Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2011: 3 (5) 183-188
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0974-248X
USA CODEN: DPLEB4

Alteration of gastric mucus secretion in rats treated with *Abelmoschus esculentus* seed mucilage

Shrikant V. Joshi[a*], Kalyani A. Kedar[b], Urvashi V. Markana[c], Sandesh R. Lodha[a], Payal D. Shah[a], Heta G. Vyas[a], Ruchi B. Vyas[a], Bhavin A. Vyas[a], Gajanan G. Kalyankar[a].

[a]Maliba Pharmacy College, Gopal Vidyanagar, Tarsadi, Dist. Surat (Gujarat), India
[b]Modern College of Pharmacy, Yamunanagar, Nigdi, Pune, (Maharashtra) India.
[c]Injectcare Parenterals Private Limited, Vapi (Gujarat) India.

ABSTRACT

In Indian folk medicine, Abelmoschus esculentus (L.) Moench seed mucilage is used for the treatment of gastric ulcer and duodenal ulcer. Among the mechanisms which protect the gastric mucosa against acute attack by necrotic agents of different types, mucus production plays crucial role. Mucilage of A. esculentus at dose of 1g/kg significantly inhibits the ulcer induced by indomethacin, ethanol and water immersion restraint stress but do not affect gastric volume, acidity and gastric pH. Pretreatment with the test significantly increased the amount of gastric mucus content in ethanol-ulcerated rats. In conclusion, cytoprotection may be because of formation of a protective layer with increase in mucous secretion form the superficial epithelial cells.

Key words: *Abelmoschus esculentus*, mucilage, gastric ulcer, gastric mucus.

INTRODUCTION

Mature *Abelmoschus esculentus* (Family-Malvaceae) fruits are cooked as a vegetable in India and known as a dietary meal good for gastric irritations, due to its high-mucilage content. A crude polysaccharide from the fruits was reported previously, as a preventive measure against *Helicobacter pylori* induced ulcer. The activity thought to be exhibited by its potent antiadhesive effects, which prevents adhesion of *H. pylori* to human gastric mucosa, due to synergic effects of both the glycan and the protein fractions of a crude polysaccharide in blocking bacterial surface receptors that coordinate the interaction between host and parasite. Moreover, okra seeds contain fixed oil is regarded as antispasmodic and stomachic [1].

The paste prepared from the crushed seeds of *A. esculentus* is being used by traditional herbal medical practitioner known well as “Vaidya” in India, for prevention and treatment of “*pitta*” or Gastric acidity and gastric and duodenal ulcers. It was found that the paste not only cures the ulcer due to *H. pylori* infection but also due to hypersecretion of acid in stomach.

The common preparation type, which is practiced in remote area of eastern parts of Maharashtra, India is to use the water based paste; the whole matured fruits of *A. esculentus* are cut into pieces and put inside a metal container (preferably of Copper) with water for about 2 or 3 weeks (cut pieces of fruit soaks the water), then they are homogenized by pressing with a hard material, i.e. mortar and pestle. This paste is stored to use for the treatment of gastric and duodenal ulcers (*pitta*), internally. A tablespoon of paste should be ingested 3-4 times a day.

In present study, efforts were made to confirm antiulcer activity of mucilage obtained from crushed seeds of *A. esculentus* which is not of *H. pylori* origin, and to comment on possible mechanism involve.

MATERIALS AND METHODS

Plant material

Fresh fruit of plant was purchased from local market, Moshi, Pune. and authenticated by Dr. P. G. Diwakar, Deputy Director, Botanical Survey of India, Pune (Voucher specimen no. UVMAE1).

Extraction and isolation of mucilage [2]

The seeds were sliced, homogenised with five times its weight of water, centrifuged at 4000g for 15 min and the clear, viscous solution decanted. The solution was heated at 70°C for 5 min to inactivate enzymes, and recentrifuged. The mucilage was precipitated with three volumes of ethanol and washed with more ethanol followed by acetone. The cream coloured solid was dried under vacuum (less than 1 Torr at 25°C for 12 h) and gave a yield of 16 g mucilage/kg of *A. esculentus*.

Purification of the mucilage [2]

The crude mucilage (1 %) were homogenised (Potter homogeniser) with cold dilute trichloroacetic acid solution (5 %). The solution was centrifuged (3500 g for 20 min), neutralised with sodium hydroxide by dropwise addition, and then dialysed for 30 h against distilled water. The mucilage was reprecipitated with ethanol (three volumes), washed successively with ethanol, acetone and diethyl ether and dried in rotary evaporator.

Animals

Wistar Strain Albino rat of either sex (120-180 gm), obtained from Animal house, Department of Pharmacology, Maliba Pharmacy College, Tarsadi were divided into group of six animals. housed in PVC cages under standard condition (12:12 hour light/ dark cycle at 25 ± 2^0C, Humidity 70-75%). Each were placed in cages with grating floor to avoid corphrophagy and fasted for 48 h allowing free access to water. Control group animals received same experimental handling as those of test groups except that the drug treatment was replaced by administration of appropriate volumes of dosing vehicle. Dried mucilage of *A. esculentus* was dissolved in water (AEaq) and administered to animals in dose 1g/kg by means of a gavage.

Ethanol induced gastric ulcer [3]

Test sample (AEaq) was administered orally 60 min before the oral administration of ethanol 96% (1 ml) to a group of six rats. Later (1 h), the animals were sacrificed with an over-dose of ether. The stomach was then removed and examined for gastric ulcers.

Indomethacin-induced gastric ulcers [4]
Test sample (AEaq) was administered orally to 48 h fasted rats 60 min prior to induction of gastric ulcers by indomethacin suspended in 0.5% carboxymethylcellulose at a single i.p. dose of 30 mg/kg. After 5 h the rats were sacrificed and examined for gastric ulcers.

Restraint water immersion stress-induced gastric ulcers [5]
Test sample (AEaq) was administered orally to 48 h fasted rats. Sixty minutes later, rats were restrained individually in stainless steel cages and immersed up to their xiphoid in a water bath maintained at 22±2 ^{0}C. After 5 h of this exposure, the rats were sacrificed and examined for gastric ulcers.

Evaluation of the gastric ulcer Lesion area [6]
Gastric ulcer lesion area was measured as described by Khan 2004. In brief the stomach samples were flattened and carefully sandwiched between the two layers of a transparent plastic folder of A4 size. The specimens within the plastic folder were scanned and using a Scion Image software lesion area was measured.

Pylorus ligation [7]
AEaq was administered orally to 48 h fasted rats. One hour later, pylorus ligation as described previously was performed. Briefly, rats were lightly anesthetized by ether. The abdomen was opened and the pylorus was ligated. The abdomen was closed by suturing. The animals were sacrificed 5 h later by an overdose of ether. The stomach was removed and its content was subjected to measurement of volume and pH and assayed for free and total acidity.

Determination of gastric wall mucus content [8]
Gastric wall mucus was determined by the Alcian blue method [9]. Briefly, the AEaq was administered orally to 48 h fasted rats 60 min prior to induction of gastric ulcers by 1.0 ml of ethanol (96%) p.o. Sixty minutes later, the animals were sacrificed and the stomach was excised and opened along the lesser curvature, weighed and immersed in 0.1% w/v Alcian blue solution for 2 h. The excessive dye was then removed by two successive rinses in 0.25M sucrose solution. Dye complexed with gastric wall mucus was extracted with 0.5M $MgCl_2$ for 2 h. The blue extract was then shaken vigorously with an equal volume of diethyl ether and the resulting emulsion was centrifuged. The optical density of Alcian blue in the aqueous layer was read against a buffer blank at 580 nm using a spectrophotometer. The quantity of Alcian blue extract per gram wet stomach was then calculated from a standard curve.

Statistical analysis of data
Results were expressed as Mean ± S.E.M. The statistical difference between the test groups and that of the control was calculated by using Student's t-test and $p < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

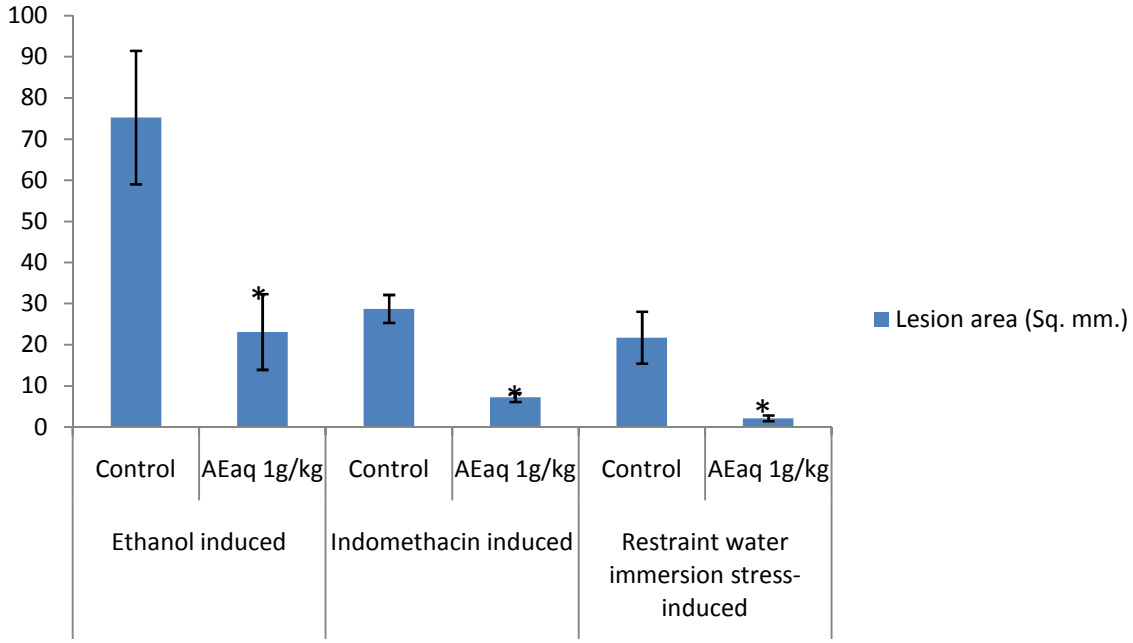
According to the experimental models used in this study, i.e. NSAIDs like indomethacin induce ulcer may inhibits cytoprotective PGs like PGE_2 and PGI_2 of gastric and duodenal mucosa responsible for mucus production and maintaining cellular integrity of the gastric mucosa. In the ethanol induced gastric ulceration model, ethanol produces necrotic lesions by direct necrotizing action which in turn reduces defensive factors, the secretion of bicarbonate and production of mucus. Whereas increased gastric acid secretion, and decreased mucosal microcirculation and mucus content were the major causes of ulcer in water immersion stress-induced ulcers [10].

Table 1 : Effect of AE_{aq} on Gastric volume, pH, free and total acidity in Pylorus ligated rats

Groups	Gastric Volume (ml)	pH	Free Acidity	Total Acidity
Control	6.87 ± 0.21	2.23 ± 0.18	42.98±1.39	80.09±1.76
AEaq (1g/kg)	6.21 ± 0.11	2.97 ± 0.07	40.67±0.94	79.35±1.22

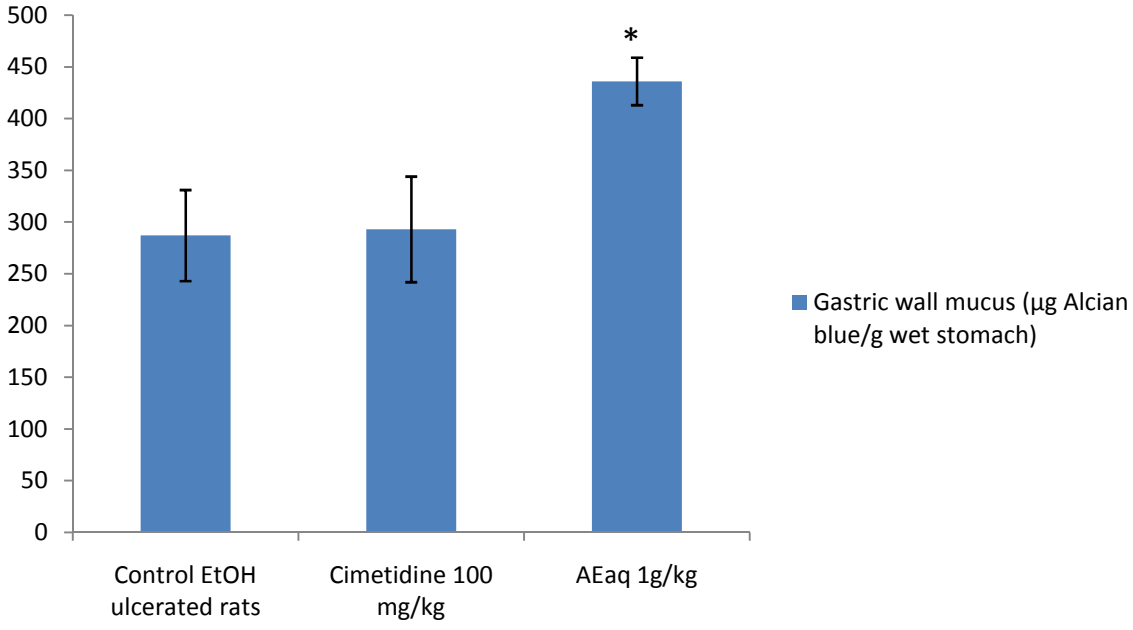
Note: Data expressed as mean±S.E.M. (n = 06).

Figure 1: Effects of AE_{aq} against experimentally induced gastric ulcer in rats.



*Data expressed as mean±S.E.M. (n = 06). * - Signifi cantly different from control rats (p < 0.01).*

Figure 2 : Effects of AE_{aq} on Gastric wall mucus content in rats.



*Data expressed as mean±S.E.M. (n = 06). * - Signifi cantly different from control rats (p < 0.01).*

In the model of gastric ulcer induced by indomethacin, ethanol and water immersion restraint stress, oral administration of the AEaq at dose of 1g/kg significantly inhibits the ulcer (Figure 1). Whereas in pylorus-ligated rats (Table 1), the test sample neither decrease the gastric volume and acidity nor the gastric pH, while cimetidine, a specific H_2-receptor antagonist could (data not shown). This suggests that the anti-gastric ulcer effect of AEaq is unlikely due to anti-secretary action. It was also found that pretreatment with the paste of *A. esculentus* significantly increased the amount of gastric mucus content in ethanol-ulcerated rats (Figure 2).

The major components of *A. esculentus* mucilage are carbohydrate-containing polymers along with proteins and some minerals. Recent studies demonstrated antiulcer activity of polysaccharide fraction from plants [11]. The polysaccharides from *A. esculentus* mucilage probably may affect the gastrointestinal mucosa regeneration or may form a protective covering on it. Ethanol tends to dissolve the components of the mucous membrane of the stomach and lowers the level of tissue proteins, bringing gastric blood flow to a standstill [12] but the damage due to ulcerogenic agent get inhibited with pretreatment of AEaq.

Ulcer healing takes place either by a regeneration process which starts from the neck cells of the glands or by a rapid process of migration of cells towards the luminal surface and their deposition on the area stripped by the ulcerogenic agent. Among the mechanisms which protect the gastric mucosa against acute attack by necrotic agents of different types, mucus production plays crucial role. This depends on a delicate balance of factors which control its synthesis and the protein, glycoprotein and lipid composition necessary to give it the right viscosity and its characteristic hydrophobicity [13].

The cytoprotective effect of AEaq can be attributed to the physico-chemical properties of mucilage. When administered as a preventive therapy, AEaq keeps the gastric mucosa under normal conditions (data not shown). In fact this treatment prevents mucus dissolution induced by ethanol. PGI_2 is known to increase the mucus production in superficial epithelial cells, which get inhibited with the use of NSAIDs like indomethacin. Cytoprotective effect of AEaq in indomethacin induced ulcer along with cytoprotection in ethanol and restraint water immersion stress-induced gastric ulcers in addition to uncertainty of anti-secretary action supports the hypothesis of formation of a protective layer as a major mechanism with increase in mucous secretion from the superficial epithelial cells.

Further studies, concerning the isolation of active fraction from AEaq and the exact mechanism of action are currently in progress in our laboratory.

REFERENCES

[1] Gurbuz, I., Ustun, O., Yesilada, E., Sezik, E., Kutsal, O. **2003**. *Journal of Ethnopharmacology* 88: 93–97.
[2] Woolfe, M.L., Chaplid, M.F. and Otchere, G. **1977**. *J. Sci. Fd Agric*.28: 519-529.
[3] Castro, G.A., Carvalho, J.E., Tinti, S.V., Possenti, A., Sgarbieri, V.C. **2010**. *J Med Food.* (1):83-90.
[4] Okokon, J.E., Antia, B.S., Umoh, E.E. **2009**. *Afr J Tradit Complement Altern Med.* 6(2):150-4.
[5] Brzozowski, T., Zwirska-Korczala, K., Konturek, P.C., Konturek, S.J., Sliwowski, Z., Pawlik, M., Kwiecien, S., Drozdowicz, D., Mazurkiewicz-Janik, M., Bielanski, W., Pawlik, W.W. **2007**. *J Physiol Pharmacol*. 58 (6):53-64.
[6] Khan, H.A. **2004**. *Journal of Pharmacological and Toxicological Methods* 49:89– 95.

[7] Sood, S. and Muthuraman, A. **2009**. *Yakugaku Zasshi* 129(12) 1523-1528.
[8] Jainu, M., VijaiMohan, K., Shyamala, C.S. **2006**. *Indian J Med Res* 123,799-806.
[9] Corne, S.J., Morrisey, S.M., Woods, R.J., **1974**. *Journal of Physiology* 242, 116–117.
[10] Rujjanawate, C., Kanjanapothi, D., Amornlerdpison, D., Pojanagaroon, S. **2005**. *Journal of Ethnopharmacology* 102 :120–122.
[11] Srikanta, B.M., Siddaraju, M.N., Dharmesh, S.M. **2007**. *World J Gastroenterol*. 13(39): 5196-5207.
[12] Galati, E.M., Monforte, M.T., Tripodo, M.M., d'Aquino, A., Mondello, M.R. **2001**. *Journal of Ethnopharmacology* 76, 1–9.
[13] Galati, E.M., Pergolizzi, S., Miceli, N., Monforte, M.T., Tripodo, M.M. **2002**. *Journal of Ethnopharmacology* 83, 229-233.