/kg). The group I was served as normal control. All the drug solutions were prepared using 0.1% Tween 80 as emulsifying agent and given 0.2 ml/200g of body weight, 10 minute prior to oral Indomethacin administration. The different groups were assigned as described below [10, 11].

Group I: Vehicle control (0.1% Tween 80)

Group II: Ulcer control, Indomethacin (20mg/kg)

Group III: Ulcer control + Omeprazole (20mg/kg)

Group IV: Ulcer control + *Abelmoschus esculentus* extract (200mg/kg)

Group V: Ulcer control + *Abelmoschus esculentus* extract (400 mg/kg)

After 6 hours Indomethacin administration, rats were sacrificed by decapitation and 2% v/v formal saline injected into totally ligated stomach for overnight storage. The next day, stomach was opened along with greater curvature, washed with warmed water and examined under a 3-fold magnifier. The length of the longest diameters of the lesions were measured and summated to give a total lesion score (in mm) for each animal. The mean count of measured lesions of each animal for each group was calculated. Inhibition of the lesion production was expressed as percentage value.

Water Immersion Stress induced Ulcer: Wistar albino rats (180 to 200 g) were randomly divided into 5 groups of 6 each. The ulcer was induced from group II to group V by fasting the animals for 24 hours and forced to swim in the glass cylinder (height 45 cm, diameter 25 cm) containing water to the height of 35 cm maintained at 25°C for 3 hours. The group I was served as normal control. All the drug solutions were prepared using

0.1% Tween 80 as emulsifying agent and given 0.2 ml/200g of body weight, 10 minute prior to forced swimming. The different groups were assigned as described below [12].

Group I: Vehicle control (0.1 % Tween 80),

Group II: Swim stress control (0.1 % Tween 80)

Group III: Swim stress + Omeprazole (20mg/kg)

Group IV: Swim stress + *Abelmoschus esculentus* seed extract (200mg/kg)

Group V: Swim stress + *Abelmoschus esculentus* seed extract (400mg/kg).

Immediately after the swim, the rats were sacrificed by decapitation and stomach of each animal was removed, opened along with greater curvature, washed with warmed water and the extent of gastric damage was assessed.

Statistical Analysis

The statistical analysis of all the results was carried out using one- way ANOVA followed by Dunnet's multiple comparisons test using graph pad in stat 3 and all the results obtained in the study were compared with the vehicle control group.

Results

In aspirin plus pylorus ligation induced gastric ulcer model, the low and high dose of methanolic seed extract of *Abelmoschus esculentus* showed significant ($P < 0.05$) and ($P < 0.001$) reduction in gastric volume, free acidity and ulcer index respectively as compared to control [Table 1, Figure 1].

Table 1: Effect of Methanolic Extract of *Abelmoschus Esculentus* Seeds on Aspirin plus Pylorus Ligation Induced Gastric Ulcer in Rats.

S.No	Treatment	Volume of gastric secretion	Total acidity meq/Lit	Ulcer index	Ulcer Inhibition %
1	Control	2.739	117 ±5.81	0.907±0.010	-----
2	Swim stress control	2.870	115.36±0.8	0.908±0.005	-----
3	Omeprazole	0.832	25.36±0.8	0.064±0.005	92.95
4	A. Esculentus(200 mg/kg)	1.542	84.4±1.87	0.478±0.021	47.45
5	A. Esculentus (400 mg/kg)	0.964	47.72±0.17	0.238±0.013	74

Values are expressed as mean ± SEM, n=6, *P<0.05, ***P<0.001 as compared to control.

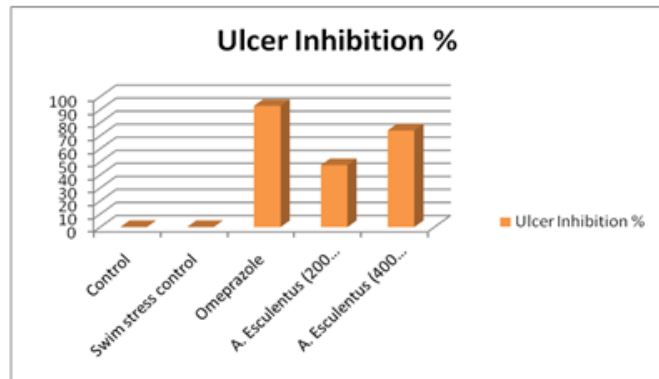


Figure 1: Ulcer inhibition of treatment groups in aspirin plus pylorus ligation induced gastric ulcer in rats.

In Indomethacin induced gastric mucosal damage model, methanolic extract of *A. esculentus* seeds significantly reduces the incidence and severity of ulceration. The extract of *A.esculentus* seeds showed ulcer protection 42.22% and 78.16% in 200 mg/kg and 400mg/kg doses respectively whereas the reference drug Omeprazole exhibited 90.15% protection [Table 2, Figure 2].

Table 2: Effect of Methanolic Extract of *Abelmoschus Esculentus* Seeds on Indomethacin Induced Gastric Ulcer in Rats.

S.No	Treatment	Ulcer index	Ulcer Inhibition %
1	Control	0.730±0.014	-----
2	Indomethacin (20mg/kg)	0.078±0.008	88.15
3	Omeprazole	0.080±0.008	90.15
4	A. Esculentus (200 mg/kg)	0.424±0.011	42.22
5	A. Esculentus (400 mg/kg)	0.174±0.008	78.16

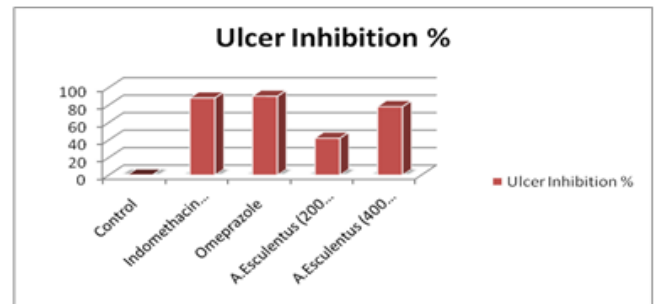


Figure 2: Ulcer inhibition of treatment groups in indomethacin induced gastric ulcer in rats. Values are expressed as mean ± SEM n=6 *P<0.05, ***P<0.001 as compared to control.

In water immersion stress induced ulcer model *A. Esculentus* showed significant reduction in severity of ulceration. *A. Esculentus* showed ulcer protection 41.17% and 87.46% in 200mg/kg and 400mg/kg doses respectively whereas standard drug Omeprazole 20mg/kg exhibited 92.42% protection [Table 3, Figure 3].

Table 3: Effect of Methanolic Extract of *Abelmoschus Esculentus* Seeds on Water Immersion Stress Induced Gastric Ulcer in Rats.

S.No	Treatment	Ulcer index	Ulcer Inhibition %
1	Control	0.782±0.054	-----
2	Swim stress control	0.790±0.003	-----
3	Omeprazole	0.068±0.003	92.42
4	A. Esculentus (200 mg/kg)	0.460±0.013	41.17
5	A. Esculentus (400 mg/kg)	0.098±0.005	87.46

Values are expressed as mean ± SEM, n=6, *P<0.05, ***P<0.001 as compared to control

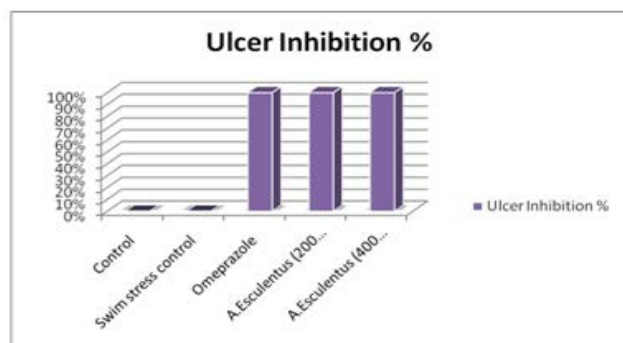


Figure 3: Ulcer inhibition of treatment groups in water immersion stress induced gastric ulcer in rats.

Discussion

The peptic ulcer results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms¹³. To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defence mechanisms by increasing mucosal protection, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis. The causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid¹⁴.

The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach. The lesions produced by this method are located in the lumen region of the stomach. The *Abelmoschus esculentus* seeds extract and Omeprazole significantly decreased the total acidity; this suggests that it having an antisecretory effect as well as markedly significant reduction in ulcer index.

Non-steroidal anti-inflammatory agents, like Indomethacin induce gastric lesions in experimental animals by inhibition of gastric cyclooxygenase resulting in less formation of endogenous prostaglandin; Indomethacin also inhibits gastro duodenal bicarbonate secretion as well as gastric mucosal blood flow. The model shows drug's effect on cytoprotection through non prostaglandin mediated mechanism. The extract shows protection against characteristic lesions produced by Indomethacin administration. This antiulcer effect of

Abelmoschus esculentus seeds extract may be due to both reductions in gastric acid secretion and gastric cytoprotection¹⁵.

Water immersion stress is one of the best models for stress induced ulcer in animals. The model provides both emotional stress as well as physiological stress to the animal. The extract showed significant ulcer inhibition. The anti ulcer effect observed in the present study might be due to a possible relationship between protection of mucosal injury, inhibition of acid secretion and the antioxidant nature of *A. Esculentus* extract. Further studies are needed for their exact mechanism of action on gastric acid secretion and gastric cytoprotection.

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