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Evaluation of Anti-ulcer activity of Ethanolic Extract of *Nymphaea Alba* Linn Flower in experimental rats.

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ABSTRACT

In the present study Anti ulcer activity of Ethanolic Extract of flower of *Nymphaea alba* Linn were investigated. The Anti ulcer activity of Ethanolic Extract of flower of *Nymphaea alba* were evaluated by Pylorus ligation & Ethanol induced ulcer model in experimental rats. In both models the common parameter determined was ulcer index. The Ethanolic Extract of Nymphaea alba (200 & 400 mg/kg) treat the Ulcer and produced significant inhibition of the gastric lesions induced by Pylorus ligation induced ulcer & Ethanol induced gastric ulcer. Preliminary Phytochemical analysis of Ethanolic Extract of Nymphaea alba revealed that the presence of various phytoconstituents Alkaloids, Carbohydrates (Polysaccharides), Glycosides, Steroids, Flavonoids and Tannin & Phenolic compound. The extract (200 mg/kg & 400 mg/kg) showed significant reduction in gastric volume, free acidity and ulcer index as compared to control. This present study indicates that Nymphaea alba Linn flower extract have potential Anti ulcer activity in the both models. These results may further suggest that Ethanolic extract was found to possess Antiulcerogenic as well as ulcer healing properties, which might be due to its Anti-secretary activity.

Keywords: Nymphaea alba Linn, Pylorus ligation, Ethanol induced ulcer model, ulcer index.

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INTRODUCTION

Gastric ulcer, one of the most widespread, is believed to be due to an imbalance between aggressive and protective factors. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (Helicobacter pylori) and drugs. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility. Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor "PAF", leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defences (mucus, bicarbonate, normal blood flow, prostaglandins(PG), nitric oxide). The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently there is no cost-effective treatment that meets all these goals. Hence, efforts are on to find a suitable treatment from natural product sources.[1-4]

Nymphaea alba is also known as the European white water lilly, white lotus, is an aquatic flowering plant of the family Nymphaeaceae. Nymphaea alba Linn (Nymphaeaceae) is Generally found in tanks and ponds throughout the warmer parts of India and Africa.. All parts of the plants are used in folk medicine. It grows in water from 30-150 centimeters deep and likes large ponds and lakes. The leaves may be up to thirty centimeters in diameter and they take up a spread of 150 centimeters per plant. It is globally distributed in Europe, North Africa, Southwest Asia, India, China and Russia. It is rich in tannic acid, Gallic acid, alkaloids, sterols, Flavonoids, glycosides, Hydrolyzable tannins and high–molecular-weight Polyphenolic compounds. All the parts of the plant have medicinal uses in traditional system of medicine. It is used as an aphrodisiac, anodyne, Anti-scrophulatic, astringent, Cardiotonic, demulcent, sedative and anti-inflammatory. Further, it also produces calming and sedative effects upon the nervous system, and is useful in the treatment of insomnia, anxiety and similar disorders. It Anti-carcinogenic action and inhibition of renal oxidative stress and hyperproliferative response were reported. It also possesses good anxiolytic activity. Gallic acid and ellagic acid are two widely occurring Phenolic compounds present in Nymphaea alba, to which many biological activities including anticancer and antiviral activity have been attributed.[5-10]

MATERIALS AND METHOD

Collection, identification and authentication of plant

The plant Nymphaea alba Linn (Flower) were collected from Sarasbag, Pune, Maharashtra, during the month ofJune-2012 The plant material was identified and authenticated by Prof. P. Jayaraman (Ph.D.), Director-Plant Anatomy Research Centre (PARC) Tambaram. The voucher specimen number is PARC/2012/1702 (a) and it was submitted to the laboratory of Department of Pharmaceutical Science, Shri Venkateshwara University Gajraula, Amroha (Uttar Pradesh) for future references.

Collection and maintenance of experimental animals

Wistar albino rats of either sex weighing between 150-250 gm of either sex were used. Institutional Animal Ethics Committee of Nagaji Institute of Pharmaceutical Science, Gwalior approved the experimental protocol; animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA Reg.No.-1498/PO/a/11/CPCSEA). The animals were housed in Polypropylene cages and maintained at 24˚C ± 2˚C under 12h light/ dark cycle and were feed ad libitum with standard pellet diet and had free access to water.

Acute Toxicity Studies

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). One-tenth of the median lethal dose (LD50) was taken as an effective dose. Acute toxicity study was done as per OECD, 2006 Guidelines. Acute oral toxicity tests found the LD50 of the Plant extract to be >2,000 mg/kg. The animals were observed for signs of toxicity such as hyperactivity, grooming, convulsions, sedation, and hypothermia continuously for 2 hours, and for mortality up to 24 hours, after administration of the doses.[11-12]

Extraction Method

The flower of Nymphaea alba were shade dried and reduced to coarse powder in a mechanical grinder. The powdered material obtained was then subjected to successive extraction by Hot Percolation Method using petroleum ether, chloroform, and Ethanol solvents in a soxhelet extractor. The different extracts obtained were evaporated at 45˚C to get a semisolid mass. The extracts thus obtained were subjected to Phytochemical analysis. The percentage yield of EENA was found to be 11 w/w %.[13]

Phytochemical Analysis of the Extracts

Preliminary Phytochemical analysis of EENA revealed that the presence of various phytoconstituents Alkaloids, Carbohydrates (Polysaccharides), Glycosides, Steroids, Flavonoids and Tannin & Phenolic compound.[14-15]

Evaluation of Antiulcer activity of *Nymphaea alba Linn*

Pylorus ligation induced ulcer model [16-17]

Simple and reliable methods for production of gastric ulceration in the rats were based on ligature of the pylorus. All the animals were fasted for 24 hours before pyloric ligation. One hour after drug or saline administration, pylorus part of the rat were ligated under light ether anesthesia. Four hours after pylorus ligation, rats were sacrificed by decapitation and their stomachs were dissected out after ligating the cardiac end. Each stomach was cut opened along the greater curvature and the content was collected. The mucosa was washed under running tap water and the extent of ulceration was scored.

Group-I were received distilled water orally and having pyloric ligated, Reference drug were administered orally for Group-II as a reference drug for Anti-ulcer activity. Groups-III and IV received Ethanolic Extract of *Nymphaea alba Linn* (EENA) in two dose (200 & 400 mg/kg). The plant extract and Reference drug are administered before 45 min of pyloric ligation**.**

(2) Ethanol induced ulcer model [18-19]

The ulcers were induced by administering ethanol. All the animals were fasted for 36 hours before administration of ethanol .The animals were divided into four groups, each consisting of six rats. Group-I represented the disease control group, which received ethanol orally. Reference drug were administered orally for Group-II as reference standard drug. Group-III and IV received Ethanolic Extract of *Nymphaea alba Linn* (EENA) in two doses (200 & 400 mg/kg) and The gastric ulcers were induced in rats by administrating absolute ethanol (90%) (1ml/200g) Orally, after 45 min of EENA and Reference drug treatment. The animal kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1h latter with anesthetic ether and stomachs were incised along the greater curvature and ulceration will be scored. A score for the ulcer was study similar to pyloric ligation induced ulcer model.

Scoring of ulcer was made as follows

Normal mucosal stomach……	(**0**)	Red coloration……	(**0.5**)
Spot ulcer……	(**1.0**)	Hemorrhagic streak……	(**1.5**)
Ulcers…….	(**2.0**)	Perforation……...	(**3.0**)

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

$$\% \text{ Protective} = \frac{\text{Control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Ulcer index were calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach.

The ulcer index was determined using the formula.

Ulcer index = 10/X

Where X = Total mucosal area / Total ulcerated area.

The collected gastric juice and gastric tissue samples were subjected for Biochemical and Histopathological evaluation. The gastric juice collected was centrifuged for 1000 rpm for 10 min and the volume of gastric juice, pH, total and free acidity was measured.

Acidity was calculated by using the formula

$$\text{Acidity} = \frac{\text{volume of NaOH x Normality of NaOH x 100}}{0.1} \text{ mEq/liter.}$$

Histopathological study [20]

Gastric tissue samples were fixed in neutral buffered formalin for 24 h. Sections of gastric tissue were histopathologically examined to study the ulcerogenic and/or anti-ulcerogenic activity of Ethanolic Extract of *Nymphaea alba Linn*. The tissues were fixed in 10% buffered formalin. The processed tissues were embedded in paraffin blocks and sections of about 5 mm thickness were cut by employing optical rotary microtome. These sections were stained with haematoxylin and eosin using routine procedures. The slides were examined microscopically for pathomorphological changes such as congestion, hemorrhage, edema and erosions using an arbitrary scale for severity assessment of these changes.

Statistical analysis

The results were reported as Mean ± SEM of different observations. Experimental data were analyzed using one-way analysis of variance (ANOVA) followed by *t*-test to compare the difference between the control and treated values. Graph Pad Prism Version was used for statistical calculations.

RESULTS AND DISCUSSION

Preliminary Phytoconstituent

The preliminary Phytochemical analysis of EENA revealed that the presence of various phytoconstituents which are represented in Table (1)

Table 1: Result of Phytochemical Investigation

Constituents	Chemical test	In Ethanolic extract
Alkaloids	Hager's Test	Present
	Mayer's Test	Present
	Dragendroff's Test	Present
	Wagner's Test	Present
	Molish's Test	Present
Carbohydrates (Polysaccharides)	Fehling's Test	Present
	Benedict's Test	Present
Glycosides	Baljet's test	Present
	Legal's test	Present
	Liebermann-Burchard Test	Present
Steroids	Salkowski test	Present
	Baljet test	Present
	Keller Killani's test	Present
Flavonoids	Shinoda test	Present
	Sodium hydroxide test	Present
Tannin & Phenolic compound	Lead acetate solution	Present
	5% $FeCl_3$ solution	Present
Fixed oil	Stain Test	Absent
Proteins and Amino acids	Millon's Test	Absent
	Biuret Test	Absent
	Ninhydrin Test	Absent
Saponin	Froth test	Present (Trace)

Effect of Ethanolic Extract of *Nymphaea alba Linn* on Pyloric ligation induced gastric ulcer

In pyloric ligated rats, Oral administration of Ethanolic Extract of *Nymphaea alba Linn* in two acidity, total acidity as compared to the pyloric ligated group. It was showing protection index of 74 % and 82 % at the dose of 200 and 400 mg/kg respectively in comparison to reference standard drug was reduction of ulcer 84 %. **(**Table 2 & Graph-1).

Effect of Ethanolic Extract of *Nymphaea alba Linn* on Ethanol-induced gastric ulcer

In control animal, oral administration of absolute ethanol produced characteristic lesions in the glandular portion of rat stomach which appeared as elongated bands of thick, black and dark red lesions. Ethanolic Extract of *Nymphaea alba Linn* has shown significant protection index of 54% and 67 % with the dose of 200 and 400 mg/kg respectively in comparison to ethanol induced group, Omeprazole as reference standard drug was reduction of ulcer 72 %. (Table 3 & Graph-2).

The animal pre-treated with Omeprazole significantly reduced the ulcer index and percentage protection when compared to the ethanol induced animal. In animal pre-treated with Ethanolic Extract of *Nymphaea alba Linn* significantly reduced the ulcer index and percent protection when compared to the ethanol treated group. A significant increase in p^H was observed on treatment with Ethanolic Extract of *Nymphaea alba Linn* when compared with ethanol treated group. The significant reduction of ulcer index and increase in pH also observed in Omeprazole treated group.

Histopathological evaluation

The pyloric ligated and ethanol treated groups showed the degeneration, hemorrhage, edematous appearance of the gastric tissue, where as Ethanolic Extract of *Nymphaea alba Linn* (400 mg/kg) and omeprazole (20 mg/kg) treated groups showed regeneration and prevents the formation of hemorrhage and edema.

Table 2: Effect of EENA on various parameters in pyloric ligation induced ulcers.

Group	Treatment	P^H of gastric juice Ulcer index Ulcer index	Protection (%)	P^H of gastric juice	Gastric Juice (ml)	Free acidity meq/ltr	Total acidity meq/ltr
I	Control (P. L.)	15.6±1.6	-----	2.4±.20	5.4±.20	97.9±1.3	116.8±.24
II	OMZ. (20 mg/kg)	2.5±.04*	84 %	4.9±.15*	2.4±.18*	32.7±2.5*	58.8±1.4*
III	EENA (200 mg/kg)	3.6±.06*	74 %	3.6±.20	4.4±.12	46.8±1.4*	69.8±.38*
IV	EENA (400mg/kg)	3.4±.03*	82 %	4.5±.18*	2.9±.15*	36.8±1.9*	61.8±1.4*

Values are expressed as mean ± SEM of 6 observations, Compaction- Group **I** Vs **II, III** and **IV**

Significant at *p<0.001 compared to control group.

Table 3: Effect of EENA on various parameters in Ethanol induced ulcers.

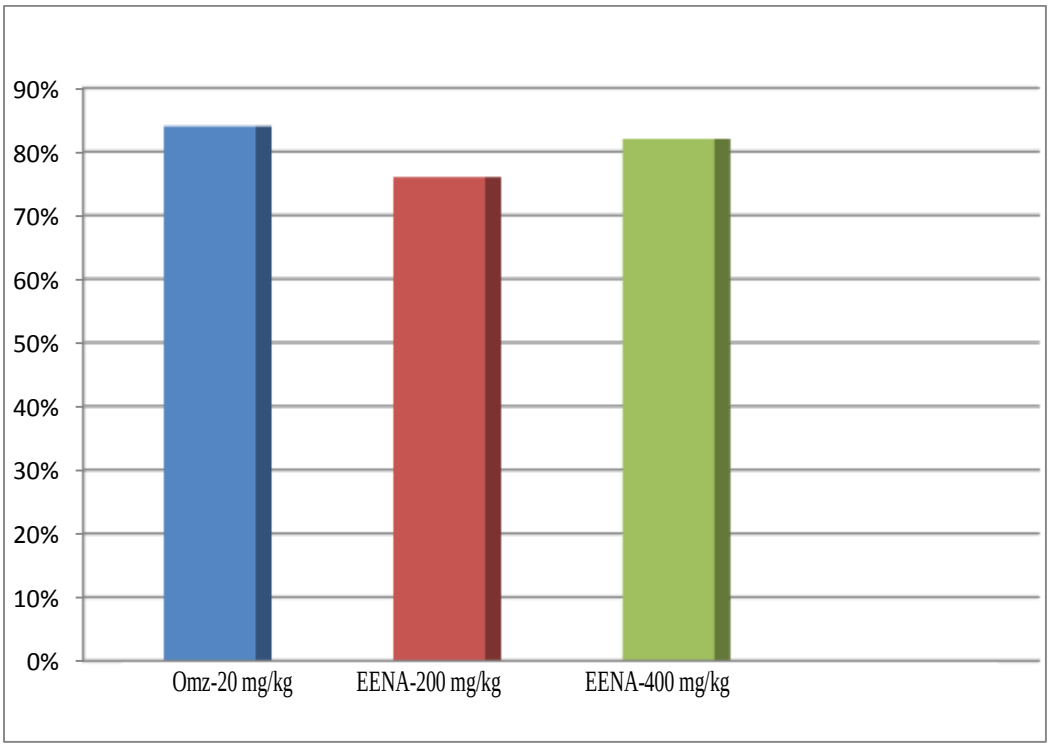
Group	Treatment	Ulcer index	% Protection	P^H of gastric juice
I	Control (Ethanol)	11.6±.08	-----	3.3±.20
II	OMZ(20 mg/kg)	3.6±.07*	72 %	5.4±.09*
III	EENA(200 mg/kg)	5.9±.05*	54 %	3.8±.15
IV	EENA(400 mg/kg)	4.4±.04*	67 %	4.8±.17*

OMZ**.** – Omeprazole. . P.L.-Pyloric Ligation, E**.**-Ethanol (1 ml/Animal)

Values are express as mean ± SEM of 6 observations,

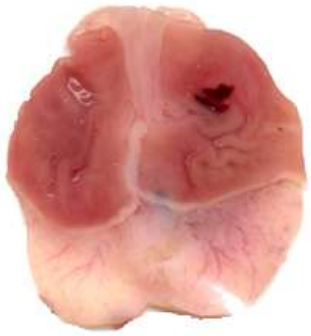
Comparison-Group **I** Vs **II, III** and **IV**

Significant at *p<0.001 compared to control group.

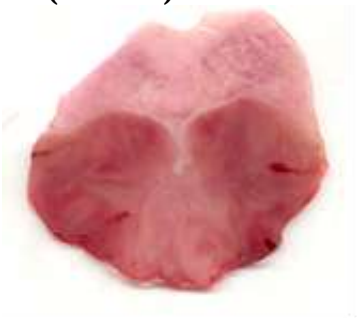


Graph 1: Percentage protection in pyloric ligation induced ulcer model.

TREATMENT GROUP (DOSES)



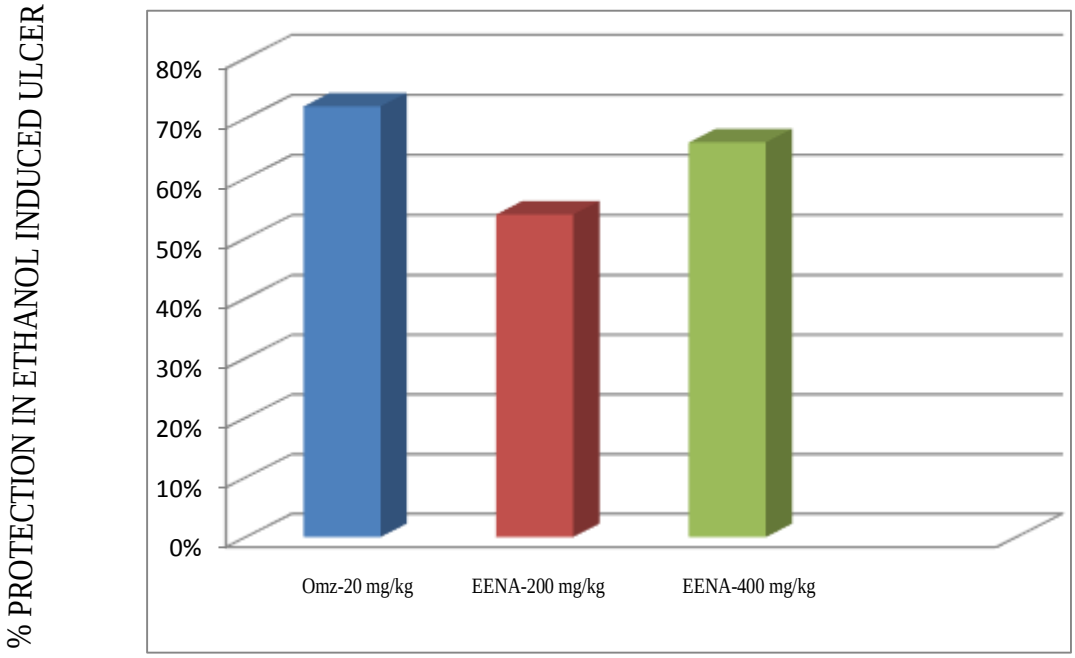
Control (P.L.)



Omeprazole (20 mg/kg)

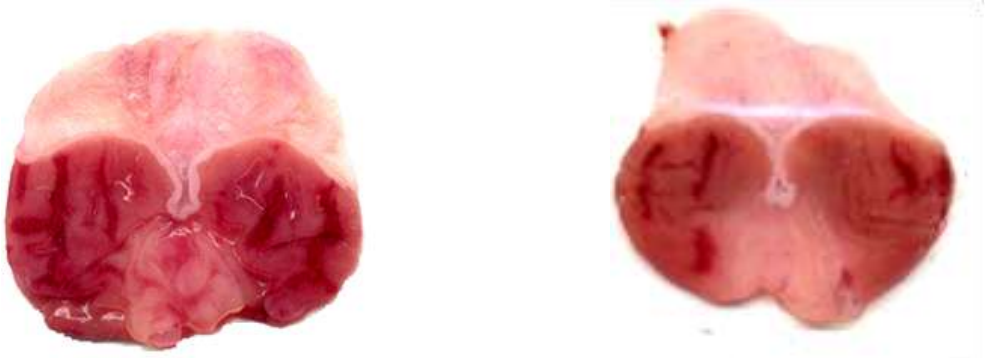
EENA (400 mg/kg)

Figure 1: - Pylorus Ligation Induced Ulcer



Graph: 2 Percentage protection in ethanol induced ulcer model.

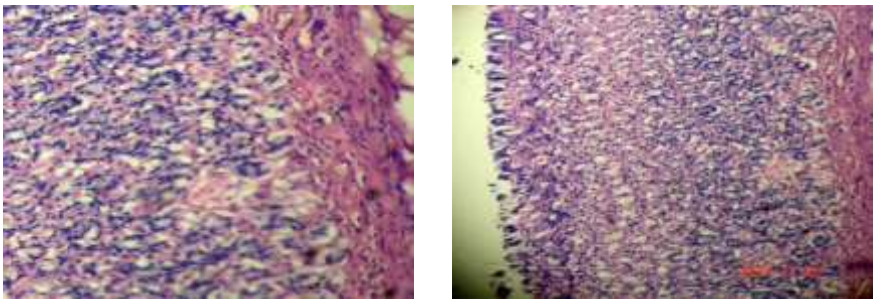
TREATMENT GROUP (DOSES)



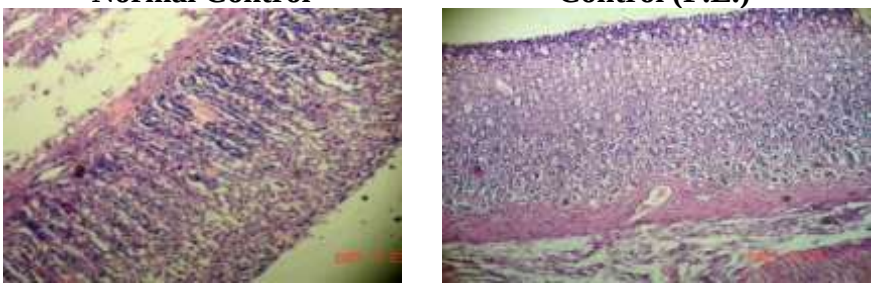
Control (Ethanol) Omeprazole (20 mg/kg)

EENA (400 mg/kg)

Figure 2: Ethanol Induced Ulcer

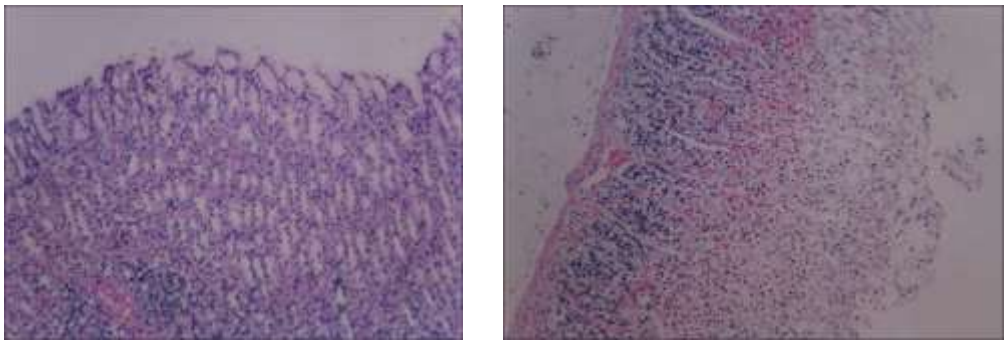


Normal Control **Control (P.L.)**

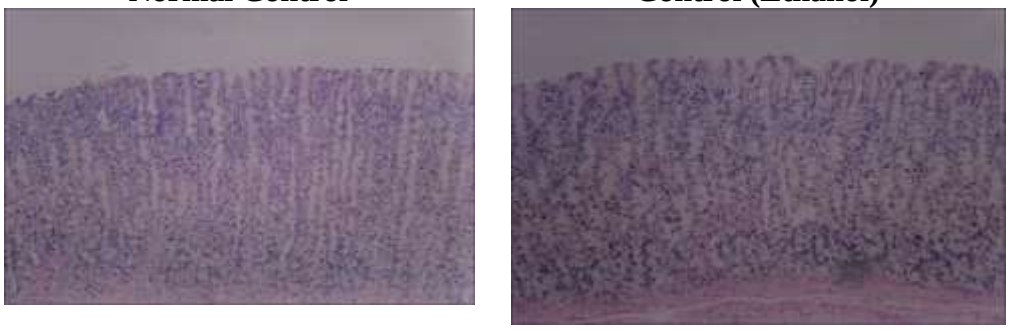


Omeprazole (20 mg/kg) **EENA (400 mg/kg)**

Figure 3: Histopathology of pyloric ligation induced ulcer model



Normal Control **Control (Ethanol)**



Omeprazole (20 mg/kg) **EENA (400 mg/kg)**

Figure 4: -Histopathology of Ethanol induced ulcer model

DISCUSSION

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanisms. To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defence mechanisms by increasing mucosal production, stabilizing the surface epithelial cells or interfering with the

prostaglandin synthesis. The causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid.

Pylorus ligation induced ulcer was used to study the effect of fruit extracts on gastric acid secretion and mucus secretion. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach. The original Shay rat model involves fasting of rats for 36 hours followed by ligation of pyloric end of the stomach. The ulcer index is determined 5 hours after pylorus ligation. The lesions produced by this method are located in the lumen region of the stomach. Many authors have modified the original model. In the present study, the Shay rat model described by Kulkarni was followed. The EENA and Omeprazole significantly decreased the total acidity and free acidity; this suggests that it having an antisecretory effect. Its antiulcer activity is further supported by histopathologically study shows that protection of mucosal layer from ulceration and inflammation.

Ethanol induced gastric ulcer was employed to study the cytoprotective effect of the extracts. Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intra cellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium. The extract shows protection against characteristic lesions produced by ethanol administration this antiulcer effect of EENA may be due to both reductions in gastric acid secretion and gastric cytoprotection. Further studies are needed for their exact mechanism of action on gastric acid secretion and gastric cytoprotection.[21-24]

CONCLUSION

The Phytochemical study revealed that the presence of alkaloids, carbohydrates, Tannins, Phytosterols, Anthraquinone, Glycosides, Saponins, Steroids and flavonoids. The results of phytochemical investigation had led to the conclusion that the compound may be Tannin & flavonoids derivative which is responsible for Antiulcer activity. EENA showed better Antiulcer activity by the use of a 'Pyloric ligation method & Ethanol Induced Ulcer Model' in Wistar rats. The EENA was effective in increasing the healing of gastric ulcers induced by Ethanol and pyloric

ligation model. The antiulcer effect of EENA may be due to both reductions in gastric acid secretion and gastric cytoprotection. The anti-ulcerogenic effect of EENA may be due to its antihistaminic effect. Further studies needed for exact mechanism of the EENA on their effect on gastric secretion and gastric cytoprotection.

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