"Pharmacological screening of *Aloevera* and *Citrus Lemon* for the treatment of ulcer."

[1]Kalyani Nirwan, [2]Dr. Chakresh Patley, [3]Dr.Rajesh Mujariya, [4]Dr. Manjeet Singh,[5]Punam Bihone

[1,2,3,4] Institute of Pharmaceutical Science and Research (IPSR), Balaghat (MP), 481331, India
Corresponding author: kalyaninirwan802@gmail.com

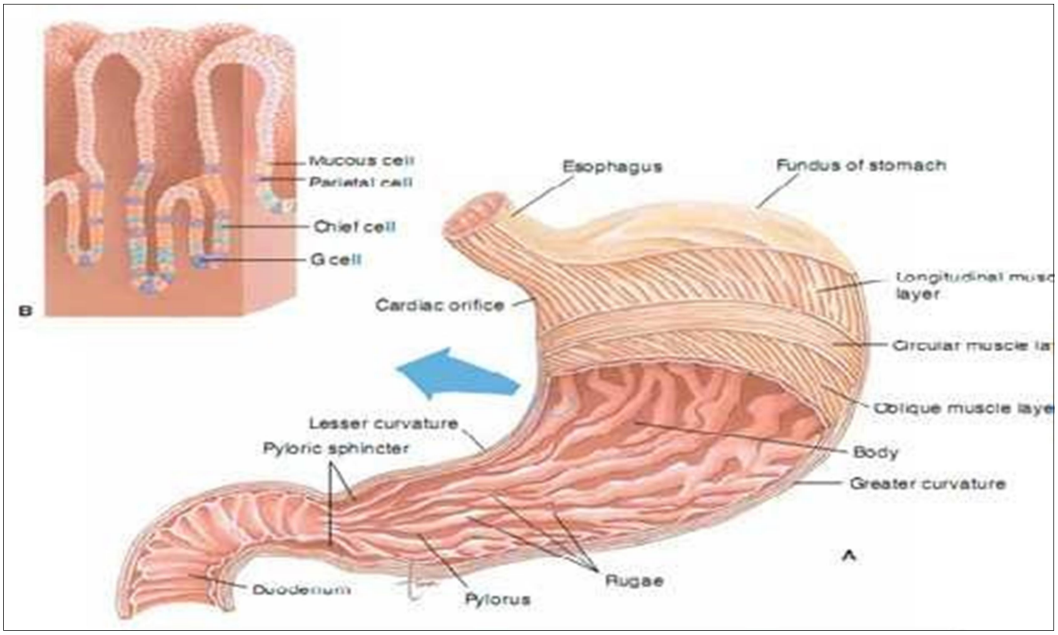
Abstract:

In this study, the anti-ulcer and anti-inflammatory activities of ethonolic extract of Aloevera & Lemon has been studied. The anti-ulcer activity was evaluated against Aspirin induced and Pylorus ligated ulcers. The anti-inflammatory study was studied using Carrageenin induced paw oedema. The standard drug showed reduced paw thickness (mm) was 0.22 ± 0.03, 0.28 ± 0.02, 0.32 ± 0.03, 0.36 ± 0.01, 0.13 ± 0.03 at 0, 30, 60,120, 240 mins. The maximum activity of the standard drug Indomethacin was observed at 240min. but the reduction of thickness of the paw was evident from 60 mins onward. The extracts 400mg/kg and 200mg/kg have showed reduced paw thickness was evident from 60 min onwards, but the amount of diminution of the inflammation was less when compared to the standard group.

Keywords: Peptic ulcers, H. pylori, Rugae, Wistar rats, Gastrointestinal tract.

INTRODUCTION

The acid-peptic diseases are those disorders in which gastric acid and pepsin are necessary, but usually not sufficient, pathogenic factors.[1] While inherently caustic, acid and pepsin in the stomach normally do not produce damage or symptoms because of intrinsic defense mechanisms. Barriers to the reflux of gastric contents into the esophagus comprise the primary esophageal defense. Peptic ulcers are craters or open sores in the lining of the upper gastrointestinal tract (GIT). [2]They include duodenal ulcers and gastric ulcers. Peptic ulcers are common and usually occur singly. But it is possible to have two or more, or even both duodenal and gastric ulcers at the same time. Although the pathogenesis of peptic ulcer disease is not fully understood, three major factors are recognized: infection with gram-negative H. pylori, increased hydrochloric acid secretion, and inadequate mucosal defense against gastric acid. Treatment approaches include (1) eradicating H. pylori infection, (2) reducing secretion of gastric acid or neutralizing the acid after it is released, and (3) providing agents that protect the gastric mucosa from damage.[3] The stomach is located in the upper left quadrant of the abdominal cavity, to the left of the liver and in front of the spleen. Although part of the alimentary tube, the stomach is not a tube, but rather a sac that extends from the esophagus to the small intestine.[4] Because it is a sack, the stomach is a reservoir for food, so that digestion proceeds gradually and we do not have to eat constantly. Both mechanical and chemical digestion takes place in the stomach.[4] The parts of the stomach are shown in Fig 1. The cardiac orifice is the opening of the esophagus, and the fundus is the portion above the level of this opening. The body of the stomach is the[32] large central portion, bounded laterally by the greater curvature and medially by the lesser curvature. The pylorus is adjacent to the duodenum of the small intestine, and the pyloric sphincter surrounds the junction of the two organs.[5] The fundus and body are mainly storage areas, whereas most digestion takes place in the pylorus. When the stomach is empty, the mucosa appears wrinkled or folded.[6] These folds are called rugae; they flatten out as the stomach is filled and permit expansion of the lining without tearing it.[7] The gastric pits are the glands of the stomach and consist of several types of cells; their collective secretions are called gastric juice. Mucous cells secrete mucus, which coats the stomach lining and helps prevent erosion by the gastric juice.



Fig, No .1: Stomach anterior view and gastric pits

PLANT COLLECTION

Aloevera and Citrus Lemon were collected from Botanical Garden Deori. It was identified and authenticated by Nirmal Institute of Agriculture Technology Gondia 441614.

Preparation of extract

Aloevera and Citrus Lemon were shade dried and then ground till they became coarse powder in a motar-pestle. The powdered material thus obtained was subjected to extraction using Petroleum Ether and Ethanol.[8] The extracts obtained were distilled to remove excess of the solvent and then evaporated at 40oC to get a semi-solid mass. These extracts were subjected to phytochemical tests which have been described below.

Animals

Wistar rats of either sex (150-200 gms) were housed in separate cages at controlled room temperature (24 ±2oC; relative humidity 60-70%) in a 12hr light- dark cycle. They were fed with standard pellet diet and water ad libitum. [9]

DETERMINATION OF ACUTE ORAL TOXICITY (LD_{50}) of *Aloevera And Lemon* .

Test substance details:

Name of the test substance: Ethanolic extract of *Aloevera and Lemon* .

Color : Greenish black, *Aloevera and Lemon*

Nature of the test Substance : gummy, *Aloevera and Lemon*

Table no 3. Experiment protocol

Name of the study	**Acute toxicity**
Guideline followed	OECD 423 method-acute toxic class method
Animals	Healthy young adult non-pregnant Swiss albino mice.
Body weight	25-30 g
Sex	Male
Administration of dose and volume	2000 mg/kg body weight, single dose in 0.2ml
Number of groups and animals	2groups and 6animals
Route of administration	Oral by using mice oral feeding needle
Vehicle	Carboxy methyl cellulose(CMC)

Housing and feeding conditions:

Room temperature	22°C ± 3°C
Humidity	40-60%
Light	12 h : 12h (light : dark cycle)
Feed	Standard laboratory animal food pellets with water *ad libitum*

Study period and observation parameters:

Initial observation	First 30 minutes
Special attention	First 1-4 hrs after drug administration
Long term observation	Up to 14 days
Direct observation parameters	Diarrhea, sitting in the corners, sniffinexcessively, standing on hind limbs,
Additional observation parameters	Skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern etc.

Study procedure:

Acute oral toxicity was performed as per Organization for Economic Co-operation for Development (OECD) guideline 423 methods. The extract was administered in a single dose by gavages using specially designed mice oral needle. Animals are fasted 24 h prior to dosing (food was withheld, but not water).[10] (OECD Guideline for testing of chemicals 423). Ethical clearance (for handling of animals and the procedures used in study) was obtained from the institutional Animal Ethics Committee before performing the study on animals.

PHARMACOLOGICAL EVALUATION:

Animal selection:

Healthy adult male Wistar albino rats weighing between 150 and 200gms were selected for the anti-ulcer studies.

Housing and feeding condition:

The temperature in the experimental animal room was kept 22±30C. Artificial lighting was provided. The animals were acclimatized to standard laboratory conditions of temperature (22 ± 30^{o}C) and maintained on 12:12 h light: dark cycle. The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding.[11] They were provided with

regular rat chow diet and distilled water *ad libitum.*

Preparation of animals:

The animals were randomly selected and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

Extracts & Standards used:

Extract used: Dried Aloevera and Lemon Ethanolic extract.

Standard used:

- Omeprazole: 20 mg/kg b.w.
- Indomethacin: 10 mg/kg b.w

Drugs, viz, Omeprazole and Indomethacin and the test extract of Aloe Vera and Lemon. Were suspended in 0.5% CMC and used for anti-ulcer and anti-inflammatory studies. Each drug suspension was freshly prepared just before administration.[12]

Preparation and administration of doses:

The extract was solubilized in 0.5% Carboxy Methyl Cellulose prior to experimental use to obtain the desired concentrations (200 and 400 mg/kg body weight) in 1 ml. The test substances were administered in a single dose using a gastric intubation tube after fasting for 3 to 4 hrs.[13]

ANTI-ULCER ACTIVITY:
Aspirin induced Gastric ulcers:

Albino rats were divided into four groups of six animals each. The weight of the animals chosen was between 150 and 180gms. The animals were fasted 36hrs prior to the commencement of the experiment but were allowed free access to water.

- Group I (control): 0.5% CMC
- Group II: 20mg/kg omeprazole.
- Group III: ethanolic extract at dose 400mg/kg.
- Group IV: ethanolic extract at dose 200mg/kg.

All the animals received 200mg/kg of aspirin orally to the rats. In the treatment

group, drugs were administered orally 1hr before the administration of aspirin. After 2hrs of treatment with aspirin, animals were sacrificed by an excess dose of ether. The stomachs were removed, opened along the greater curvature and examined for lesions.[14]

Normal coloration – 0

Red coloration – 0.5

Spot ulcer – 1

Hemorrhagic streaks – 1.5

Ulcer - 2

Perforation - 3

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows:-

$$\%protection = \frac{\text{control mean ulcer index} - \text{test mean ulcer index}}{control\ mean\ ulcer\ index} * 100$$

Statistical Analysis:

Statistical analysis was carried out using Graph Pad Prism5 software version 5.04 (Graph Pad prism software Inc.) The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's t-test. P values< 0.05 were considered significant.

Animals in all the groups were fasted for 36 h after the respective assigned treatment and were anaesthetized with anaesthetic ether. The abdomen was opened by a small midline incision below the xiphoid process and pylorus portion of stomach was lifted out and ligated. Precaution was taken to avoid traction to the blood supply. The stomach was sutured with interrupted sutures. Animals were allowed to recover and stabilize in individual cages and were deprived of water during post-operative period. Four hours after the pyloric ligation, the animals were sacrificed by an excess dose of ether. The stomach was carefully removed and the gastric

contents were collected. The gastric juice was centrifuged at 1000rpm and gastric volume was measured. Free and total acidites of the supernatant were determined by titration with 0.1 N NaOH and expressed as mEq/ L /100 gms. The stomach was cut open along the greater curvature and pinned onto a soft board for evaluating the gastric ulcers and to calculate ulcer index. Ulcer scoring is done according to the scale mentioned below.

Ulcer Index:

After the incision of the stomach at the greater curvature the ulcers were observed. And the number of ulcers was counted using a magnifying glass and the diameter of the ulcers were measured using vernier calipers. The following arbitrary scoring system was used to grade the incidence and severity of lesions.

Normal coloration -0

Red coloration – 0.5

Spot ulcer – 1

Hemorrhagic streaks – 1.5

Ulcer - 2

Perforation - 3

Determination of Free Acidity and Total Acidity:

The gastric contents were centrifuged at 1000rpm for 10mins. 1ml of supernatant was diluted with 9ml distilled water. A volume of 2ml diluted gastric juice was treated with 0.1 N sodium hydroxide run from a micro burette using 3-4 drops of Topfer's reagent as indicator until a canary yellow colour was observed. The volume of NaOH run down was noted. This corresponds to free acidity. Further, 2-3 drops of phenolphthalein was added and titrated with NaOH until pink colour was restored. This gives total acidity. Free acidity and Total acidity are expressed in terms of ml of 0.1N HCl per 100 gms of gastric contents. This is the same as mEq/lit. Acidity may be calculated by using

The following formula:

Acidity=(Vol of NaoH*Normality of NaoH)/0.1*mEq/L

Histopathological Evaluation:

The gastric tissue samples were fixed in neutral buffered formalin solution for duration of 24 hrs. Sections of tissue from stomachs were examined histopathologically to study the ulcerogenic and/ or anti-ulcerogenic activity of ethanolic extract of Aloevera and Lemon. The tissues were fixed in 10% buffered formalin and were processed using a tissue processor. These sections were stained with hematoxylin and eosin using routine procedures. The slides were examined microscopically for Patho morphological changes such as congestion, haemorrhage, oedema and erosions using an arbitrary scale for the assessment of severity of these changes.

ANTI-INFLAMMATORY ACTIVITY:

Carrageenin Induced Paw oedema in Rats:

Male wistar rats weighing between 150-180gms were used for the study. The animals were divided into 3groups (n=5). The first group received 0.5% Carboxy Methyl Cellulose (1ml/kg p.o) served as control, while the second group received reference drug Indomethacin (20mg/kg p.o). The third and fourth group of animals were administered with 400mg/kg and 200mg/kg of ethanolic extract of Aloevera and Lemon respectively by oral route. Acute inflammation was produced by the sub-plantar administration of 0.1% Carrageenin (in 0.9% normal saline) in the right hind paw of the rats. The paw thickness was measured at 0min, 30min, 60min, 120min and 240min after carrageenin injection by using vernier calipers. The animals were pretreated with the test drugs 1hour before the administration of Carrageenin.

Toxicological evaluations of ethonolic extract of *Aloevera and Lemon:*

Table No: 5 Acute oral Toxicity study (423) observations.

S.NO	Response	
1	Alertness	N
2	Grooming	A
3	Anxiety	A
4	Roaming	N
5	Sniffing	N
6	Tremors	A
7	Convulsion	A
8	Depression	N
9	Gripping strength	N
10	Scratching	A
11	Defecation	A
12	Writhing	N
13	Pupils	N
14	Urination	N
15	Salivation	N
16	Skin colour	N
17	Lacrimation	N

N-Normal, A- Absent

Anti-Ulcer Evaluation:

Aspirin Induced gastric ulcers:

Table no: 5 shows the apparent effect of Omeprazole and Extract on the Ulcer Index and extent of mucosal damage in the stomach. In Group I i.e. control animals, oral administration of aspirin produced characteristic lesions in the glandular portion of rat stomach which appeared as elongated bands of thick, black & dark red lesions. Group II animals, pretreated with the standard drug, Omeprazole, showed considerable protection from ulcer in gastric mucosa and in case of Group III and IV, EESP significantly reduced

the ulcer Index at 400mg/kg and 200mg/kg doses to and respectively. Omeprazole as reference had the ulcer protection of. The intensity of heamorrhage and lesions was significantly reduced upon pretreatment, revealing the protective effect of EESP.

Table No. 6. Ulcer Index

Groups	Ulcer Index	% Protection
Group I- Control	12.24±0.06	--
GroupII- Standard(omeprazole)	3.49±0.08***	71.48 %
Group III – Aloevera & Lemon Extract 400mg	4.5±0.10***	63.23%
Group IV – Aloevera & Lemon Extract 200mg	5.72±0.05*	53.26 %

All values represent Mean ± SEM, n=6 in each group. $P < 0.05$. Control group (Group I) is compared with standard and extract doses, * represents significance.

Conclusion

The anti-ulcer activity was evaluated against Aspirin induced and Pylorus ligated ulcers. The anti-inflammatory study was studied using Carrageenin induced paw oedema. Gastric ulcer disease is an imbalance between mucosal defense factors (bicarbonate, mucin, prostaglandin, nitric oxide, and other peptides and growth factors) and injurious factors (acid and pepsin). Ulcer caused by pylorus ligation is due to increased accumulation of gastric acid and pepsin leading to auto digestion of gastric mucosa and break down of the gastric mucosal barrier. The activation of the vagus-vagal reflux by stimulation of pressure receptors in the antral gastric mucosa in the hyper secretion model of pylorus ligature is believed to increase gastric acid secretion. NSAIDs are also frequently associated with peptic ulcers Topical injury by the luminal presence of the drug appears to play a minor role in the pathogenesis of these ulcers, as evidenced by the fact that ulcers can occur with very low doses of aspirin (10 mg) or with parenteral administration of NSAIDs. The effects of these drugs are instead mediated systemically; the critical element is suppression of the constitutive form of cyclooxygenase-1 (COX-1) in the mucosa and decreased production of the cytoprotective prostaglandin (PGE_2andPGI_2). Antiulcer effect is supported

by the decrease in the aggressive factors like gastric volume, decrease in free and total acidity and an increase in the resistance factors like pH showing the anti-secretary mechanism. It is significant to note when the pH was nearing 5.2 (Std) and 3.5 (Ext 400mg/kg), the ulcer score appeared less. The antiulcer agent may protect the mucosa from acid effects by selectively increasing prostaglandins. Prostaglandins have a vital protective role. The mucosal defense mechanism may be due to the epithelial cells of the gastric mucosa, which are impermeable to H+ ions thereby forming a physical barrier. The ethonolic extract of Aloevera & Lemonwas evaluated by using aspirin induced ulcer model, Oral administration of ethanol extract of *Aloevera & Lemon* at doses of 200 and 400mg/kg exhibited dose dependent inhibition percentage of 53.26% and 63.23% ($p<0.001$) respectively compared to the ulcer control, proving the anti-ulcer activity. The standard drug omeprazole (20mg/kg) exhibited percentage inhibition of 71.48% when compared with ulcer control. Extract treated and ulcer control group was compared with normal control group.

Reference

1. Akter S, Begum T and Begum R: Phytochemical analysis and investigation of anti-inflammatory and anti-ulcer activity of *Terminalia bellirica* leaves extract. International Journal of Pharmacognosy 2019; 6: 54-65.
2. Bhajoni PS, Meshram GG and Lahkar M: Evaluation of the antiulcer activity of the leaves of *Azadirachta indica*: An experimental study. Integrative Medicine International 2016; 3(1-2): 10-16.
3. E.O. Farombi, O. Owoeye, Antioxidative and chemopreventive properties of Vernonia amygdalina and Garcinia biflavonoid, Int. J. Environ. Res. Publ. Health 8 (2011) 2533–2555.
4. G.O. Avwioro, F.A. Awoyemi, T. Odoula, A novel natural collagen and muscle stain from Morinda lucida extracts 4 (2) (2005) 44–48.
5. H.D. Neuwinger, African ethnobotany: poisons & drugs: chemistry pharmacology, Toxicology (1996).
6. L. Muhammad, D. Sani, W. Sumayya, S.M. Abdallah, Phytochemical screening and antibacterial activity of bitter leaf (vernonia amygdalina) 2 (2020) 1-7.
7. M. Azamthulla, M. Asad, and V. S. Prasad, “Antiulcer activity of *Allium sativum*bulb juice in rats,” *Saudi Pharmaceutical Journal*, vol. 17, no. 1, pp. 70–77, 2009.

8. M. C. Divakar, S. B. Rao, G. R. N. Nair, and A. Hisham, “The role of fatty acids on the ulcer healing property of the nimbidin fraction of the neem oil,” *Journal of Medicinal and Aromatic Plants Science*, vol. 23, no. 3, pp. 404–408, 2001.
9. N. R. Pillai andG. Santhakumari, “Toxicity studies on nimbidin, a potential anti-ulcer drug,” *Planta Medica*, vol. 50, no. 2, pp. 146–148, 1984.
10. O.A. Olajide, S.O. Awe, J.M. Makinde, The effect of Morinda lucida Benth (Rubiaceae) extract on the gastrointernational tract of rodents, Phytotherapy : Int. J. Pharmacol. Toxicol Eval. Nat. Product 12 (6) (1998) 439-441.
11. Pal A and Chinnaiyan SK: Anti-ulcer activity of leaves of *Averrhoa carambola* Linn. International Journal of Pharmacology Research 2019; 9(05): 1-6.
12. Panchawat S and Pradhan J: Comparative Evaluation of Antiulcer Activity of *Ficus religiosa* (Stem Bark) Extracts Prepared by Different Methods of Extraction. Current Traditional Medicine
13. R.U.B. Ebana, U.O. Edet, D.E. Atang, L.A. Iyere, Antimicrobial Studies and Phytochemical Analysis of the Fruits and Leaves of Cnestis ferruginea, 25, World News of Natural Sciences, 2019, pp. 188-198.
14. S. K. Borra, R. K. Lagisetty, and G. R. Mallela, “Anti-ulcer effect of Aloe vera in non-steroidal anti-inflammatory drug induced peptic ulcers in rats,” *African Journal of Pharmacy and Pharmacology*, vol. 5, no. 16, pp. 1867–1871, 2011.