Indian Journal of Research in Pharmacy and Biotechnology
Volume 6, Issue 3, 2018
Journal homepage: http://www.ijrpb.com

ISSN:
2321-5674 (Print)
2320-3471
(Online)

Research article

Indexed in CAS and CABI
Impact factor:0.64

Phytochemical studies and anti-ulcer activity of *Limonia acidissima* linn. leaf in treating ethanol induced ulcer Albino rats

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Keywords:
Limonia acidissima, Ethanol, Gastric ulcer, Ranitidine

Article Info:
Received: 15-05-2018
Revised: 30-05-2018
Accepted:10-06-2018

ABSTRACT

The preliminary phytochemical analysis of *Limonia acidissima* plant leaf, showed the presence of alkaloids, flavonoids, steroids, saponins, glycosides, phenols, gum and mucilage, fixed oils and fats, resins and tannins. The objective of the present investigation is to elucidate the anti-ulcer activity of ethanolic leaf extract of *Limonia acidissima* in ethanol induced GIT damage in albino rats. The lyophilized extract was given by oral gavages (200mg/kg and 400mg/kg) before administering ethanol at 1ml/kg. Pre-treatment with extract significantly decreased the ulcerated area. The volume and acidity of the gastric juice decreased in the pre-treated rats. In conclusion, *L. acidissima* was able to decrease the acidity and increase the mucosal defense in the gastric areas, there by justifying its use as an anti-ulcerogenic agent.

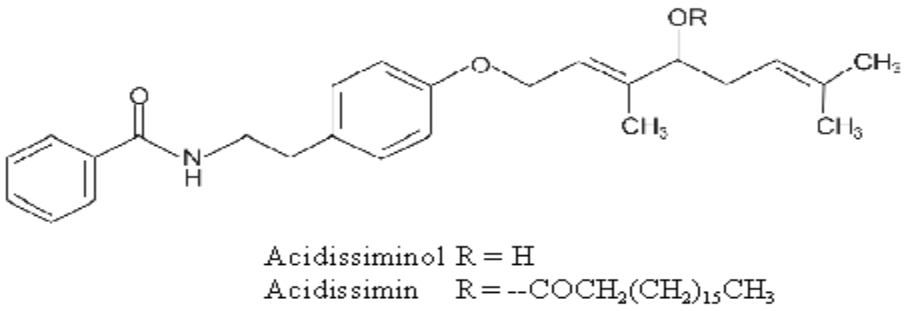
1. INTRODUCTION

Ulcer is a common disorder of the gastrointestinal system, which causes much discomfort in patients, disrupting their daily routines and causes mental agony. Peptic ulcer disease can be characterized by inflamed lesions or excavations of the mucosa and tissue that protect the gastrointestinal tract. A peptic ulcer is a sore in the lining of stomach or duodenum, the first part of small intestine. If peptic ulcers are found in the stomach, they are called gastric ulcers. If they are found in the duodenum, they are called duodenum ulcers. Gastric ulcers are also associated with considerable morbidity related to chronic epigastric pain, nausea, vomiting, and anemia. Rarely, an ulcer can lead to a gastric or duodenal perforation. This is extremely painful and requires immediate surgery. Recently, *Helicobacter pylori* have been implicated in the antral gastritis, peptic ulcer, gastric malignancy and the non-ulcer dyspepsia. With the increasing use of non-steroidal anti-inflammatory drugs and alcohol consumption.

Ethanol induced gastric ulceration (EIGU) in rats is considered to be a reliable tool to study the pathogenesis of acute gastric mucosal ulceration. The gastric sub-mucosal micro vascular disturbance resulting in local ischemia is an important early reaction following the use of ethanol. The endogenous mediators for the early vascular damage of the gastric mucosa include: nitric oxide, leukotrienes, histamine, adenosine, TNFα and endothelins. The release of endothelins plays a role not only in the pathogenesis of EIGU but also in the process of ulcer healing.

Limonia acidissima is one of the medicinally important plants belonging to Rutaceae, commonly known as wood apple. *Limonia*, confined to India, Pakistan, Sri Lanka and Southeast Asia. It is also known as wood apple, elephant-apple, monkey fruit, curd fruit, kathbel and kaitha. This plant is given as a medicine for the treatment of various disorders. *L. acidissima* is a deciduous, slow-growing, erect tree with a few upward-reaching branches bending outwards near the summit where they are subdivided into slender branchlets drooping at the tips.

Chemical Constituents: Major chemical constituents present in the ethanolic extract of leaves of *L. acidissima* are Acidissimin and Acidissiminol. Various parts of wood apple have been used against various ailments in ethnomedicine. Juice of young leaves is mixed with milk and sugar candy given as remedy for biliousness and intensive troubles of children.



Acidissiminol R = H
Acidissimin R = --$COCH_2(CH_2)_{15}CH_3$

Figure.1.Molecular structure of Acidissiminol and Acidissimin

2. MATERIALS AND METHODS

Collection of Plant material: Fresh and disease free leaves of *Limonia acidissima Linn*. were collected in the month of September- October 2017 from the Battiprolu, Repalle, Guntur (Dist), Andhra Pradesh.

Experimental animals: Healthy female albino rats of 110-200g were used throughout the study. Animals were housed under standard environmental conditions at temperature (25±20C) and light and dark (12:12h) Rats were feed standard pellet diet and water.

Preparation of plant extract for phytochemical screening: Leaves of *L. acidissima* were shade dried separately at room temperature and the dried leaves were powdered in a Wiley mill. 50gms of powered *L. acidissima* leaves were packed in a soxhlet apparatus separately and extracted with ethanol. The ethanol extracts were subjected to qualitative test for the identification of various phytochemical contstituents as per the standard procedures.

Preparation of ethanol extracts of *L. acidissima* leaves for antiulcer activities: Leaves of *L. acidissima* were cut separately into small pieces and shade dried for the experimental studies. Dried leaves were powdered separately and then extracted in ethanol. Then it was filtered with the help of whatmann paper No.1 filter paper and filtrate was lyophilized. The lyophilized samples were stored at dry place.

Phytochemical studies: Qualitative Phytochemical Analysis of Ethanolic Extract of *Limonia acidissima* leaves.

Table.1.Phytochemical constiuents of Etahanolic extract of *L.acidissima*

Name of the chemical constituent	Ethanolic extract of *L. acidissima*
Alkaloids	Present
Sterols	Present
Triterpenoids	Absent
Flavanoids	Present
Saponins	Absent
Glycosides	Present
Tannins	Present
Carbohydrates	Present
Proteins and Aminoacids	Absent
Fixed oils and Fats	Absent
Gums and mucilage	Absent

Induction of ulcer: Ethanol was used as the ulcerogenic agent at the dose of 1ml/kg body weight.

Experimental design: All the animals were grouped into three groups. Each group had 6 animals.

Group I: Served as control animals, without any treatment fed with normal water.

Group II: Served as negative control, the animals are treated only with the ethanol.

Group III: Animals were pre-treated with ranitidine (20mg/kg) and then treated with Ethanol following the dose and mode of administration.

Group IV: Six animals of this group were further divided into two groups each of 3 animals based on dose to be administered.

Group IVA: Initially animals were given the test dose of 200mg/kg body weight and after that ethanol were administered with a time gap of 1hour.

Group IVB: Initially animals were given with the test dose of 400mg/kg body weight and after that ethanol were administered with a time gap of 1hour.

Experimental procedure: Standard antiulcer drug Ranitidine are used at the rate of 20 mg/kg of body weight. All the groups of animals were kept overnight

fasting, fed only with the drinking water. The animals of group III was treated with the sample extract at the doses of 200 mg/kg and 400mg/kg of body weight. Animals of Group II were treated with ranitidine simultaneously. After one hour of last administration of sample extract, the Ethanol was given by oral gavages to the Group II and Group III animals. After 2 hours of Ethanol administration, the animals were sacrificed by cervical dislocation.

The animals were dissected and the stomach was taken out. Finally the ulcers were observed macroscopically. The observation was made for any bulging or inflammation in the stomach. The stomach was opened along the greater curvature and the mucosa was exposing for evaluation. By sacrificing the rat, stomach was removed and opened along the greater curvature and washed it slowly under tap water, but it on the glass slide and observed with naked eye.

The stomach was carefully keeping the oesophagus closed, opened along the greater curvature and the gastric contents were removed. The mucosa was flushed with saline and observed for gastric lesions using the microscopic structure.

3. RESULTS AND DISCUSSION

The leaves of *Limonia acidissima* were found to be rich in phytochemical constituents who have a variety of pharmacological actions. The literature survey revealed the presence of Alkaloids, Tannins, and Glycosides in the entire plant. The result of the present study indicates that ethanolic extracts of leaves of *Limonia acidissima* exhibited anti-ulcer activity against ethanol induced ulcer in rats. Table no.1 show the results obtained from ethanolic extract of leaf of *Limonia acidissima* on Albino rats at doses 200 mg, 400 mg/ kg body weight and had shown significant increase in pH of gastric juice of ethanol induced ulcer in rats.

Table.2.Effect of ethanolic extract of *Limonia acidissima* (Linn) leaves on gastric secretion of Ethanol Induced ulceration in rats

Group No	Body weight (gm)	Treatment	Volume of Gastric juice (ml)	pH
I Control	130.83±16.55	Control	2.95±0.22	3.27±0.27
II Negative control	132.5±12.94	Negative control (Ethanol)	3.3±0.4	2.75±0.37
III Standard	140.83±12.41	Standard (Ethanol+ Ranitidine)	3.13±0.43	4.28±0.37
IV(A) Low dose	52.5±11.29	Ethanolic extract of leaves of *L.acidissima* (200mg/kg)	2.03±0.44	4.28±0.31
IV(B) High dose	161.66±18.61	Ethanolic extract of leaves of *L. acidissima* (400mg/kg)	1.65±0.20	4.86±0.10

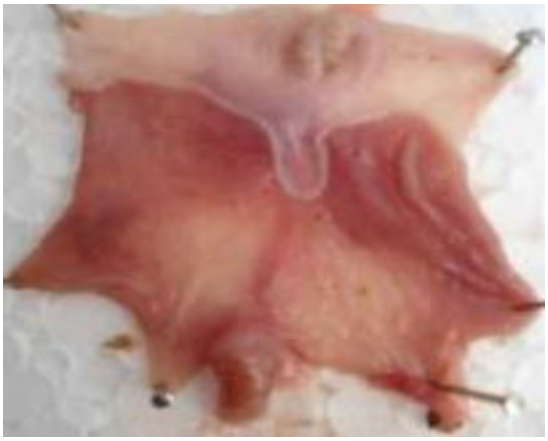


Figure.2.Effect of vehicle on ethanol induced method in rats

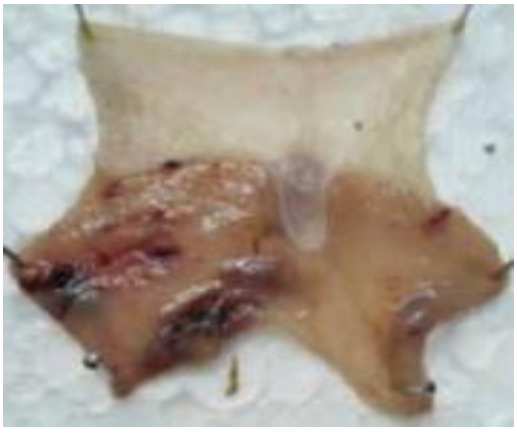


Figure.3.Effect of negative control ethanol induced method in rats

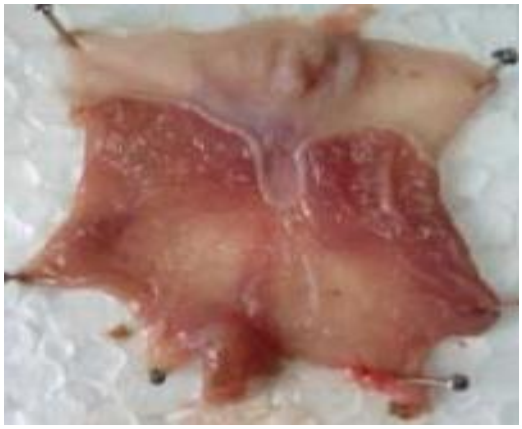


Figure.4.Effect of Ethanolic extract of *L.acidissima* leaves (200mg/kg)

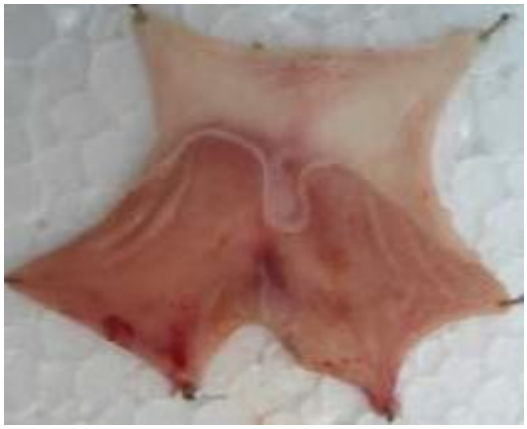


Figure.5.Effect of Ethanolic extract of *L.acidissima* leaves (400mg/kg)

Figure.6.Effect of standard drug (Ranitidine) on ethanol induced rats

Histopathological studies: Histopathology refers to the microscopic examination of tissue in order to study the manifestations of disease. Specifically, in clinical medicine, histopathology refers to the examination of a biopsy or surgical specimen by a pathologist, after the specimen has been processed and histological sections have been placed onto glass slides.

Collection of tissues: Histopathological examination of tissues start with surgery, biopsy or autopsy. The tissue is removed from the body or plant, and then often following expert dissection in the fresh state placed in a fixative which stabilizes the tissues to prevent decay. The most common fixative is formalin (10% neutral buffered formaldehyde in water).

Preparation of tissue for histology: The tissue is then prepared for viewing under a microscope using either chemical fixation or frozen section.

If a large sample is provided e.g. from a surgical procedure then a pathologist looks at the tissue sample and selects the part most likely to yield a useful and accurate diagnosis - this part is removed for examination in a process commonly known as grossing or cut up. Larger samples are cut to correctly situate their anatomical structures in the cassette. Certain specimens (especially biopsies) can undergo agar pre-embedding to assure correct tissue orientation in cassette & then in the block & then on the diagnostic microscopy slide. This is then placed into a plastic cassette for most of the rest of the process.

Staining of processed histology slides: This can be done to slides processed by the chemical fixation or frozen section slides. To see the tissue under a microscope, the sections are stained with one or more pigments. The aim of staining is to reveal cellular components; counter stains are used to provide contrast.

Discussion: Peptic ulcers are caused when the natural balances between the aggressive factors of acid, pepsin, defensive mechanisms of mucus, bicarbonate, mucosal turnover and blood supply (mucosal barrier) are disturbed. It is reported that acid and pepsin are relatively less important as causative agents and that a defect in the defensive mechanism of gastric mucosa is the first step toward ulcer formation. Although in most cases the etiology of ulcer is unknown, it is generally accepted that it is the result of an imbalance between aggressive factors and maintenance of the mucosal integrity through the endogenous defense mechanism. It is known that gastric lesions produced by ethanol administration appeared as multiple hemorrhagic red bands of different sizes along the glandular stomach.

Ethanol is commonly used for inducing ulcers in experimental rats and leads to intense gastric mucosal

damage. Studies suggest that the ethanol damage to the gastrointestinal mucosa starts with micro vascular injury, namely disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting. Ethanol produces necrotic lesions in the gastric mucosa by its direct toxic effect, reducing the secretion of bicarbonates and production of mucus. Exposure to ethanol increases the extension of cellular damage in a dose-dependent way. Oxidative stress plays an important role in the pathogenesis of various diseases including gastric ulcer, with antioxidants being reported to play a significant role in the protection of gastric mucosa against various necrotic agents.

Antioxidants could help to protect cells from damage caused by oxidative stress while enhancing the body's defence systems against degenerative diseases. Administration of Anti-oxidants inhibits ethanol-induced gastric injury in rat. *L.acidissima* leaf extracts possess a broad spectrum of biological activities and the plant extract have been shown to contain a relatively large quantity of antioxidant compounds. Results of the present study also revealed protection of gastric mucosa and inhibition of leucocytes infiltration of gastric wall in rats pre-treated with *L.acidissima* extract. Absolute alcohol would extensively damage the gastric mucosa leading to increased neutrophils infiltration into the gastric mucosa.

Oxygen free radicals derived from infiltrated neutrophils in ulcerated gastric tissues have an inhibitory effect on gastric ulcer healing in rats. Neutrophils mediate lipid per oxidation through the production of superoxide anions. Neutrophils are a major source of inflammatory mediators and can release potent reactive oxygen species such as superoxide, hydrogen peroxide and myeloperoxidase derived oxidants. These reactive oxygen species are highly cytotoxic and can induce tissue damage. Suppression of neutrophil infiltration during inflammation was found to enhance gastric ulcer healing.

L.acidissima extract has been shown to contain anti-inflammatory activity and it is speculated that the gastroprotective effect exerted by *L.acidissima* extract could be attributed to its anti-inflammatory activity. This anti-inflammatory activity could also be a key factor in the prevention of gastric ulcer as reported. In the present study, we also observed flattening of the mucosal folds which suggests that the gastroprotective effect of *L.acidissima* extract might be due to a decrease in gastric motility. It is reported that the changes in the gastric motility may play a role in the development and prevention of experimental gastric lesions. Relaxation of circular muscles may protect the gastric mucosa through flattening of the folds. This will increase the mucosal area exposed to necrotizing agents and reduce the. Ethanol produces a marked the gastric irritants on rugal cresti and contraction of the circular muscles of rat fundic strip. Such a contraction can lead to mucosal compression at the site of the greatest mechanical stress, at the crests of mucosal folds leading to necrosis and ulceration.

In conclusion, *L.acidissima extracts* could significantly protect the gastric mucosa against ethanol induced injury. Such protection was shown to be dose dependent as ascertained by the reduction of ulcer areas in the gastric wall as well as the reduction or inhibition of edema and leucocytes infiltration of submucosal layers and protection was most prominent at a dose of 400 mg/kg leaf extract. Further studies are required to determine the active ingredients responsible for the mechanism of anti-ulcer of *L.acidissima* extracts.

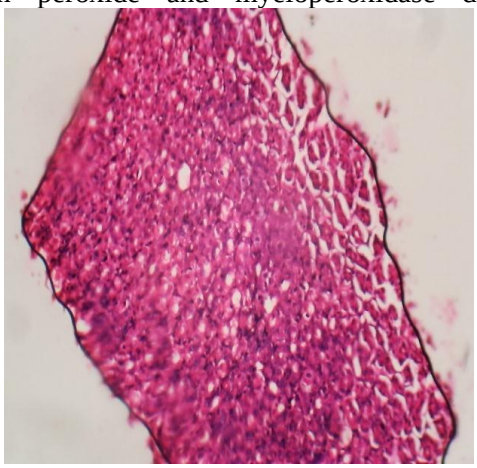


Figure.7.Section of gastric mucosal layer showing Normal appearance without any tissue damage when induced with control

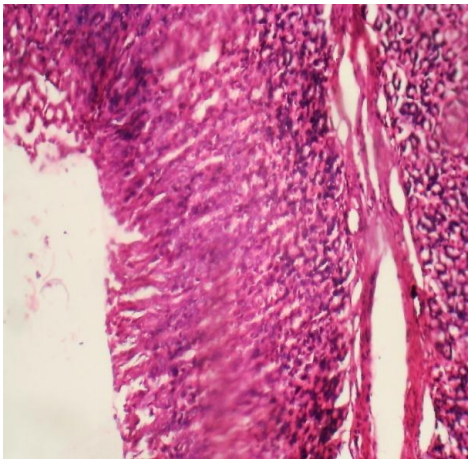


Figure.8.Section of gastric mucosal layer when induced with negative control ethanol Shows damage of epithelial cells

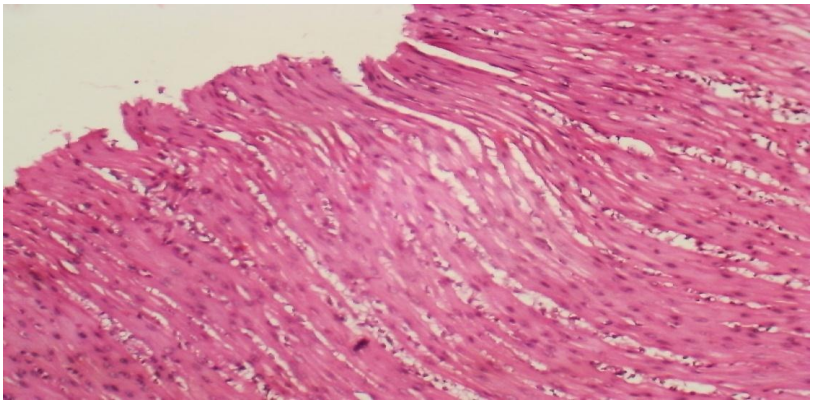


Figure.9.Section of gastric mucosal layer when induced standard (Ranitidine 20mg/kg) shows No significance change in the histopathology almost normal appearance

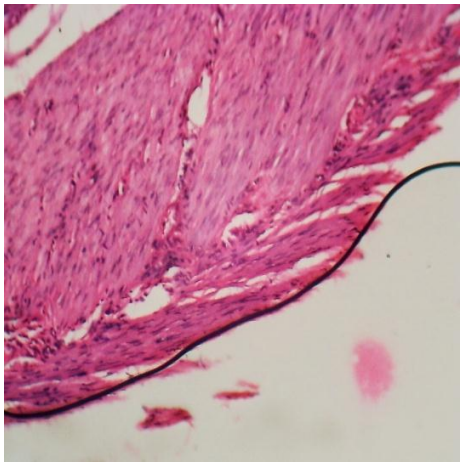


Figure.10.Section of gastric mucosal layer in a rat pre-treated with 200mg/kg of *L.acidissima* extract. There is a slight Disruption to the surface epithelium

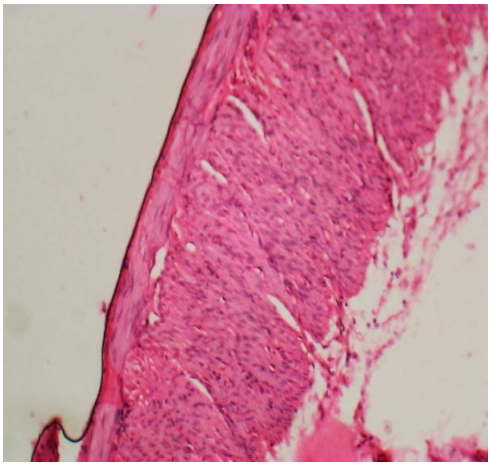


Figure.11.Section of gastric mucosal layer in a rat pre-treated with 400mg/kg of *L.acidissima* extract. There is no Disruption to the surface epithelium.

4. CONCLUSION

The present study showed that, pre-treatment with leaf extract of *L.acidissima* caused a beneficial effect on Ethanol-induced gastric ulcer in rats as evidenced by the reduction in the mucosal lesions. Further studies are required to establish its exact mode of action and the active principles involved in its antiulcer effect.

In conclusion that, *L.acidissima extracts* could significantly protect the gastric mucosa against ethanol induced injury. Such protection was shown to be dose dependent as ascertained by the reduction of ulcer areas in the gastric wall as well as the reduction or inhibition of edema and leucocytes infiltration of submucosal layers and protection was most prominent at a dose of 400 mg/kg leaf extract. Further studies are required to determine the active ingredients responsible for the mechanism of anti-ulcer of *L.acidissima* extracts.

ACKNOWLEDGEMENT

All the authors of this article like to take the privilege of expressing cordial thanks to management of Chalapathi Institute of Pharmaceutical Sciences and also convey sincere gratitude for enabling to prepare the article and for providing the laboratory facility and encouraging our work.

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