



Pharmacological Evaluation of Phytochemical and Cyathocline Lyrata Ethanolic Plant Extract

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Abstract

In recent years, many studies have been conducted to investigate the antiulcerogenic properties of plants containing flavonoids. The antioxidant activity of flavonoids has recently piqued the interest of researchers due to mounting evidence that oxidation processes are implicated in the causes of various gastrointestinal illnesses, including ulcerogenesis. Photo-constituents such as flavonoids, tannins, terpenoids, and saponin have been identified as potential gastro protective agents in numerous anti-ulcer literatures. Flavonoids, tannins, and triterpenes are examples of cytoprotective active ingredients whose antiulcerogenic activity has been thoroughly demonstrated. The ethanolic extract contains these phytoconstituents, as evidenced by phytochemical screening for antiulcer activity. The ethanolic extract of Cyathocline Lyrata was tested utilizing a water immersion stress caused ulcer. Oral administration of ethanol extract of Cyathocline Lyrata at doses of 200 and 400 mg/kg resulted in dose dependent inhibition percentages of 55.12% and 63.03%, respectively, as compared to the ulcer control, demonstrating anti-ulcer action. When compared to the ulcer control, the conventional medicine omeprazole (20mg/kg) revealed a percentage inhibition of 70.58%. The extract-treated and ulcer-control groups were compared to the normal control group. It demonstrates that Cyathocline Lyrata has superior antiulcer action.

Key Words: Cyathocline Lyrata, Flavonoids, tannins, ethnobotanical.

INTRODUCTION

Peptic ulcer disease (PUD) is defined by a break in the inner lining of the GI tract caused by stomach acid secretion or pepsin. It penetrates the stomach epithelium's muscularis propria layer. It is most commonly found in the stomach and proximal duodenum. It can affect the lower esophagus, the distal duodenum, or the jejunum. In patients with a stomach ulcer, epigastric pain normally develops within 15-30 minutes of eating; whereas, discomfort with a duodenal ulcer usually occurs 2-3 hours after eating. Helicobacter pylori testing are now recommended

in all individuals with peptic ulcer disease. Endoscopy may be required in certain patients to confirm the diagnosis, particularly those with serious symptoms.¹

Peptic ulcer disease is caused by an imbalance between offensive (acid, pepsin, and Helicobacter pylori) and defensive (mucin, prostaglandin, bicarbonate, nitric oxide, and growth hormones) components. Peptic ulcers were once thought to be caused by spicy foods and stress; however, these were discovered to be merely aggravating factors, with the true causes being bacterial

infection (*Helicobacter pylori*) or a reaction to various medications, particularly NSAIDs (nonsteroidal anti-inflammatory drugs). The primary etiological factors related with peptic ulcer are *Helicobacter pylori*, NSAIDs medicines, emotional stress, alcohol misuse, and smoking. *Helicobacter pylori*, Gram-negative bacteria, remain present between the mucous layer and the gastric epithelium and are specifically built to thrive in the stomach's hostile environment. *Helicobacter pylori* begin in the antrum and migrate to the more proximal parts of the stomach throughout time.²

The current study was carried out to analyze medicinal plants used as gastro protective and therapeutic agents on ulcers in ayurvedic resources, as well as to acquire evidence for their efficacy and biological mechanisms in modern research.³ To achieve this goal, the Indian ayurvedic book *Materia Medica* and electronic databases such as science direct, PubMed, Scopus, and google scholar were searched for each of the medicinal plants for peptic ulcers, and all retrieved articles were evaluated for in vitro, in vivo, or clinical evidence for efficacy and possible mechanisms. The researchers found either clearly illustrate the usefulness of these herbs or indirectly demonstrate their efficacy on the implicated processes in the treatment of peptic ulcers.⁴

Materials and Methods^{5, 6, 7, 8}

Extraction of Plant:

Cyathocline Lyrata fresh plant was washed with distilled water, shade dried at room temperature, and then pulverized into coarse powder in a laboratory grinder. With the maceration method, 100 gm of dried powder was extracted with alcoholic (ethanol) and other solvents. The plant was immersed in an alcoholic solvent (ethanol) for 24 hours to obtain an extract, which was then evaporated in a water bath at 55°C to obtain crude as a semisolid mass.

Weight of extract

$$\% \text{ yield} = \frac{\text{Weight of extract}}{\text{Weight of plant material used}} \times 100$$

Tests for Carbohydrates

Molish Test

In a test tube, 2 ml of extract was treated with 2 drops of alcoholic -naphthol solution before 1 ml of concentrated sulphuric acid was gently applied around the edges of the test tube. The presence of carbs is shown by the formation of a violet ring at the junction.

Fehling's Test

In a test tube, 1 ml of extract, 1 ml of Fehling's A and 1 ml of Fehling's B solutions were mixed and heated in a water bath for 10 minutes. The presence of reducing sugar is indicated by the formation of red precipitate.

Benedict's test

In a test tube, an equal volume of Benedict's reagent and extract were combined and heated in a water bath for 5-10 minutes. The test solution turns green, yellow, or red depending on the amount of reducing sugar present, indicating the presence of reducing sugar.

Barfoed's Test

In a test tube, 1 ml of extract and Barfoed's reagent were combined and heated on a water bath for 2 minutes. The presence of monosaccharide is shown by the red color caused by the production of cupric oxide.

Tests for Protein and Amino acids

Biuret's Test

In a test tube, 1 ml of 10% sodium hydroxide solution was added to the extract and heated. To the aforesaid mixture, a drop of 0.7% copper sulphate solution was added. The presence of proteins is indicated by the production of violet or pink color.

Tests for Glycosides

Borntrager's Test

Dilute sulphuric acid was added to 3 ml of test solution, heated for 5 minutes, and filtered. An equal volume of benzene or chloroform was added to the cooled filtrate and wellled. Ammonia was added to the organic solvent layer after it was separated. The presence of anthraquinone glycosides is indicated by the formation of pink to red color in the ammonical layer.

Legal's Test

In pyridine, 1 mL of test solution was dissolved. 1 mL of sodium nitropruside solution was added, and the solution was alkalized with 10% sodium hydroxide solution. The presence of Cardiac glycosides is indicated by the formation of pink to blood red color.

Keller-Killiani Test

In a test tube, 2 ml of test solution was mixed with 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride. By the side of the test tube, carefully add 0.5 mL of strong sulphuric acid. The presence of Cardiac glycosides is shown by the formation of blue color in the acetic acid layer.

Tests for Alkaloids

Concentrated hydrochloric acid was added to the extract, shaken thoroughly, and filtered. The following tests were performed.

Mayer's Test

A few drops of Mayer's reagent were applied around the walls of the tube to 2-3 ml of filtrate. The presence of alkaloids is indicated by the formation of white or creamy precipitate.

Hager's Test

A few drops of Hager's reagent were applied to 1-2 ml of filtrate in a test tube. The presence of alkaloids can be detected by the formation of yellow precipitate.

Wagner's Test

A few drops of Wagner's reagent were applied to 1-2 ml of filtrate in a test tube. The presence of alkaloids is indicated by the formation of a reddish brown precipitate.

Tests for Saponins Froth Test

The extract was diluted with distilled water and agitated for 15 minutes in a graduated cylinder. The presence of saponins is indicated by the creation of a foam layer.

Tests for Flavonoids Lead

Acetate Test

A few drops of lead acetate solution were added to the extract. The presence of flavonoids may be indicated by the formation of yellow precipitate.

Alkaline Reagent Test

In a separate test tube, the extract was treated with a few drops of sodium hydroxide. The presence of flavonoids is indicated by the formation of a

bright yellow hue that fades when a few drops of dilute acid are added.

Tests for Triterpenoids and Steroids

Salkowski's Test

The extract was chloroform-treated and filtered. The filtrate was agitated and allowed to stand after a few drops of strong sulphuric acid were added. Sterol is present if the lower layers turn crimson. The presence of triterpenes is indicated by the presence of a golden yellow layer at the bottom.

Lieberman-Burchard's Test

The extract was chloroform-treated. A few drops of acetic anhydride were added to this solution before it was heated and cooled. Through the test tube's sidewalls, concentrated sulphuric acid was added. production of a brown ring at the intersection of two layers, if the upper layer turns green, indicates the presence of steroids, while production of a deep red color indicates the presence of triterpenoids.

Tests for Tannin and Phenolic compounds Ferric Chloride Test

A small amount of extract was dissolved in distilled water. 2 ml of 5% ferric chloride solution was added to this solution. Blue, green, or violet color formation indicates the presence of phenolic chemicals.

Lead Acetate Test

Distilled water was used to dissolve some of the extract. A few drops of lead acetate solution were added to this solution. The presence of phenolic compounds is shown by the formation of white precipitate.

Gelatin Test

Some extract dissolved in distilled water. 2 ml of 1% gelatin solution containing 10% sodium chloride was added to this solution. The formation of white precipitate suggests the presence of phenolic chemicals.

Tests for Fats and Oils

Solubility test

Solubility was discovered after adding a few mL of chloroform to 2-3 mL of the extract's alcoholic solution.

Solubility was detected after adding a few mL of 90% ethanol to 2-3 mL of the extract's alcoholic solution.

Determination of total phenolic

One aromatic ring with one or more hydroxyl groups is a phenolic defining characteristic. Due to their strong propensity to bind metals, phenolic compounds have antioxidant properties. Phenolic compounds have hydroxyl and carboxyl groups and can bind metals, most notably iron and copper. When phenols and phosphomolybdic acid interact in the Folin-Ciocalteu reagent in an alkaline media, a blue-colored complex is created that may be measured spectrophotometrically.^{9,10}

Folin-ciocalteu colorimetric assay

The FolinCiocalteu reagent was used to determine the quantity of total phenolic in extracts. As a benchmark, gallic acid was employed, and total phenolics were reported as mg/g gallic acid equivalent (GAE). Gallic acid concentrations of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml were produced in methanol. Plant extracts at concentrations of 0.1 and 1mg/ml were produced in methanol, and 0.5ml of each sample was placed into the test and combined with 2.5ml of a 10 fold dilute folinCiocalteu reagent and 2ml of 7.5% sodium carbonate. The tubes were wrapped in parafilm and left at room temperature for 30 minutes before being spectrophotometrically read at 760 nm. All tests were carried out in triplicate. The folin-Ciocalteu reagent reacts with reducing substances such as polyphenols. When they react; they produce a blue colour. This blue colour was spectrophotometrically measured.

A line of regression from Gallic acid was utilized to estimate the unknown phenol concentration.

$$y = 0.005x + 2.569 \text{ and } R^2 = 0.991$$

Thus, the goodness of fit for the selected standard curve was found to be good. By plotting the absorbance of the test sample ($y = \text{absorbance}$) along the regression line of the previously indicated Gallic acid.

Determination of Total Flavonoid Content

Principal

Flavonoids are polyphenolic substances found in nature that are classified according to their

chemical structure as flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins, and chalcones. Flavonoids react with AlCl_3 to create a colored product that may be quantified spectrophotometrically.

An aliquot of diluted rutin sample or standard solution was stirred for 6 minutes in a 75 μl NaNO_2 solution before adding 0.15 mL AlCl_3 (100 g/L). 0.5 mL of NaOH was added after 5 minutes. With distilled water, the final volume was adjusted to 2.5 ml and carefully mixed. The absorbance of the combination was measured at 510 nm in comparison to the identical mixture without the sample as a blank. The total flavonoid concentration was reported as mg Rutin/g dry weight (mg rutin/g DW) using the Rutin calibration curve. Three replications were performed on all samples.

A line of regression from rutin was utilized to estimate the unknown flavonoid concentration.

$$y = 0.001x - 0.020 \text{ and } R^2 = 0.994$$

Thus, the goodness of fit was found to be good for the specified standard curve. By inserting the absorbance of the test sample ($y = \text{absorbance}$) in the line of regression of the previously indicated rutin.^{11, 12, 13}

Pharmacological Activity

Experimental Animals: Strain: Albinorats (180-200gm)

Animals were chosen The animals were then randomly divided into different treatment groups and housed in propylene cages with sterile husk as bedding. The animals were kept in a 30.7% relative humidity environment with a 22:20 C temperature and a 12:12 light and dark cycle. The animals were fed normal pellets (Golden feeds, New Delhi, India) and had free access to water (additional components were added to the water according to the methodology outlined below).^{14, 15, 16}

Acute oral toxicity

The acute oral toxicity test was carried out in accordance with the OECD 423 standards. The Organization for Economic Cooperation and Development (OECD) specifies guidelines for oral acute toxicity studies. It is an international association that strives to reduce both the number

of animals used and the level of discomfort associated with acute toxicity testing. Animals were given single doses of the medication in an acute toxicity investigation of Cyathocline Lyrata plant extract. The albino rats were divided into groups. All animals fed the conventional rat pelleted diet had unlimited access to tap water. The study's doses were 2000 mg/Kg, 400 mg/Kg, and 200 mg/Kg.

The animals were monitored for death in the 72 hours following sample injection. Screening on the basis of an acute toxicity research, the test was chosen for the pharmacological on the basis of maximum tolerated dosage limit (MTD), as there was no mortality seen up to 2000 mg/Kg. Finally, two dosages (200 mg/kg and 400 mg/kg) were chosen for additional pharmacological research.

**Anti-Ulcer Activity
Preparation of animals**

The animals were chosen at random and kept in their cages for at least 5 days before treatment to allow them to acclimate to laboratory environment.^{18, 19}

Extracts & Standards used

Dried plant of Cyathocline Lyrata extract.
Omeprazole (20mg/gb.w.)

Assessment of gross mucosal damage

The glandular lesion was viewed with a 10 x magnifying glass, the length was measured with a divider and scale, and the gastric lesion was rated as follows:

Scoring of ulcer was made as follows:

- Normal stomach (00)
- Red coloration.....(0.5)
- Spot ulcer.....(01)
- Hemorrhagic streak.....(1.5)
- Ulcers.....(02)
- Perforation.....(03)

The ulcer index of each animal has been calculated by adding the values, determining their mean values, and calculating the percentage inhibition.¹⁷

Formula for Ulcer Protection

(Ulcer index Control - Ulcer index Test)

$$\% \text{ Protection} = \frac{\text{Ulcer index Control} - \text{Ulcer index Test}}{\text{Ulcer index Control}} \times 100$$

Ulcer index Control

Water Immersion Stress induced ulcer

Five groups of six albino rats (180-200gm) were formed. From group II to group V, the ulcer was generated by fasting the animals for 24 hours and forcing them to swim for 7 hours in a glass cylinder (ht. 45 cm and diameter 25 cm) containing water to a height of 35 cm and kept at 25°C. All medication solutions were administered one hour before the forced swimming. Cervical decapitation was used to sacrifice the animals.

Group I: Normal control (healthy).

Group II: Swim stress control given normal saline.

Group III: Swim stressed along with standard 20mg/kg omeprazole.

Group IV: Swim stressed along with ethanolic extract of Cyathocline Lyrata at dose 200mg/kg.

Group V: Swim stressed along with ethanolic extract of Cyathocline Lyrata at dose 400mg/kg.

Histological observation.

The animals were sacrificed, and their stomachs were opened along the greater curvature. Gastric tissue specimens were preserved in 10% buffered formalin. Sections of the stomach were cut at 5µm intervals and stained with hematoxylin and eosin for histological analysis. The efficacy of medicines was determined by assessing inflammatory and necrotic changes in mucosal tissue.

RESULTS AND DISCUSSION

Plant Extraction yield

$$\% \text{ yield} = \frac{\text{Weight of extract}}{\text{Weight of plant material used}} \times 100$$

The plant material was extracted by maceration process and the percentage yield calculated.

Table: Percent yield for different solvent extract of Cyathocline

Sr. No.	Solvent for extraction	Percent Yield
1.	Petroleum Ether	2.4
2.	Chloroform	12.6
3.	Benzene	8.9

4.	Ethanol	16.2
5.	Water	14.6

Phytochemical screening:

Table: Chemical test for extract of *Cyathocline Lyrata*

S. No.	Test	Petroleum Ether	Ethanol	Benzene	Chloroform	Water
1.	Carbohydrate a) Fehling test b) Benedict test	-	-	-		-
2.	Protein a) Xanthoprotein test	-	+	+		-
3.	Alkaloid a) Mayer test	+	+	-	+	-
4.	Tanin (5% FeCl ₃)	-	+	-	-	-
5.	Amino Acid a) Ninhydrin test	-	-	-		+
6.	Steroid a) Salkowski reaction	+	-	+	+	-
7.	Glycoside a) Keller Killanin test	+	+	+	+	-
8.	Flavonoid a) Alkaline reagent test	+	+	+	+	+
9.	Phenolics a) FeCl ₃ test	+	+	+	+	+
10.	Terpenoids a) Salkowski	-	+	-	+	+

test					
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Acute oral toxicity:

The acute oral toxicity research was carried out in accordance with OECD 423 standards. Toxicology tests were conducted using four dose ranges: 100mg/kg, 200 mg/kg, 400 mg/kg, and 2000 mg/kg. Individual animals were examined for the next 4 hours after dosage for the presence of death, as well as 72 hours after sample administration.

Table: Acute oral toxicity of ethanolic extract of *Cyathocline Lyrata*

S.No.	Groups	Observations/Mortality
1.	100mg/kg	0/3
2.	200mg/kg	0/3
3.	400mg/kg	0/3
4.	2000mg/kg	0/3

In an acute oral toxicity trial, rats were given doses of 100mg/kg, 200mg/kg, 400mg/kg, and 2000mg/kg during a 72-hour period. There was no mortality and no behavioural alterations were observed, thus dosages of 200 and 400 mg/kg were chosen for the pharmacological investigation for antiulcer activity.

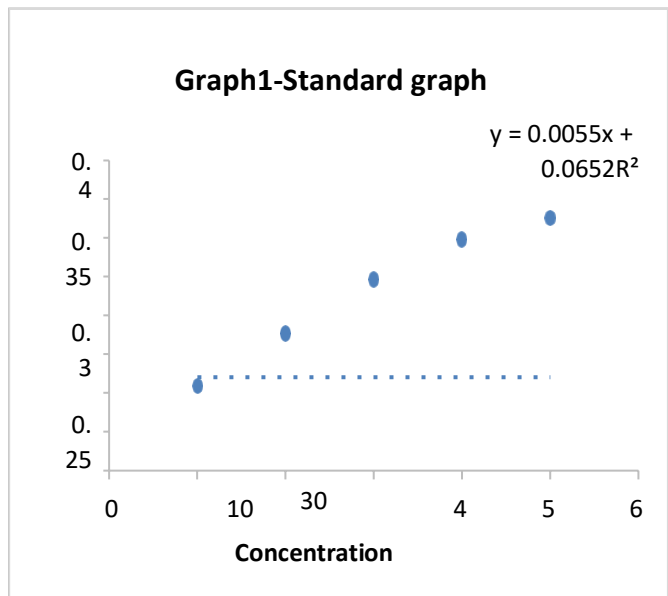
Quantitative Analysis

Total Phenolic Content determination:

Table 6: Standard Table of Gallic acid

S.No.	Concentration	Absorbance
1	10 µg/ml	0.1098
2	20 µg/ml	0.1763
3	30 µg/ml	0.2468
4	40 µg/ml	0.2981
5	50 µg/ml	0.3258

3	30 µg/ml	0.165
4	40 µg/ml	0.177
5	50 µg/ml	0.201



Graph 1: Standard curve of Gallic acid

Total Phenolic Content determination:

Table: Total phenolic content in Cyathocline Lyrata

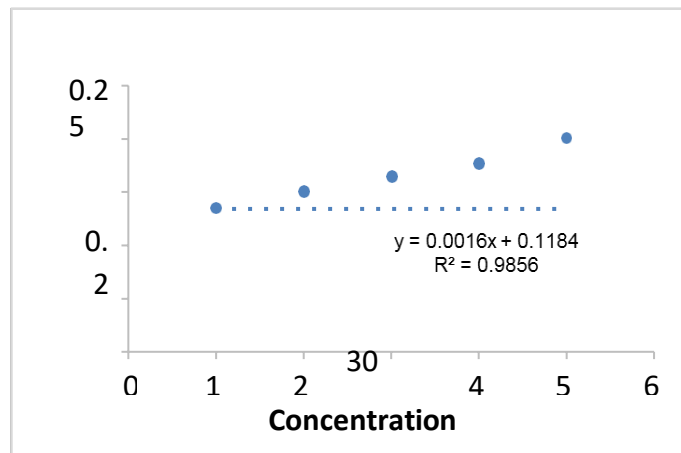
Sr.No.	Absorbance of extract	Concentration of extract	Total phenolic content mg/g equiv. to Gallic acid
1	0.353	1mg/ml	28.0
2	0.358	1mg/ml	30.0
3	0.354	1mg/ml	29.0
	Mean±SD		29±.05

The phenolic content of the ethanolic extract of Cyathocline Lyrata was determined to be 29.0 mg/g comparable to Gallic acid.

Total Flavonoid Content determination:

Table: Standard curve of Rutin

S. No.	Concentration	Absorbance
1	10 µg/ml	0.135
2	20 µg/ml	0.151



Graph 2: Standard Graph of Rutin

Table: Total flavonoid in extract of Cyathocline Lyrata

S.No	Concentration (mg/ml)	Absorbance	Total flavonoid content mg/g equiv. of rutin
1	1mg/ml	0.129	10.0±0.6846
2	1mg/ml	0.127	9.5±0.6916
3	1mg/ml	0.124	9.1±0.7516
		MEAN±S.D	9.60 ±0.7216

The flavonoid content of Cyathocline Lyrata ethanolic extract was determined to be 9.60 mg/g comparable to rutin.

In vivo antiulcer activity:

Water immersion induced ulcer model:

In a water immersion induced ulcer model, treatment of Cyathocline lyrata at two distinct doses (200mg/kg and 400mg/kg) resulted in a substantial reduction in ulcer index when compared to the control group. In comparison to the control, Cyathocline lyrata demonstrated a protection index of 63.03% and 55.12% at doses of 400mg/kg and 200 mg/kg, respectively,

whereas Omeprazole demonstrated a protection percentage of 70.58%.

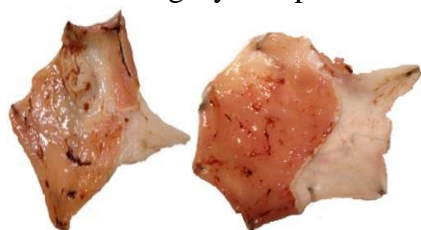
Table: Ulcer Index of cyathoclineyrata on gastric lesion induced by water immersion in rat.

Groups	Ulcer Index	%Inhibition
Group I- Normal Control (healthy)	0.00	100.0%
Group II- Experimental Control (diseased)	5.67±0.057	-
Group III Standard Omeprazole (20mg/kg)	1.68±0.032	70.58%
Group IV- Cyathocline lyrata (200mg/kg)	2.07±0.024	55.12.%
Group V Cyathocline lyrata (400mg/kg)	2.49±0.014	63.03%

All values are Mean ± SD, with n=6 in each group. The variations in mean values across treatment groups are more than would be predicted by chance; there is a statistically significant difference (P 0.001).

Morphological investigation of the stomach in swim stress-induced ulcers

In the normal group, stomach integrity was preserved and appeared normal. Severe bleeding, perforation, and spot ulcers were detected in the control group, whereas animals in the standard and extract-treated groups had less ulceration and stomach integrity was preserved.



a. Normal control b. Experimental Control



c. Omeprazole treated group (20mg/kg)
d. Extract treated group (200)

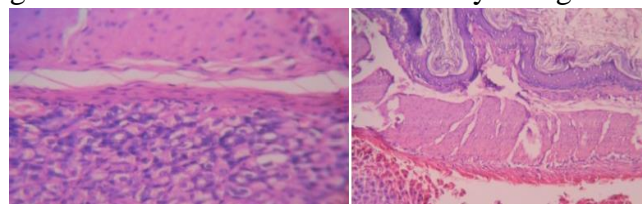


e. Extract treated group (400 mg/kg)

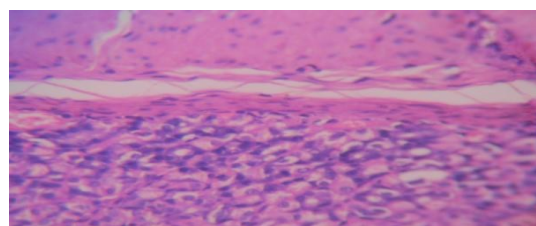
Fig: Morphological Features of Stomach in Stress Induced Ulcer

Histology of Stomach in swim Stress induced Ulcer:

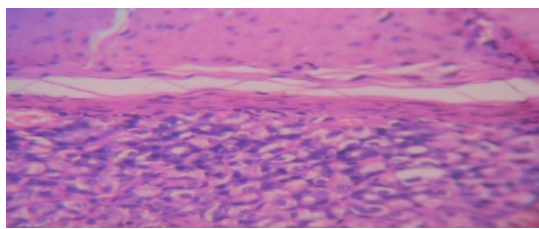
Histopathological analysis of gastric mucosa in the normal control group revealed intact gastric mucosa and a continuous epithelial surface. Mucosal ulceration was observed in the experimental control group. At a dose of 200mg/kg, Cyathocline Lyrata extract caused superficial erosions and a few ulcers, as well as minor inflammatory reactions. Sections of intact mucosa with minimal irritation were found in the 400mg/kg group with Cyathocline Lyrata extract. The omeprazole (20mg/kg) group had intact gastric mucosa with no inflammatory changes.



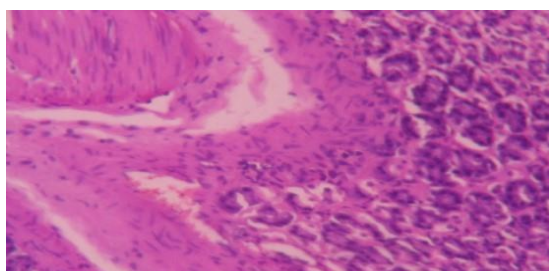
a. Normal control b. Experimental Control



c. Omeprazole treated group 20mg/kg



d. Extract treated group (200 mg/kg)



e. Extract treated group (400 mg/kg)

Fig: Histology of Stomach in Stress Induced Ulcer
SUMMARY AND CONCLUSION

In recent years, Many Indian herbs are utilized in traditional methods to treat a variety of human diseases. Cyathocline Lyrata is an essential medicinal plant that can be used to treat inflammation, discomfort, and ulcers. Furthermore, isolated principles from Cyathocline Lyrata should be evaluated scientifically in the future using various innovative experimental models and clinical trials to understand its mechanism of action, in search of other active constituents, so that its other therapeutic uses can be widely explored.

Flavonoids and phenolic are cytoprotective substances that can boost mucus, bicarbonate, and prostaglandin secretion, improve the gastric mucosal barrier, and scavenge free radicals, all of which are useful in preventing ulcerative and erosive gastrointestinal lesions.

Flavonoids are polyphenolic substances found throughout nature that are classified into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins, and chalcones based on their chemical structure. Flavonoids' pharmacological actions were closely tied to their functional group. Furthermore, there may be some interference from other chemical components present in the extract. Furthermore,

flavonoids may protect cell structures through a variety of processes, one of which is their capacity to boost levels of glutathione, a powerful antioxidant. Many studies have been conducted to investigate the antiulcerogenic properties of plants containing flavonoids. Plants rich in flavonoids were discovered to be useful in preventing this type of lesion, owing to their antioxidant characteristics. The antioxidant activity of flavonoids has recently piqued the interest of researchers due to mounting evidence that oxidation processes are implicated in the causes of various gastrointestinal illnesses, including ulcerogenesis. Phyto-constituents such as flavonoids, tannins, terpenoids, and saponin have been identified as potential gastro protective agents in numerous anti-ulcer literatures. Flavonoids, tannins, and triterpenes are examples of cytoprotective active ingredients whose antiulcerogenic activity has been thoroughly demonstrated. Tannins may inhibit ulcer development due to their protein precipitating and vasoconstriction actions. Their astringent activity can aid in the precipitation of micro proteins at the ulcer site, generating an impermeable coating over the lining that inhibits gut secretions and protects the underlying mucosa from toxins and other irritants. These phytoconstituents were found in the ethanolic extract using phytochemical screening for antiulcer activity.

The ethanolic extract of Cyathocline Lyrata was tested utilizing a water immersion stress caused ulcer. Oral administration of ethanol extract of Cyathocline Lyrata at doses of 200 and 400 mg/kg resulted in dose dependent inhibition percentages of 55.12% and 63.03%, respectively, as compared to the ulcer control, demonstrating anti-ulcer action. When compared to the ulcer control, the conventional medicine omeprazole (20mg/kg) revealed a percentage inhibition of 70.58%. The extract-treated and ulcer-control groups were compared to the normal control group. It demonstrates that Cyathocline Lyrata has superior antiulcer action.

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