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Research

Evaluation of Anti-Ulcer Activity of *Aegle Marmelos* Leave Extract In Experimental Animal Model

Ramana hechhu[1*], B.Sravani[2], Avanapu Srinivasa Rao[3], R.Ramya Krsishna[4] A.Prasad[5]

[1] Professor & Academic Co-ordinator, Department of Pharmaceutical Chemistry, Bhaskar Pharmacy College, Yenkapally, Moinabad, Ranga Reddy, Hyderabad, Telangana, India – 500075.
[2] Research Scholar, [3] Professor & Principal, [4,5] Assistant Professor, Department of Pharmacology, Bhaskar Pharmacy College, Yenkapally, Moinabad, Ranga Reddy, Hyderabad, Telangana, India-500075

*Author for Correspondence: Dr. Ramana Hechhu
Email: hechhu.ramana@gmail.com

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Abstract

The cause of ulceration in patients is mainly due to hypersecretion of gastric juice and also due to hypersecretion of pepsin. In traditional system of medicine a number of herbal preparations have been used for the treatment of peptic ulcers. There are various medicinal plants has been used for the treatment of gastrointestinal disorders. In view of this, in present study we have to evaluate the anti-ulcer activity of *Canthium Dicoccum*. Study was carried out, by using three methods i.e.,alcohol, paracetamol and stress induced ulcers in rats pretreated with the doses of 250 mg/kg AQCR and ALCR, 10mg/kg Omeoprazole and 50 mg/kg Ranitidine. In alcohol induced ulcer model, paracetamol induced ulcer model and stress induced model the ulcer index was determined. Where as in stress induced ulcers stress plays an important role in ulcerogenesis. In alcohol-induced ulcers, It was also effective in decreasing ulcer index in paracetamol-induced ulcers. All the results obtained with *Canthium Dicoccum* were dose dependent. The results suggest that AQCR and ALCR possesses significant and dose dependent antiulcer activity. The antiulcer activity of AQCR and ALCR can be attributed to its cytoprotective and antisecretory action.

Keywords: *Canthium Dicoccum*, antisecretory, cytoprotective, gastric ulcer, alcohol induced ulcers, paracetamol-induced ulcers and stress induced ulcers.

INTRODUCTION

GASTROINTESTINAL (GI) TRACT

In order to digest food, absorb nutrients and excrete unabsorbed waste products, the GI tract has to perform a no.of coordinated activities and the tract has to provide the whole body with a continual supply of water, electrolytes and nutrients[1]. In order

to achieve these objects, several organs have to integrate with each other and are regulated by nervous rand hormonal systems, as well as the central nervous system[2]. Thus for fully understanding physiological functions of GI tract and its various pathological states including peptic ulcer, diarrhea and constipation, etc., a brief description of some important headings is given below.

Control and Co-ordination of GI Tract

In addition to the main function of assimilation of food, the GI tract has to perform endocrine function and also gut has its own integrative neuronal network, the enteric nervous system(ENS) that shares about the same no.of neurons as those of spinal cord. Many of the neurotransmitters or neuromodulators and hormones in GI tract are peptides. The smooth muscles, blood vessels and glands (exocrine, endocrine and pancrine) are main elements under the neuronal and hormonal control.

a) Neuronal Control

The gut wall contains millions of enteric neurons, which are organized into two separate plexuses (myoenteric plexus also called Auerbach's plexus) in between the outer or longitudinal and the middle or circular muscles layers and the Meissner's plexus (submucous plexus) on the luminal side of the circular muscle layer. The former is continous from the upper oesophagus to the anus while the later is located continuosly only in small and large intestines[3,4]. These plexuses are interconnected and their ganglia are innervated with preganglionic parasympathetic fibres from vagus nerve that are mostly chol,inergic and often excitatory. The sympathetic fibres are mainly postganglionic which have endings in the plexuses where they inhibit the secretion of acetylcholine. The enteric nervous system has not only the sympathetic and parasympathetic neurons but also non-adrenergic and non-cholinergic neurons that secrete norepinephrine, acetylcholine, serotonin(5-HT), purines and certain peptides including substance-p, Vasoactive Intestinal Peptiode(VIP), somatostatin, enkephalins, bombesin, cholecystokinin and neurotensin[5,6]. In addition, Angiotensin II may also be secreted in its function. The sensory neurons that respond to chemical and mechanical stimuli are also present in enteric plexus. Therefore, both local reflexes as well as reflexes mediated through the celiac plexus and central nervous system (CNS) are involved to regulate activity of GI tract. The mucosal glands have also been found to possess many peptides, for example gastrin[7,8,9].

b) Hormonal Control

Gastrointestinal hormones include the peptides synthesized by mucosal cells e.g. gastrin and gastric inhibitory peptide (GIP). Gastric mucosa and wall of whole GI tract release paracrine secretions or local hormones of upper GI tract, which stimulate secretions and motility[10]. Neurotensin, peptide YY and enteroglucagon are general inhibitors of the secretion and motility that are released from ileum and colon in response to the nutrients (chime) into distal intestine. The glucose homeostasis is regulated by release of insulin and glucagon from pancreas[11,12,13].

Although pharmacologically impotant functions of GI tract include gastric secretions, motility of bowel, emesis (vomiting) and exreation of bile but gastric secretions are more relevant to the present work and have, therefore, been described in details on the following:

Gastric Exocrine secretions

Normally human stomach secretes about 2.5 litre of gastric juioce in 24 hours. The principle exocrine secretions include pepsinopgens, which are released from zymogens, or peptic or chief cells and hydrochloric acid (HCL) in addition to the intrinsic factor from the parietal cells. The gastric mucosa contains many deep as well as surface glands. Chief and parietal cells are located in the body fundus of stomach, while glands in the pyloric and cardiac regions secrete the mucosa. Thus, mucus is secreted throughout the gastric mucosa. In addition to mucus, bicarbonate ions (HCO_3^-) are secreted by mucus cells on the surface of epithelium in between the gastric glands. The gastric secretions released in response to food involve both neuronal and humoral mechanisms. They can be divided into two phases:

a) Gastric phase

It comprises of local neuronal reflexes in response to chemical stimuli (mucosal receptor mediated), eg. Effect of distension on receptors in the stomach wall. The presence of food in the stomach has been found to accelerate the increase in gastric secretions. The stretch and chemical receptors located in the walls of stomach and mucosa are activated. The Meissner's plexus receives the fibres from these receptors where their cell bodies are located. They synapse on postganglionic parasympathetic neurons, which innervate parietal cells and increase the acid secretions[14,15].

b) Intestinal phase

Chemical stimuli initiate this phase in the duodenum. The passage of food into duodenum inhibits the gastric acid and pepsin secretions as well as gastric motility through neural and hormonal mechanisms. Enterogastrone (an intestinal hormone) has been found to be mainly responsible for inhibition of gastric secretions. Apart from these mechanisms, the gastric secretions are stimulated by other factors as well, e.g. hypoglycemia, but some basal gastric secretion occurs even in the absence of food and other stimuli[16,17,18].

Gastric Motility and Emptying

The fundus and upper portion of body of stomach relax and accommodate the food upon its entry with little increase in pressure, which is known as "receptive relaxation". In the lower portion of body of stomach, motility begins which mixes and grinds

the food. The small portions of semisolid food are then passed through the pylorus and enter intoi duodenum. This receptive relaxation is triggered by pharynx and oesophageal moivements and is mediated by vagus nerve. The gastric basic electrical rhythm is controlled by the peristaltic waves, which initiaqte soon thereafter and sweeps towards the pylorus. This distal stomach contraction or "systole" lasts upto 10 seconds, which occurs 3-4 times per minute. Therefore, the antrum, pylorus and duodenum work apparently as a unit. So, the contraction waves of antrum pass down to pylorus and then duodenum in a sequence and propelling the crushed, well-mixed and semisolid food into the intestine[19].

Gastroduodenal Physiology

Food after mastication is swallowed into the stomach after passing through the oesophagus. The stomach stores, mixes and empties the chyme into the duodenum for digestion and absorption into the small intestine. The secretion of HCl, pepsin, gastrin, intrinsic factor and mucus in stomach helps in digestion of proteins mainly. The parietal cells are responsible for secretion of HCl and maintenance of acidic pH of stomach ranging from 1-2 reaching up to 6-7. The gastric epithelium is constantly exposed to acids/pepsin/bile salts in addition to exogenous medications, bacteria and alcohol. Thus a powerful defensive mechanism is required. This is provided by mucus bicarbonate layer which serves as a mucus gel impeding diffusions of harmful substances. A net imbalance in mucosal offensive and defensive factors play a major role in ulcer production[20]. Surface epithelial cells provide the next line of defence including mucus production, maintenance of pH and bicarbonate production. Any breach in pre epithelial barrier will cause migration of gastric epithelial cells to help in restitution. An alkaline pH and growth factors help in restitution. Epithelial cell regeneration is regulated by Prostaglandins (PG) and growth factors (GF). An adequate oxygen and uninterrupted blood supply is essential which is provided by angiogenesis activated by GF. PG plays a central role in providing this defence[21].

MATERIALS AND METHODS

The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goal(s) under the prescribed guidelines and recommendations. It includes in it all the steps from field trip to the observation including selection and collection of the medicinal plant, selection of dose value, standardization of protocol, usage of instruments, preparation of reagents, selection of specific solvents for extraction, formation of protocols and final execution of the standardized protocol. All this requires good build of mind and a good and soft technical hand to handle the materials and procedure in a true scientific manner.

Drugs and Chemicals

Drugs and Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India.

Table 1: Drugs and Chemicals

S.No	Materials	Company Name
1.	Cimetidine	Taj pharmaceuticals Ltd
2.	Omeprazole	Aurobindo pharma Ltd.
3.	Ranitidine	Aurobindo pharma Ltd.
4.	Alcohol	ChangshuYangyuan Chemicals, China.

Instruments

Following instruments were required for the study:

Table 2: List of Instruments used for study

Name of the instrument	Source
Centrifuge	Dolphin
Digital weighing balance	Horizon
Heating mantle	ASGI®
Disection box	Camel
Refrigerator	Videocon
Actophotometer	Dolphin
Glass cylinder	ASGI®
Adhesive tape	YVR medivision Pvt Ltd
Thread	YVR medivision Pvt Ltd
Stop watch	ASGI®
Syringes	YVR medivision Pvt Ltd
Needles	YVR medivision Pvt Ltd

Soxhlet extractor	ASGI®
Condenser	ASGI®
Burette stand	Dolphin
Round bottom flask	ASGI® , Amar
Mixer	Videocon
Oven	ASGI®
Water bath	ASGI®
Stirrer/glass rod	ASGI®
Watch glass	ASGI®
Whatmann filter paper	Manipore microproducts, Ghaizabad.
Butter paper	ASGI®
Spatula	ASGI®
Rubber pipes	ASGI®

Experimental animals

Wistar rats (150-200 g) and were procured from Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. All the animals were maintained under standard conditions, that is room temperature 26 ± 1°C, relative humidity 45 - 55% and12:12 h light – dark cycle. The animals were housed in large spacious hygienic cages during the course of the experimental period. Animal studies had approval of IAEC.

Plant Material Collection

The leaves of *Aegle marmelos* was collected from the Botanical garden and was identified and authenticated from Department. The plant material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.

Preparation of plant extracts

Preparation of Aqueous Extract

Fresh leaves of *Aegle marmelos* were collected and washed under tap water. The leaves extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of water. The contents were mixed well and then the mixture was boiled up to 80-100^{0}C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

Preparation of Alcoholic Extract

Fresh leaves of *Aegle marmelos* were collected and washed under tap water. The leaves extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of alcohol. The contents were mixed well and then the mixture was boiled up to 50-60^{0}C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

Selection of dose for animal study

The dose considered for the experiment on rats was obtained from conversion of human dose of *Aegle marmelos* (3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats (Ghosh 1984). Hence the calculated dose for the rats (considering human dose3 and 5 g/kg) is 200 mg/kg. Acute toxicity was done at dose of 2000mg/kg body weight.

Pharmacological evaluation

Preparation of extracts

The aqueous and alcoholic extracts of *Aegle marmelos* suspended in water in presence of 3%v/v Tween-80 solution. All the drugs were administered orally for experimental purpose. Each time preparations of the extracts were prepared when required. The drugs were administered at a constant volume of 10ml/kg for each animal.

ACUTE ORAL TOXICITY

The acute oral toxicity of aqueous and alcoholic extracts of *Aegle marmelos* was determined by using rats which were maintained under standard conditions. The animals were fasted 12 hour prior to the experiment, up and down procedure OECD guideline no. 425 were adopted for toxicity studies. Animals were administered with single dose of individual extract up to 2000mg/kg and observed for its mortality during 7days and 21days study period (long term) toxicity and observed up to 7days for their mortality, behavioral and neurological profiles.

RESULTS

Administration of the *Aegle marmelos* extracts *in* rats at doses of 250 mg/kg by oral gavage did not reveal any adverse effects or signs of toxicity. Observations twice daily for fourteen days also did not reveal any drug related observable changes or mortality. Accordingly, the acute oral LD50 of the extractives was concluded to exceed 2000 mg/kg body weight, the highest dose tested in the study.

Effect on alcohol induced gastric ulcers

Oral administration of 80% alcohol produced haemorrhagic gastric lesions in glandular portion of stomach. Pretreatment with AQAM and ALAM at the dose of 250 mg/kg and Omeprazole (10 mg/ kg) significantly ($p<0.001$) protected the gastric mucosa as shown by reduced values of lesion index (19.3 ± 0.35 and 27.47± 0.75 respectively) against alcohol challenge as compared to solvent control (26.14 ± 0.24).

Table 3: Effect of *Aegle marmelos* at various doses on alcohol induced gastric ulcer in rats.

Treatment (n=6)	Dose mg/kg (p.o.)	Lesion index	% Inhibition of ulcer	Mucus content (μg Alcian blue/g wet tissue)
1% CMC	-	26.14 ± 0.24	-	0.50 ± 0.01
Ulcer control	-	35.94±0.36	-	0.57±0.02
Omeprazole	10	27.47± 0.75	20.12	0.66 ± 0.01
AQAM	250	30.21 ± 0.43	7.63	0.51 ± 0.02
ALAM	250	19.3 ± 0.35	45.01	0.86 ± 0.01

Values are mean ± S.E.M. n=number of animals in each group. Significant differences with respect to solvent control group were evaluated by Student's t – test. ($p<0.05$, $p<0.01$ and $p<0.001$).

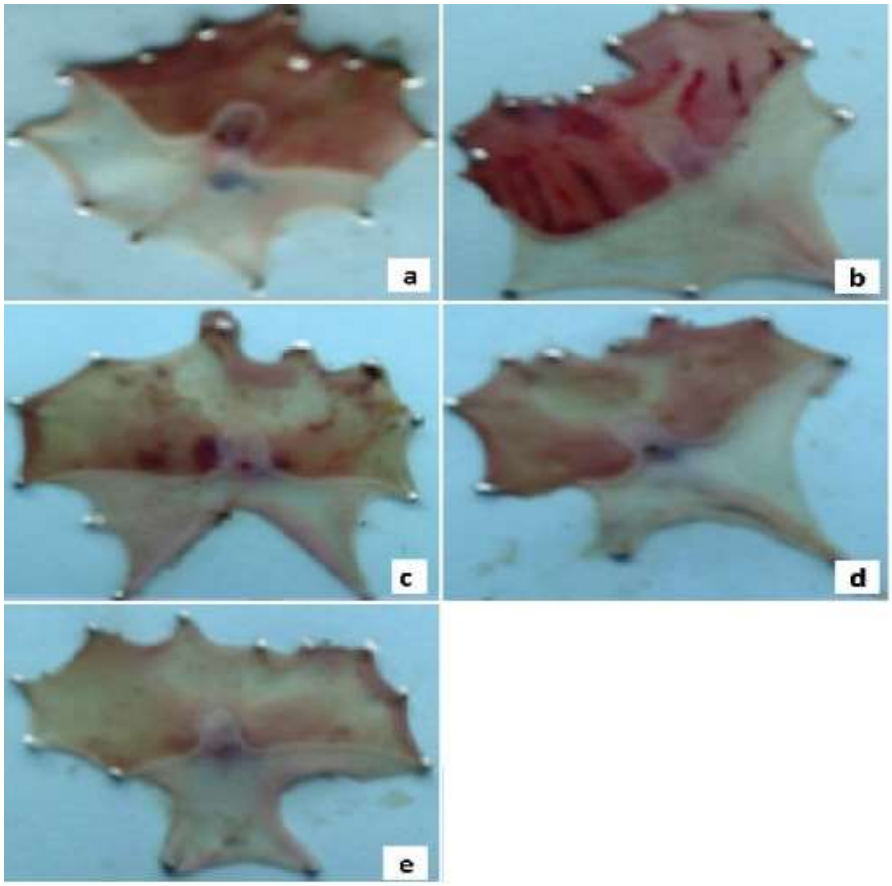

Fig 1: Effect of *Aegle marmelos* on alcohol induced ulcers in the rats in the study (a) Normal Control (b) Ulcer Control (c) *AQAM* (250 mg/kg) treated (d) *ALAM* (250 mg/kg) treated (e) Omeprazole (10 mg/kg treated)

Effect on Paracetamol induced gastric ulcers

In *Aegle marmelos* treated groups (250 mg/kg), the ulcer index values (0.43 ± 0.02 respectively) were significantly reduced ($p<0.001$) when compared to solvent control (0.70 ± 0.02), while the ulcer index for ranitidine treated group was 0.25 ± 0.02 ($p<0.001$). The %inhibition of ulcer showed by AQAM and ALAM (250mg/kg) and ranitidine was 51.3%, 37.2% and 53.2% respectively.

Table 4: Effect of *Aegle marmelos* at various dose levels on paracetamol induced gastric ulcer in rats.

Treatment (n=6)	Dose mg/kg (p.o.)	Ulcer index	% Inhibition of ulcer
1% CMC	-	0.70 ± 0.02	-
Ulcer control	-	0.84±0.01	--
Ranitidine	50	0.25 ± 0.02	51.3
AQAM	250	0.43 ± 0.02	37.2
ALAM	250	0.30 ± 0.02	53.2

Values are mean ± S.E.M. n=number of animals in each group; Significant differences with respect to solvent control group were evaluated by Student's t - test. ($p<0.001$).

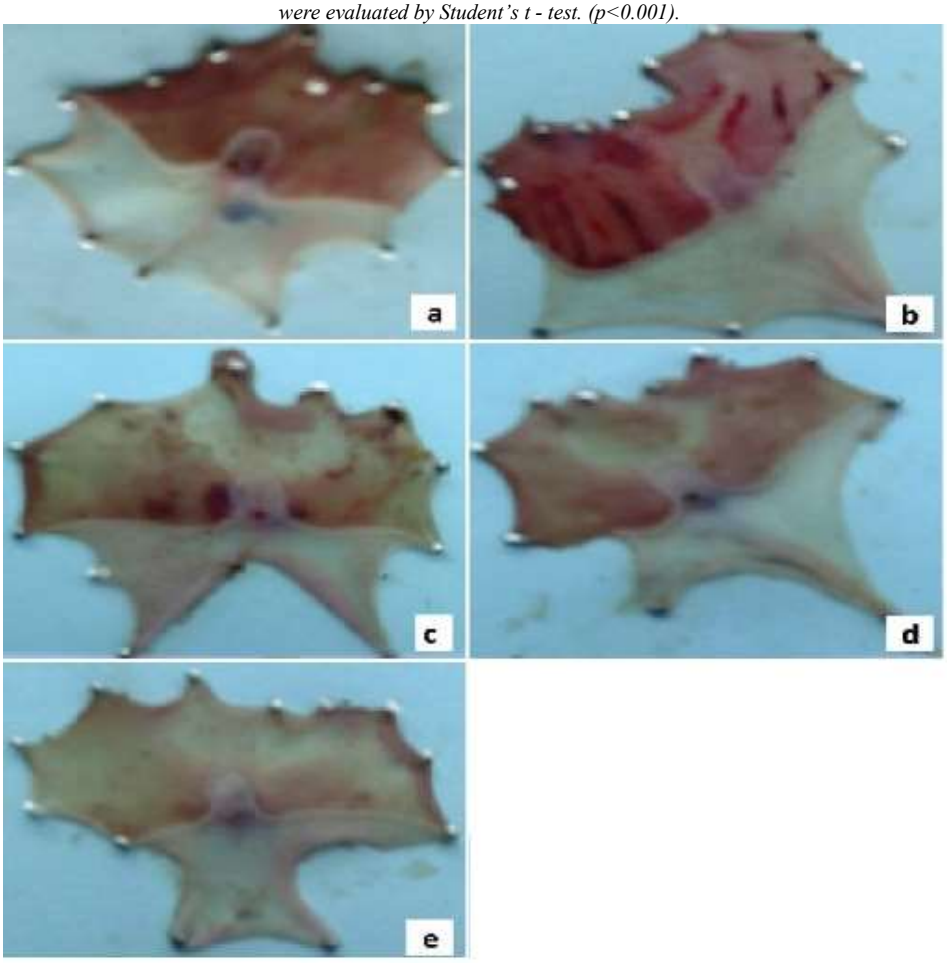

Fig 2: Effect of *Aegle marmelos* on paracetamol induced ulcers in the rats in the study (a) Normal Control (b) Ulcer Control (c) *AQAM*(250 mg/kg) treated (d) *ALAM*(250 mg/kg) treated (e) Ranitidine (50 mg/kg treated).

Stress-induced ulcers

In water immersion stress induced ulcers, the mean score value of ulcer inhibition was found to be significant ($P<0.001$) for 250 mg/kg of the extract. The percentage ulcer inhibition was 75.29 and 84.55 for 250 mg/kg for both aqueous and alcoholic extracts, and that of the standard was found to be 91.42.

Table 5: Effect of *Aegle marmelos* at various dose levels on Stress induced gastric ulcer in rats.

Group	Ulcer index	Percentage inhibition
Normal Control	00.00±0.00	-----
Ulcer control	22.73±4.31	------
Standard	2.86±0.13	91.42
AQAM	6.90±3.02	75.29
ALAM	4.34±2.87	84.55

Values are mean ± S.E.M. n=number of animals in each group; Significant differences with respect to solvent control group were evaluated by Student's t - test. ($p<0.001$).

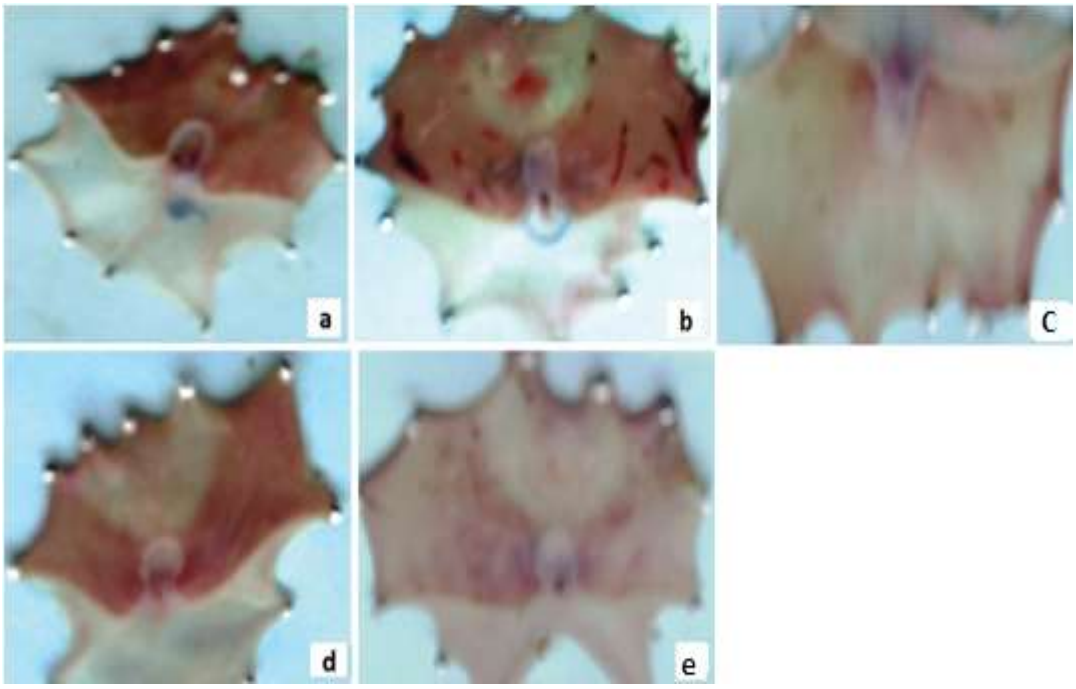

Fig 3: Effect of *Aegle marmelos* on stress induced ulcers in the rats in the study (a) Normal Control (b) Ulcer Control (c) *AQAM* (250 mg/kg) treated (d) *ALAM* (250 mg/kg) treated (e) Omeprazole (10 mg/kg treated)

DISCUSSIONS

The anti-ulcer activity of *Aegle marmelos* was evaluated by employing alcohol/paracetamol/acetic acid/stress induced gastric ulcers in rats. Alcohol and paracetamol induced ulcer models were used because they represent some of the most common causes of gastric ulcer in humans. Many factors and mechanisms are implicated in the ulcerogenesis and gastric mucosal damage induced by different models employed in the present study involving the increase of gastric acid output, vascular injury, depletion of gastric wall mucin, mucosal damage induced by non-steroidal anti-inflammatory drugs and free radical production.

Alcohol induced gastric injury is associated with significant production of oxygen free radicals leading to increased lipid peroxidation which causes damage to cell and cell membranes. *Aegle marmelos* has significantly protected the gastric mucosa against alcohol challenge as shown by reduced values of lesion index as compared to solvent control group suggesting its potent cytoprotective effect. This is further substantiated by increase in gastric mucus content produced by *Aegle marmelos* extract.

NSAID's like paracetamol, aspirin, Indomethacin cause gastric mucosal damage by decreasing prostaglandin levels through inhibition of PG synthesis. *Aegle marmelos* extract was significantly effective in protecting gastric mucosa against paracetamol induced ulcers at all the dose level studied. Hence *Aegle marmelos* extract affords effective protection to gastric mucosa against various insults by increasing gastric mucus content and decreasing the acid volume, free and total acidity in rats.

Stress plays an important role in ulcerogenesis. The pathophysiology of stress-induced gastric ulcers is complex. Stress-induced ulcers are probably mediated by histamine release with enhancement in acid secretion and a reduction in mucus production. The aqueous and alcoholic extracts of *Aegle marmelos* were effective in reducing the ulcers induced by stress.

The effects in all the 3 models studied were dose dependent. In conclusion, to the best of our knowledge for the first time, we have demonstrated that Hence *Aegle marmelos* extract has gastro protective activity against experimentally induced ulcers in rats. The mechanism of gastro protective action can be attributed to its antisecretory and cytoprotective property. However further experiments are required to establish and elaborate the molecular mechanism(s) of its Anti-ulcer activity.

SUMMARY AND CONCLUSION

The anti-ulcer activity of the plant *Aegle marmelos* was evaluated by employing paracetamol, alcohol and stress induced ulcer models. These models represent some of the most common causes of gastric ulcer in humans. Many factors and mechanisms are implicated in the ulcerogenesis and gastric mucosal damage induced by different models employed in the present study involving, depletion of gastric wall, mucin mucosal damage induced by nonsteroidal anti-inflammatory drugs and free radical production.

NSAID's like aspirin and paracetamol causes gastric mucosal damage by decreasing prostaglandin levels through inhibition of PG synthesis. Alcohol and Aqueous extract of the plant of *Aegle marmelos* was significantly effective in protecting gastric mucosa against paracetamol induced ulcers at all the dose level studied.

Alcohol induced gastric injury is associated with significant production of oxygen free radicals leading to increased lipid peroxidation, which causes damage to cell and cell membrane. The extracts of the *Aegle marmelos* has significantly protected the gastric mucosa against alcohol challenge as shown by reduced values of lesion index as compared to control group suggesting its potent cytoprotective effect. It has been proposed that in pyloric ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for induction of ulceration.

The antiulcer activity of *Aegle marmelos* extracts in stress induced model is evident from its significant reduction in gastric volume, ulcer index and increase in pH of gastric juice. Because of animals treated with *Aegle marmelos* extracts significantly inhibited the formation of ulcer in the stomach and also decreased both acid concentration, gastric volume and increased the pH values.

It is suggested that *Aegle marmelos* extracts can suppress gastric damage induced by aggressive factors. It is generally accepted that gastric ulcers result from an imbalance between aggressive factors and the maintenance of the mucosal integrity through endogenous defence mechanisms. The excess gastric acid formation by prostaglandin (PG) includes both increase in mucosal resistance as well as a decrease in aggressive factors, mainly acid and pepsin. Inhibitions of PG synthesis by aspirin coincide with the earlier stages of damage to the cell membrane of mucosal, parietal and endothelial cells.

The preliminary phytochemical studies revealed the presence of flavonoids in aqueous and alcoholic extracts of *Aegle marmelos* various flavonoids have been reported for its anti-ulcerogenic activity with good level of gastric protection. So the possible mechanism of antiulcer action of *Aegle marmelos* may be due to its flavonoids content. In this study we observed that *Aegle marmelos* provides significant anti-ulcer activity against gastric ulcers in rats.

On the basis of the present results and available reports, it can be concluded that the anti-ulcer activity elucidated by *Aegle marmelos* could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection and partly due to acid inhibition.

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