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A Research Article on Evaluation of Anti-Ulcer Activity of Methanolic Leaf Extract of Jacquemontia Caerulea Against Experimentally Induced Ulcers in Wistar Rats

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ABSTRACT

Objective: To evaluate the anti-ulcerogenic effect of Jacquemontia caerulea Leaf Methanolic Extract (JCLME) against aspirin, ethanol, cold stress induced and pylorus ligation induced gastric ulcers in Wistar rats.

Methods: The methanolic extract of fresh leaves of the plant, Jacquemontia caerulea (Family-Convolvulaceae) was prepared and assessed for anti-ulcer effect with different doses and the standard drugs (Risperidone and Amitriptyline respectively) by using aspirin, ethanol, cold stress induced and pylorus ligation methods.

Results: JCLME was assessed and compared statistically with the anti-ulcer effects in the control rats which was treated with saline (NaCl, 0.9%). It was found that JCLME at doses of 200 and 400 mg/kg reduced the ulcer index significantly when compared to control group ($p< 0.05$). It was found that JCLME significantly reduced the free acidity, total acidity and ulcer index ($p<0.05$) and increased the gastric content when compared with the control group. Hence, the current study shows that the JCLME has potent anti-ulcer, cytoprotective and antisecretory property thereby making it a great ulcero-protective agent.

Keywords: Jacquemontia caerulea, Anti-ulcerogenic effect, Risperidone, Amitriptyline, Aspirin, Ethanol, Cold stress and Pylorus ligation

1. Introduction

Ulcers are open sores of the skin or mucus membrane distinctive of sloughing of inflamed dead tissue [1]. Ulcers are lesions on the surface of the skin or a mucous membrane characterized by a superficial loss of tissue. The two most common types of peptic ulcer are called "gastric ulcer" and "duodenal ulcer. The name refers to the site of ulceration. Gastric ulcers are located in the stomach, characterized by pain, ulcers are common in older age group .Gastric ulcers have normal or diminished acid production yet ulcers may occur even in complete absence of acid [2].Duodenal ulcers are found at the beginning of small intestine and are characterized by severe pain with burning sensation in upper abdomen that awakens patients from

sleep Generally pain occurs when the stomach is empty and relieves after eating A duodenal ulcer is more common in younger individuals[3]. The pathophysiology of peptic ulcer disease involves an unbalance between offensive (acid pepsin and Helicobacter pylori) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors) [4].

Recent attention has focused on mucosal factors in ulceration, leading to the formation of the term "cytoprotection." This term encompasses physiological processes that shield the protection of the stomach lining from acid and pepsin digestion. Numerous protective mechanisms against gastric issues are linked to the natural secretion of prostaglandins. Traditional medical strategies involve either suppressing acid secretion or neutralizing acid. While antacids can temporarily neutralize gastric acid, their efficacy is short-lived. Muscarinic antagonists like atropine or pirenzepine effectively inhibit acid production. Histamine H2-receptor antagonists such as cimetidine, ranitidine, and famotidine act as robust inhibitors (70-80%) of acid secretion [5].

Acute ulcers involve tissues to the depth of the submucosa, appearing as single or multiple lesions in various stomach sites and the first few centimeters of the duodenum. Chronic ulcers penetrate through the epithelial and muscle layers of the stomach wall, potentially involving adjacent organs like the pancreas or liver. Typically occurring singly in the pyloric antrum of the stomach, both acute and chronic ulcers exhibit similar pathological findings, aiding in diagnostic efforts [6].

In this modern era also 75-80% of the world populations still use herbal medicine mainly in developing countries for primary health care because of better cultural acceptability, better compatibility with the human body, and lesser side effects. Jacquemontia caerulea, commonly known as Sky-blue cluster vine or blue morning glory, is a flowering plant belonging to the Convolvulaceae family, originating from tropical regions in the America, particularly Central and South America. It holds potential medicinal value. Traditional knowledge suggests that various plant parts, including leaves and stems, have been employed in traditional medicine for purposes such as wound healing, anti-inflammatory effects, and treating digestive disorders [7]. It has also been used in ancient times to lower fever, blood glucose levels, in respiratory tract infections like cough, cold, bronchitis and to support women's health [8].

Present study was conducted to evaluate the antiulcer activity of dried Leaves of "Jacquemontia caerulea". The present study was aimed to assess the anti-ulcer effect of Jacquemontia caerulea using dried leaves soaked in methanol as a solvent.

2. Material and Methods

2.1. Plant Material and Preparation of Extract

The leaves of Jacquemontia caerulea (Family- Convolvulaceae) were collected and authenticated by a plant taxonomist, Dr. Madhava Shetty at the Department of Botany, Sri Venkateswara University, Tirupathi. The plant sample deposited had voucher no. 0778. 300g of leaves of Jacquemontia caerulea were collected, washed thoroughly and dried in shade. Leaves were made into coarse powder. The powder of the leaves was extracted in a Soxhlet extractor with successive methanol solvent. The extract then subjected to distillation and heated on water bath for semisolid consistency and then placed in the refrigerator.


2.2. Drugs & Chemicals

Diethyl ether, Methanol AR, Chloroform, Phenolphthalein pH indicator solution and Sodium Hydroxide pellets were obtained from SD Fine-Chem Limited, Mumbai while pure Aspirin was procured from Divis Laboratories, Hyderabad. Absolute Ethanol was obtained from Changshu Yangyuan Chem, China and RIZE 3 (Risperidone) was taken from Lifecare Ltd., Himachal Pradesh, OMITRIP (Amitriptyline tablets), Omicron Pharma, Surgical spirit from Hyderabad Chemicals, Hyderabad. Distilled water and Topfer's Reagent was obtained from Stangen Fine Chemicals and Nice Chemicals, Hyderabad respectively.

2.3. Animals

Healthy adult Wistar rats of either sex, 8-10 weeks old, weighing about 200-250 gm, obtained from the animal house of Shadan College of Pharmacy were used in the experiment. Animals were placed in polypropylene cages maintained under standard conditions and provided with standard diet and water. All the animals were acclimatized to the laboratory conditions for a week before the commencement of the experiment. The entire study was carried out in compliance with the guidelines set forth by the CPSCEA. It was approved by IAEC Shadan College of Pharmacy.

2.4. Assessment of Anti-ulcer activity by Invivo Assays

2.4.1. Aspirin Induced Peptic Ulcers in Rats

All the animals (n=6) were fasted for 24 hours and were given free access to water ad libitum. To prevent cannibalism and coprophagy, the animals were housed in cages with raised bottoms of wide wire mesh. After fasting for 24 hours aspirin was administered orally at a dose of 250 mg/kg. After 4 hours the animals were sacrificed. Anesthesia effect was given by using diethyl ether in induction chamber (E. Williamson et.al., 1986). Then the stomachs were incised along the greater curvature and examined for ulcers. The gastric juice was collected for the determination of volume of gastric content, total acidity and free acidity [9].

Ulcer Index (UI): Mean Ulcer score for each animal is known as Ulcer Index. The stomachs were washed thoroughly in running water to check the ulcers present in glandular portion of the stomach. The number of the ulcers per stomach were noted and the severity of the ulcers was scored microscopically with the help of hand lens (10X) and scoring was done as per Kulkarni (1999) as follows [10]

0 = normal stomach,
0.5 = red coloration,
1 = spot ulcers,

1.5 = hemorrhagic streaks,
2 = ulcers > 3 mm but < 5 mm,
3 = ulcers > 5 mm.
The mean ulcer score for each animal was expressed as the ulcer index.
% Ulcer Inhibition = Mean ulcer index of control- Mean ulcer index of test X 100
Mean ulcer index of control

Determination of pH

A sample of 1 ml of gastric juice was diluted with 1 ml of distilled water, and pH of the solution was measured using pH meter.

Determination of total acidity

1 ml of gastric juice was diluted with 1 ml of distilled water and was taken into a 50 ml conical flask and two drops of phenolphthalein indicator was added and titrated with 0.01N NaOH until a permanent pink color was observed. The volume of 0.01N NaOH consumed was noted. The total acidity was expressed as mEq/L and calculated by the following formula[10]

Acidity=V NaOH×N×100mEq/L0.1, where V is volume and N is normality.

Histopathological Studies. The isolated stomachs were preserved by using 15% formalin solution and then were transferred to the laboratory for histopathological examination by using haematoxylin and eosin as staining agent. After examination the morphological changes were observed and recorded with 100x lenses [11].

2.4.2 Ethanol-Induced Peptic Ulcers:

Procedure:

Wistar rats (150-200 gm) will be used for the experiment. To induce ulcers with ethanol, animals were fasted for 24 hours before the Operative procedure. They were given free access to water ad libitum. To prevent cannibalism and coprophagy, the animals were housed in cages with raised bottoms of wide wire mesh. After fasting for 24 hours absolute ethanol (95-99%) was administered orally at a dose of 1mg/kg. After 1 hour the animals were sacrificed. Anesthesia effect was given by using diethyl ether in induction chamber (G.Seneret al.,2005). Then the stomach was incised along the greater curvature and examined for ulcers. The gastric juice was collected for the determination of volume of gastric content, pH of liquid, total acidity and free acidity [12].

Grouping of Animals:

Group 1: Control (n=6) treated with vehicle.
Group 2: Test I (n=6) with test drug P.O. (200mg/kg).
Group 3: Test II (n=6) with test drug P.O. (400mg/kg).
Group 4: Standard (n=6) with standard drug and inducing agent (20mg/kg) P.O.
(Omeprazole 20mg/kg+ Ethanol 1 ml/body weight).

2.4.3. Cold Restraint Stress Induced Ulcers:

Procedure:

Wistar rats (150-200 gm) will be used for the experiment. They were given free access to water ad libitum. To prevent cannibalism and coprophagy, the animals were housed in cages with raised bottoms of wide wire mesh. The ulcer was induced by subjecting the animals to cold restraint stress, the animal were fasted for 24 hours before the operative procedure. The animals were kept in stress wire meshed cylindrical cage.

(D.A. Brodie et al., 1962). The cage will be kept at a temperature of 4°C for 2 hours. After 2 hour the animals were sacrificed (E.C. Senay et al., 1967). Anesthesia effect was given by using diethyl ether in induction chamber (G. Sener et al.,2005). Then the stomach was incised along the greater curvature and examined for ulcers. The gastric juice was collected for the determination of volume of gastric content, pH of lid. total acidity and free acidity. [13]

Grouping of Animals:

Group 1: Normal (n=6) treated with vehicle P.O.
Group 2: Test I (n=6) with test drug P.O. (200mg/kg).
Group 3: Test II (n=6) with test drug P.O. (400mg/kg).
Group 4: Standard (n=6) with standard drug and inducing agent (20mg/kg) P.O.
(Amitriptyline 100mg/kg+ Cold stress 4°C).

2.4.4. Pylorus -Ligation induced ulcer model

Secure the rat on the operating table. Give an incision of 1 cm long in the abdomen just below the sternum. Expose the stomach. Pass a thread around the pyloric sphincter and apply a tight knot While putting the knot care should be taken so that no blood vessel is tied along the knot. Close the abdomen wall by putting the sutures. Clean the skin from any blood spots and bleeding. Apply collodion over the wound. Keep the rat in a separate cage and allow it to recover [14].

To another rat inject cimetidine (10 mg/kg, i.p). After 15 min perform pyloric ligation as described in step 1.

After 4 hr. of pyloric ligation sacrifice both the animals by decapitation. Open the abdomen and tie the esophageal end (cardiac end) of the stomach. Cut and remove the entire stomach from the body of the animal.

Give a small cut to the pyloric region just above the knot and collect the contents of the stomach in a graduated centrifuge tube.

Open the stomach along the greater curvature and wash it slowly under the running tap water. Put it on the slide and observe under 10X magnification for ulcers. Score the ulcers as below:

0= Normal colored stomach, 0.5= Red coloration, 1= Spot ulcers 1.5= Hemorrhagic streaks, 2= Ulcers ≥ 3 but ≤ 5, 3= Ulcers > 5

Mean ulcer score for each animal is expressed as ulcer index.

7. Centrifuge the gastric content at 1,000 rpm for 10 min. Note the volume. Pipette out 1 ml of supernatant liquid and dilute it to 10 ml with distilled water. Note the pH of this solution with the help of pH meter. Titrate the solution against 0.01N sodium hydroxide using Topfer's reagent as indicator. (It is dimethyl-amino-azo-benzene with phenolphthalein and used for detection and estimation of hydrochloric acid and total acidity in gastric fluids). Titrate to end point when the solution turns to the free acidity. Titrate further till the solution regains pink color. Note the total volume of NaOH which corresponds to the total acidity. Acidity (mEq/I/100 g) can be expressed as:

Acidity= Vol. Of NaOH ×Normality × 100/ 0.1. m. Eq /I/100g

8. Compare the gastric volume, acidity and ulcer index of control animal and the animal treated with cimetidine.

Inference Cimetidine reduces gastric secretion and inhibits ulcer formation in pyloric ligated animals.

Grouping of Animals:

Group 1: Control (n=6) treated with vehicle.

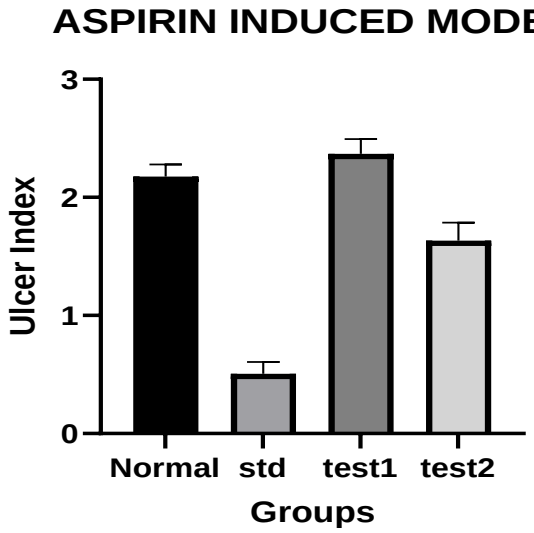
Group 2: Test I (n=6) with test drug P.O. (200mg/kg).
Group 3: Test II (n=6) with test drug P.O. (400mg/kg).
Group 4: Standard (n=6) with standard drug and inducing agent (20mg/kg) P.O.
(Omeprazole 20mg/kg+ Ethanol 1 ml/body weight).

RESULTS:

The data were studied with the help of one-way ANOVA followed by Dunnet's multiple comparison test. The mean ± S.E.M values were calculated for all groups. P< 0.05 was considered to be statistically significant.

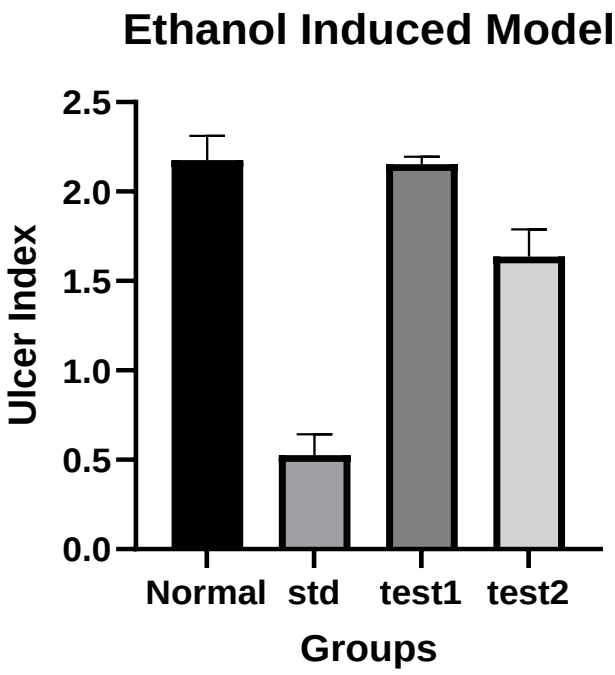
EFFECT OF JACQUEMONTIA CAERULEA ON ASPIRIN INDUCED MODEL

S.NO	GROUPS	Dose	Ulcer Index
1	**Control**	10ml/kg	2.17±0.10****
2	**Std Misoprostol**	20mg/kg	0.50±0.09****
3	**Test 1**	200mg/kg	2.37±0.12****
4	**Test 2**	400mg/kg	1.63±0.15****



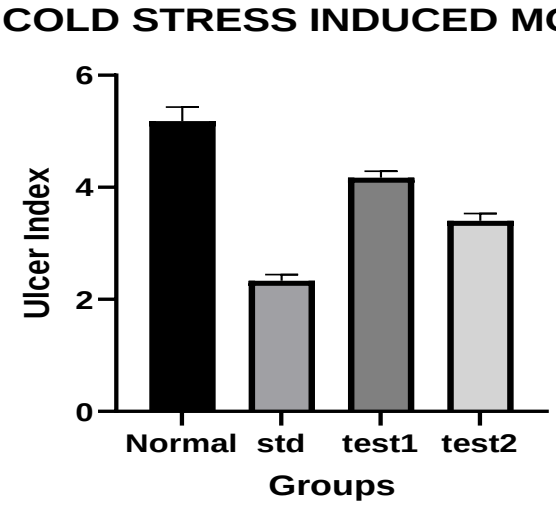
EFFECT OF JACQUEMONTIA CAERULEA ON ETHANOL INDUCED MODEL

S.NO	GROUPS	Dose	Ulcer Index
1	Control	10ml/kg	2.17±0.13****
2	Std Omeprazole	20mg/kg	0.52±0.11****
3	Test 1	200mg/kg	2.15±0.04****
4	Test 2	400mg/kg	1.63±0.15****



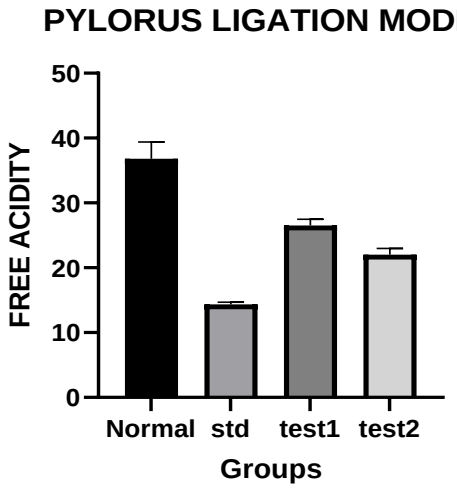
EFFECT OF JACQUEMONTIA CAERULEA ON COLD STRESS INDUCED MODEL

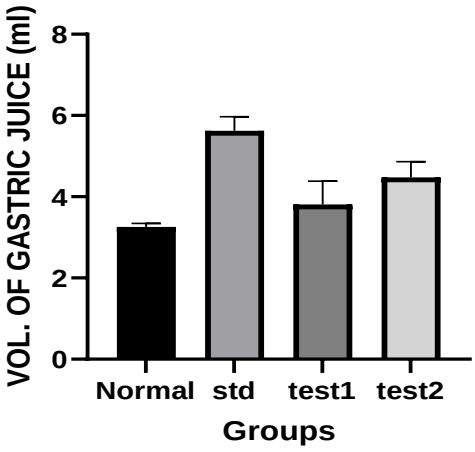
S.NO	GROUPS	Dose	Ulcer Index	% of ulcer protection
1	Control	10ml/kg	5.18 ±0.25****	---------
2	Std Amitriptyline	20mg/kg	2.33±0.10****	73%
3	Test 1	200mg/kg	4.17±0.11****	45%
4	Test 2	400mg/kg	3.40±0.13****	68%

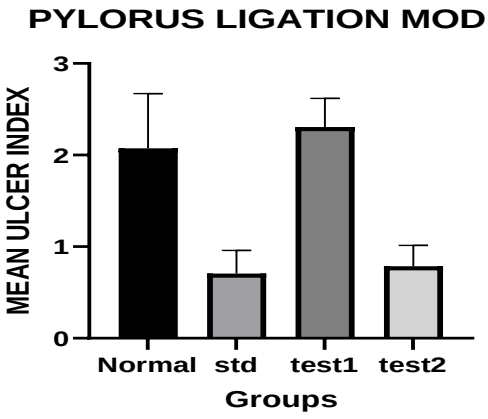


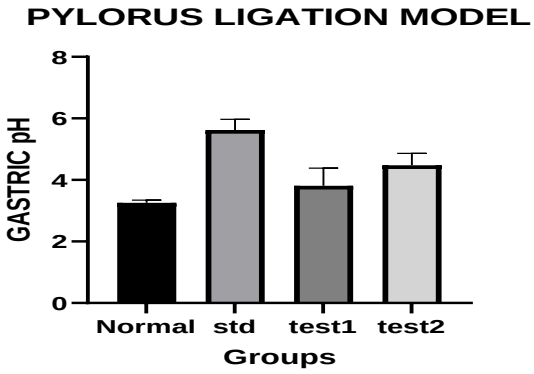
EFFECT OF JACQUEMONTIA CAERULEA ON PYLORUS LIGATION MODEL

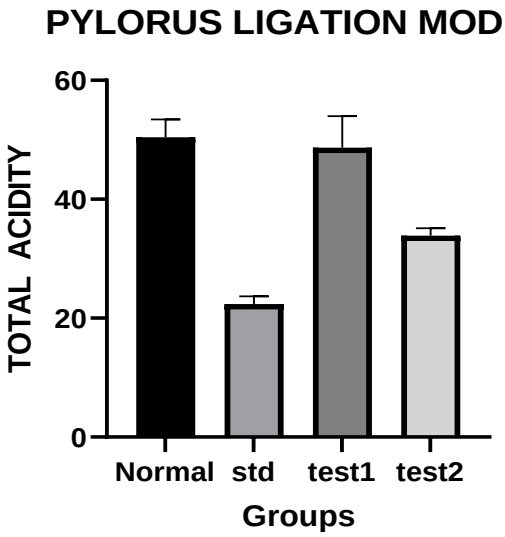
S.NO	GROUPS	Dose	VOL. OF GASTRIC JUICE (ml)	GASTRIC pH	FREE ACIDITY	TOTAL ACIDITY	MEAN ULCER INDEX
1	Control	10ml/kg	3.37±0.32****	3.25± 0.09****	36.80± 2.59****	50.45± 2.97****	2.07±0.59****
2	Std Omeprazole	20mg/kg	1.78±0.18****	5.62± 0.34****	14.35± 0.34****	22.38± 1.30****	0.70±0.25****
3	Test 1	200mg/kg	2.49±0.22****	3.810± 0.57****	26.52± 0.95****	48.66± 5.29****	2.30±0.31****
4	Test 2	400mg/kg	1.87±0.36****	4.48± 0.38****	22.01± 0.98****	33.89± 1.21****	0.78±0.22****









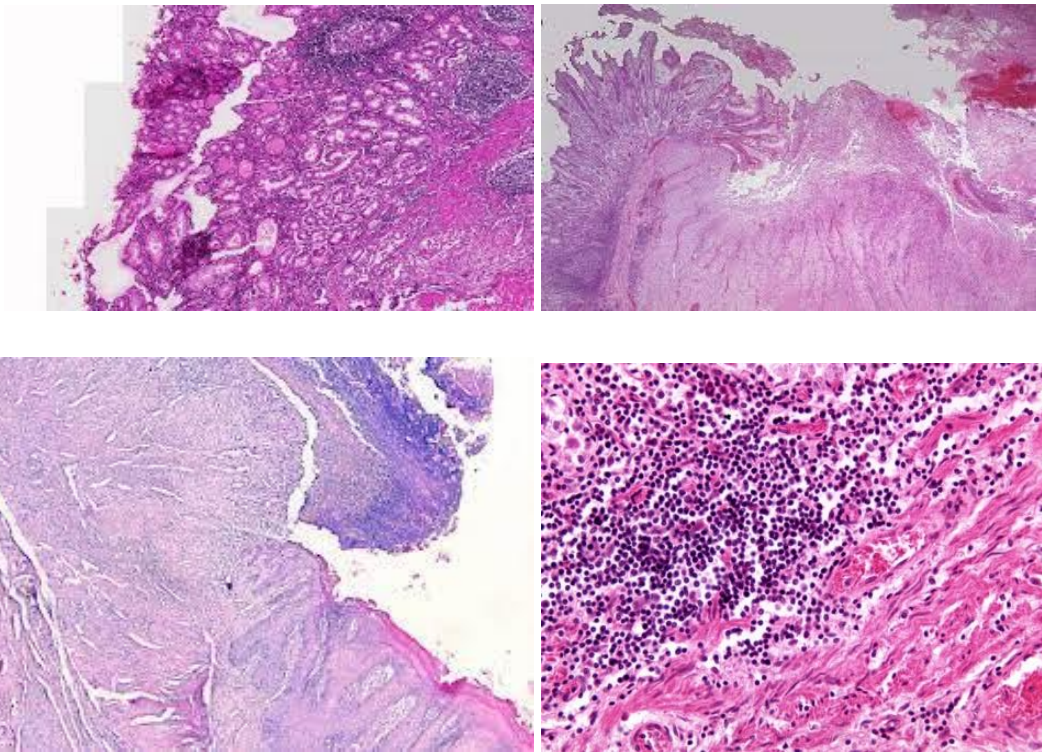


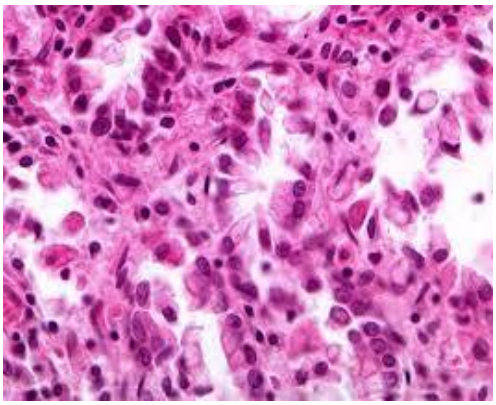
The data were studied with the help of one-way ANOVA followed by Dunnet's multiple comparison test. The mean ± S.E.M values were calculated for all groups. P< 0.05 was considered to be statistically significant.

Statistical Analysis:

Data obtained will be evaluated using ANOVA test followed by Dunnet's t-test.

Histopathological Studies of Ulcers :





DISCUSSION & CONCLUSION

In this study it is observed that the methanolic extract of Jacquemontia caerulea at certain concentrations showed significant reduction in releasing gastric acid. Hyperchlorhydria is a problem characterized by uncontrolled hyper secretion of hydrochloric acid from parietal cells of gastric mucosa through proton pump. H^+/K^+ -ATPase is a key enzyme in inducing acidity, it is located on apical secretory membrane of parietal cells. In H^+/K^+ -ATPase inhibition activity, the methanolic extract of Jacquemontia caerulea showed maximum percentage inhibition.

The investigation into the anti-ulcer activity of Jacquemontia caerulea on experimental rats has yielded noteworthy insights into its potential therapeutic efficacy for managing gastric ulcers. The following discussion encompasses key findings, their contextualization, potential mechanisms of action and considerations for future research:

The results of this study demonstrate a significant reduction in gastric ulcers in rats treated with Jacquemontia caerulea extract. This outcome aligns with the primary objective of assessing the plant's anti-ulcer properties and underscores its potential as a gastroprotective agent.

The observed reduction in gastric ulcers is consistent with trends reported in studies investigating the anti-ulcer activities of various medicinal plants. While each plant's specific mechanisms may differ, the overall trend supports the exploration of botanical extracts for their gastroprotective potential. Comparisons with existing literature reinforce the validity of Jacquemontia caerulea as a subject of interest.

Although the precise mechanisms underlying Jacquemontia caerulea's anti-ulcer effects remain to be fully elucidated, we propose that its bioactive compounds may contribute to anti-inflammatory and antioxidant activities. These properties could promote mucosal defense, inhibit ulcerogenic factors, and enhance the overall resilience of the gastric mucosa. Future studies employing molecular and biochemical analyses are essential to unravel the specific pathways involved.

The absence of observable adverse effects or signs of toxicity in rats treated with Jacquemontia caerulea extract at the tested dosage is a promising indication of its safety. This aspect is crucial for the potential translation of the plant's use into clinical applications. However, comprehensive safety assessments, including chronic toxicity studies, are warranted before considering its long-term use.

CONFLICT OF INTEREST

All authors approve the final manuscript and declare that there are no conflicts of interests.

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