e-ISSN: 2320-4230, p-ISSN: 2961-6085

Journal of Drug Discovery and Therapeutics

Available Online at www.jddt.in

CODEN: - JDDTBP (Source: - American Chemical Society)

Volume 12, Issue 04; 2024, 5-17

Phytochemical and Pharmacological Evaluation of Anti-ulcer Activity of *Nymphaea Alba* Linn Flower

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Received: 11-05-2024 / Revised: 15-06-2024 / Accepted: 10-07-2024

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Conflict of interest: No conflict of interest.

Abstract:

In the present study Phytochemical and 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.

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.

Extraction Method

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. A total of 50 gm of powder of flowers of the Nymphaea alba was taken and mixed with 250 ml distilled water (1:5) in a round bottom flask and gentle refluxed for 1.5 hour separately. The residue was removed by filtration through Whatmann No. 1 filter paper and the aqueous extract was concentrated on rotary evaporator to get solid yield extract.

Physicochemical Investigation

Determination of pH range

The pH of different formulations in 1 % w/v (1g:100 ml) of water soluble portions of plants fruit powder of Nymphaea alba, were determined using standard simple glass electrode pH meter.

Loss of Drying/ Moisture Content (Gravimetric determination)

Separately place about 1.0 gm of whole fruit powder of the *Nymphaea alba* in an accurately weighed moisture disc. For estimations of loss of drying, it was dried at 105˚C for 5 hours in an oven, cooled in desiccators for 30 minutes, and weighed without delay. The loss of weight was calculated as the content in mg per gm of air-dried material.

Determination of total Ash value

Incinerate 2gm. of powdered drug in a tarred silica dish at a temp. Not exceeding 4500c until

free from carbon. Cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ash less filter paper, incinerate the residue and filter paper add the filtrate, evaporate to dryness and ignite at a temp. Not exceeding 450˚c. Calculate the % of ash with respect to the air dried drug.

Determination of acid insoluble ash

Place the ash, as described earlier, in a silica dish, Add 25ml. Hydrochloric acid (2 N), cover with a watch glass, and boil for 10 min. and allow cooling. Collect the insoluble matter on an ash less filter paper, wash with hot Distilled water until the filtrate is neutral, dry, ignited to dull redness, allow cooling in a desiccator and weighing. Repeat until the difference between two successive weighing is not more than .1 g. calculate the %of acid insoluble ash with reference to the air dried drug.

FLUORESCENCE STUDIES OF POWDER DRUGS

A pinch of dried and powdered plant material was taken in a clean slide with about 1-2ml of solvent like acetone, benzene, petroleum ether chloroform, ethanol, glacial acetic acid, HCl, HNO3, methanol and distilled water. All the slides were shaken well and incubated for about 30 min. The colors of the drug solutions thus obtained were observed for their characteristic color reaction under the visible light (fluorescent tube) and ultra violet light (UV366nm) .

Preliminary Phytochemical Screening

Preliminary screening of phytochemicals is a valuable step, in the detection of bioactive principles present in medicinal plants and subsequently may lead to drug discovery and development. It refers to extraction , screening and identification of the medicinally active substances found in plants. The preliminary screening of the ethyl acetate, methanol and water extracts of plant powder of *Nymphaea alba* were carried out using standard laboratory procedures to detect the presence of different secondary metabolites such as alkaloids, flavonoids, saponins, tannins, steroid glycosides, phenols, coumarins, reducing sugars , protein, fixed oils and fats.

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.

Evaluation of Antiulcer activity of *Nymphaea alba Linn*

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]

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.

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

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

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:

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

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

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 Nymphaea alba inn flower revealed that the presence of various phytoconstituents which are represented in Table (1)

Sr. No.	Nameof the Chemical test	Aqueous Extract	Methanolic Extract	Ethanolic Extract	Chloroform Extract
1.	**TestforFlavonoids**				
(a)	Shinodatest	+	+	+	-
(b)	Alkalinereagenttest	+	+	+	-
(c)	Zinchydrochloridetest	+	+	+	-
2.	**TestforSaponins**				
(a)	Foamtest	+	+	+	
3.	**TestforAlkaloids**				
(a)	Dragendroff'stest	-	+	-	-
(b)	Mayer'stest	-	+	-	-
4.	**TestforSteroid**				
(a)	Salkowskireaction	+	+	+	+
(b)	Liebermann-Burchard reaction	+	+	+	+
5.	**Testforamino-acids**				
(a)	Ninhydrin test	+	+	+	+
6.	**TestforCarbohydrate**				
(a)	Molish'stest	+	+	+	+
7.	**TestforProteins**				
(a)	Biurettest	+	+	+	+
(b)	Million'stest	+	+	+	+
8.	**TestforTannins**				
(a)	Drug+5%$FeCl_3$	+	+	+	+
(b)	Drug+leadacetate solution	+	+	+	+
9.	**Testforvit. C**	+	+	+	+

Table no. 2: Physicochemical Parameters of *Nymphiea Alba Linn* Flower

S.N.	Parameters	Values
1	pH range	4.25±0.01
2	Loss on drying	8.25±0.10
3	Methanol soluble extractive value	18.54±0.40
4	Water Soluble extractive value	23.15±0.50
5	Total ash value	8.5%
6	Water soluble ash	5.5%
7	Acid insoluble ash	1.0%
8	Sulphated ash	1.04±0.30

Table 3: Fluorescence properties of *Nymphiea Alba Linn*

Sr. No.	Materials/Treatment	Visible	Short UV	Long UV
1.	Drug Powder as such	Yellowish-Brown	LightGreen	Creamy
2.	Drug Powder rubbed on filter paper	Yellow	Green	Black
3.	Powder treated with1M NaOH in water	Brown	Dark-Green	Dark-Green
4.	Powder treated with1M HCl	Yellowish-Brown	Green	Dark-Green
5.	Powder treated with Pet.Ether	Brown	Light-Green	White
6.	Powder treated with HNO_3	Light-Brown	Light-Green	Dark-Brown
7.	Powder treated with 5% $Fecl_3$	Yellowish-Brown	Dark-Green	Black
8.	Powder treated with dil.Ammonia	Light-Brown	Light-Green	Dark-brown
9.	Powder treated with Methanol	Light-Brown	Light-Brown	White
10.	Powder treated with 1M H_2SO_4	Light-Brown	Green	Gray
11.	Powder treated with Ethanol	Light-brown	Light-Green	White
12.	Powder treated with KOH	Brown	Dark-Green	Dark-Brown
13.	Powder treated with Chloroform	Light-Brown	Light-Brown	White

Table 4: Extractive value of *Nymphiea Alba Linn*

Sr. No.	Solvents Values	(W/W)
1	Chloroform water	10.8%
2	Methanol	9.0%
3	Ethanol	6.8%
4	Chloroform	1.0%
5	Pet. Ether	0.2%

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 5 & 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 6 & 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 5: 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 (200mg/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 6: Effect of EENA on various parameters in Ethanol induced ulcers

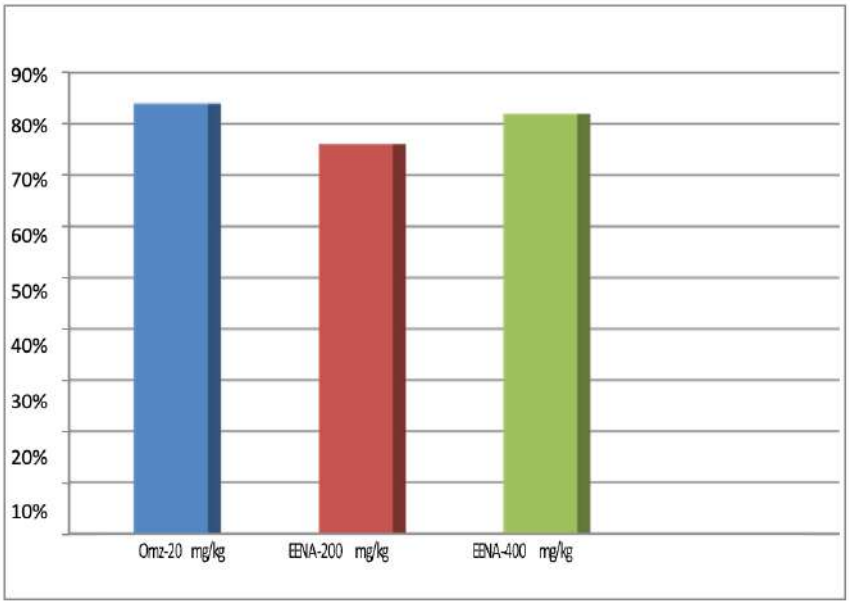
roup	Treatment	Ulcer index	% Protection	p^H of gastric juice
I	Control (Ethanol)	11.6±.08	-----	3.3±.20
II	OMZ(20 mg/kg)	3.6±.07*	72 %	5.4±.09*
III	EENA(200 mg/kg)	5.9±.05*	54 %	3.8±.15
IV	EENA(400 mg/kg)	4.4±.04*	67 %	4.8±.17*

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.

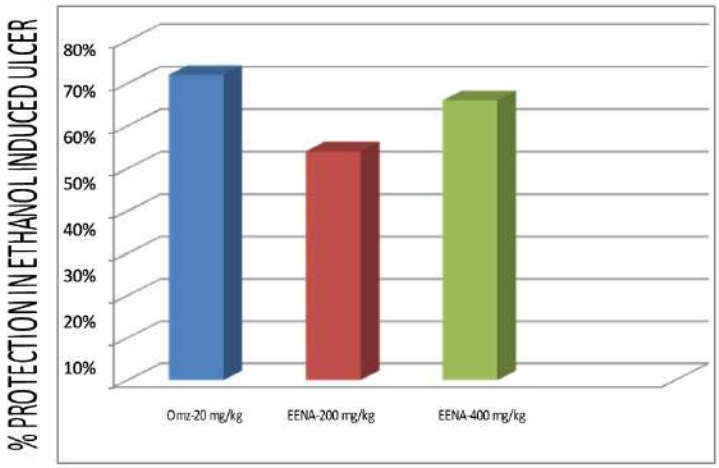


Graph 1: Percentage protection in pyloric ligation induced ulcer model.

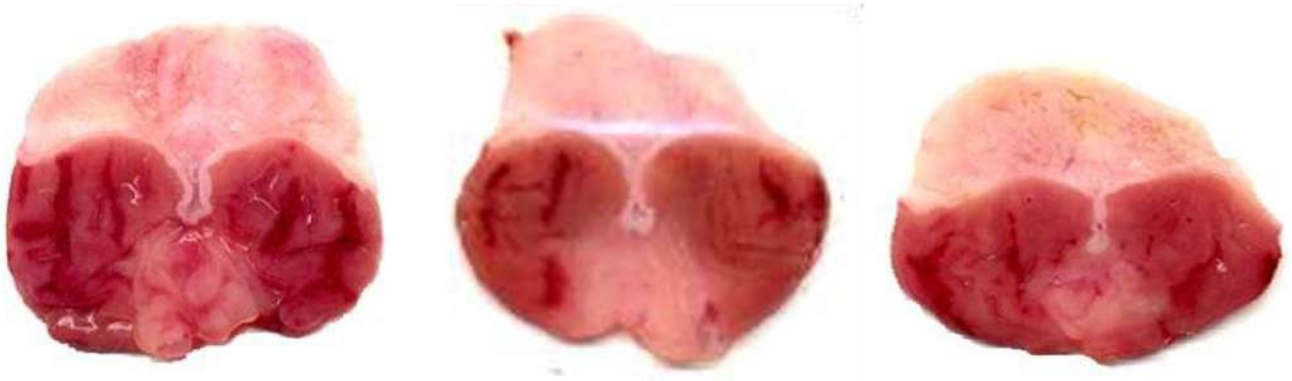
TREATMENT GROUP (DOSES)

Control (P.L.) **Omeprazole(20 mg/kg)** **EENA (400 mg/kg)**

Figure 1: Pylorus Ligation Induced Ulcer

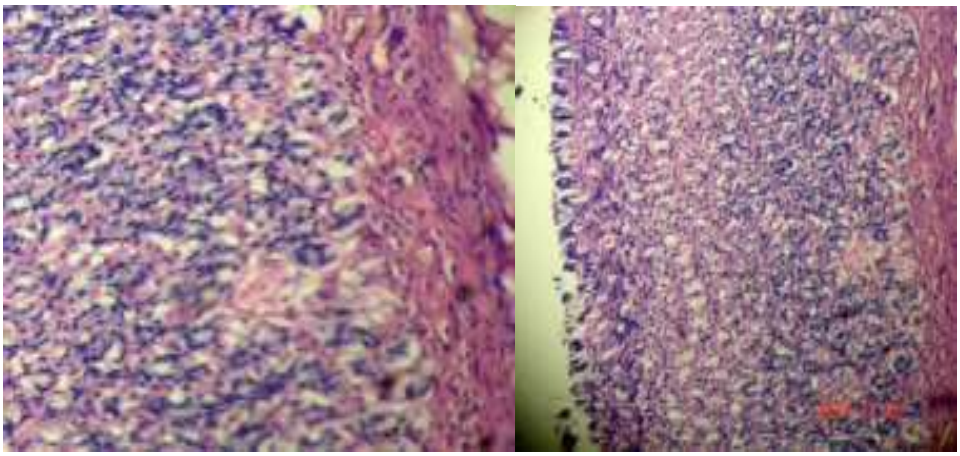


Graph: 2 Percentage protection in ethanol induced ulcer model.

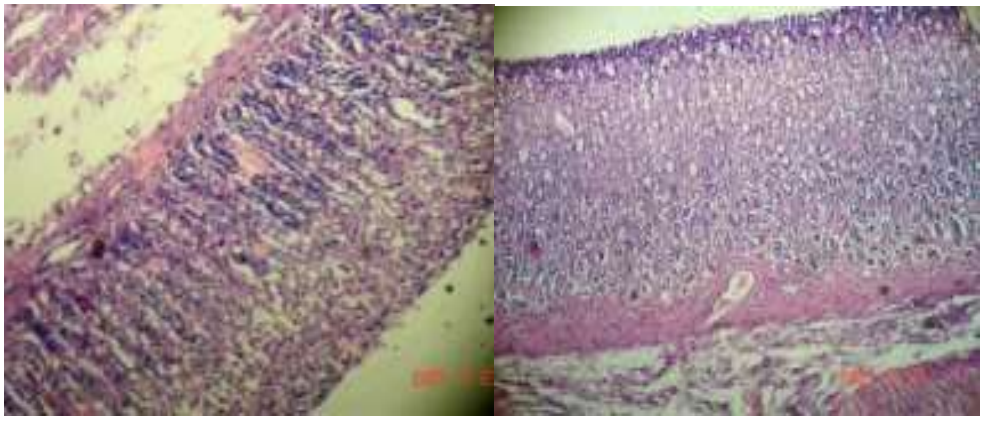


Control (Ethanol) **Omeprazole(20 mg/kg)** **EENA (400 mg/kg)**

Figure 2: Ethanol Induced Ulcer

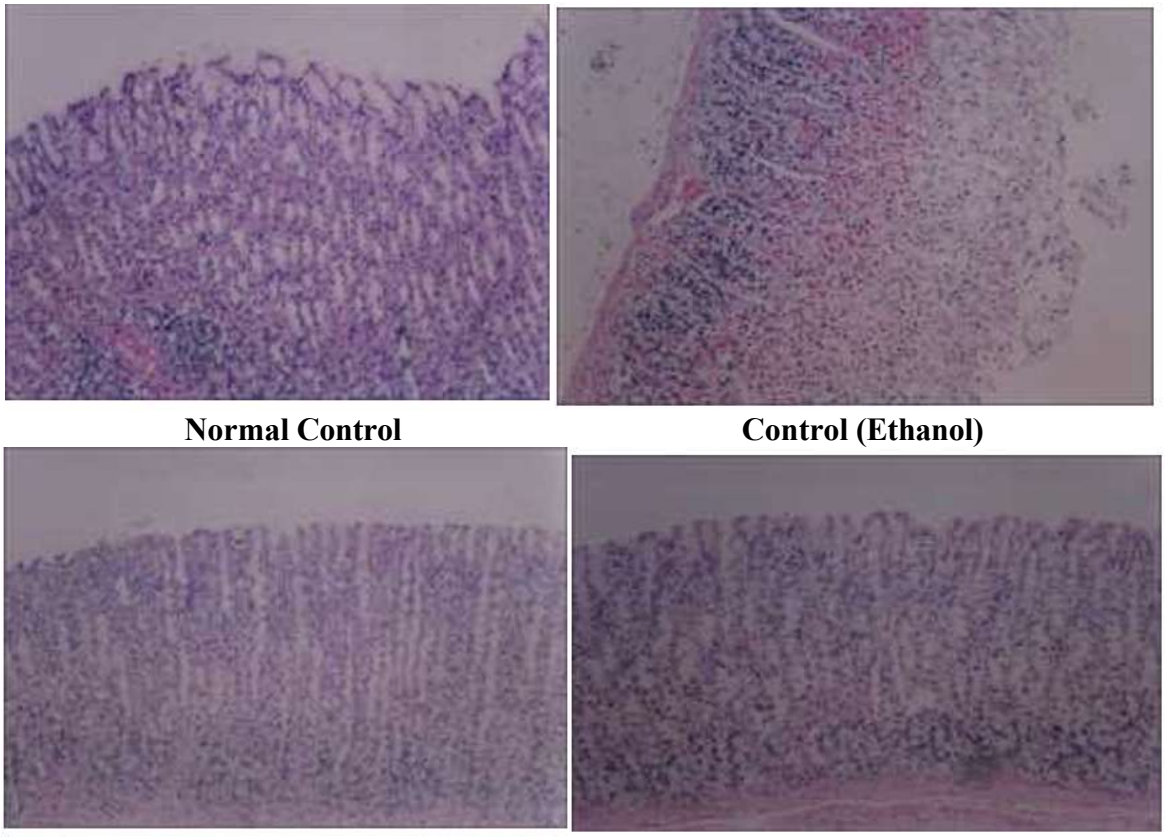


Normal Control **Control (P.L.)**



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 physico-chemical study is a major and reliable criterion of identification of plant drugs. The physico-chemical parameters are necessary for confirmation of identity and determination of quality and purity of crude drugs. To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus, in recent years there has been an emphasis on standardization of medicinal plants, and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and inexpensive means. Physico-chemical studies on different plants have been done by various workers.According to WHO, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.

The physico-chemical parameters helps in judging the purity and quality of the drug. The powder drugs were evaluated for its physico-chemical parameters like foreign matter, loss of drying, total ash, acid insoluble ash and different extractive values. The current study establishes not only physicochemical characterizations of plants but also phytochemical characters of all the plant extracts. These characeristics can be used further as identification and authentication parameters of the plant extracts.All the plant extracts are found to be rich in flavonoid and saponins having wide spectrum of bioactivity.the plants studied here can be seen as a potential source of useful therapeutics. Further studies are going on these plant extracts in order to isolate, identify, characterize and elucidate the structure of bioactive compounds along with their pharmacological activity. In other words, the physicochemical features examined in the current study may serve as a tool for

identification of the plant for validation of the raw material and for standardization of its formulations at herbal industrial level in the coming days.

The present study on Pharmacognostical Qualitative and Quantitative Phytochemical evaluation of flowers of *Nymphaea alba Linn* family Cucurbitaceae will provide useful information for its identification. Physiochemical standards discussed above can be considered as the identifying parameters to substantiate and authenticate the drug. *Nymphaea alba Linn* flowers have presence of flavonoids, saponins, carbohydrates, tannins, amino acids which have therapeutic value.

In the present work, the acute toxicity carried out based on OECD-423 rules for chloroform extract of *Nymphaea alba Linn* prove that, the doses of 250 and 400 mg/kg did not indication any sign of toxicity and mortality. Hence these doses of the concentrate were chosen for assessment of anti-ulcer activity. *Nymphaea alba Linn* in the highest dose tested (400 mg/kg), shows increased in Gastric pH and Gastric juice pH, whereas decrease in Gastric content, Gastric juice volume and Total acidity. Therefore, as per histopathological evaluation studies, it was concluded that, *Lagenaria siceraria*, at the highest dose of 400 mg/kg, found to be safe and more effective in eradicating gastric ulceration. In Conclusion, based on the results obtained the chloroform extract of *Nymphaea alba Linn* treated groups demonstrates a critical impact when contrasted with control group animals which showing that the plant having the having the anti-ulcer activity. The anti ulcer action of *Nymphaea alba Linn* was assessed by pylorus ligation instigated ulcer models. These models cause the gastric ulcer in people. Numerous variables and instruments are associated with the ulcerogensis and gastric mucosal harm. Pylorus ligation induced ulcer was utilized to note the impact of *Nymphaea alba Linn* extract on gastric acid secretion and bodily fluid emission. The ligature of the opening finish of the abdomen causes accumulation of internal organ acid within the abdomen. This increase within the internal organ acid secretion causes ulcers within the abdomen. Ligation of pyloric end of the stomach is made in 24 h fasted rats; the UI is resolved 4 h after pylorus ligation. The lesions created by this methodology are placed inside the lumen area of abdomen. The Chloroform extract of *Nymphaea alba Linn* and ranitidine altogether diminished the complete acidity, free acidity and significantly enhance the pH; this proposes it is having an anti-secretory effect. Its antiulcer activity is any supported by histopathological study demonstrates that protection of tissue layer from ulceration and inflammation. Pylorus ligature induced lesion management rats shown perforated lesion, deep ulceration of granular epithelial tissue and nearly reducing the sub-mucosa. The chloroform extract of *Nymphaea alba Linn* at 250 mg/kg dose has shown mucosal erosion, the partial healing of ulcer with few inflammatory cells and the dose 400 mg/kg has shown the healed ulcer, normal mucosa and no inflammatory cells. *Nymphaea alba Linn* extracts have been reported to possess antioxidant activity and to contain various types of compounds such as flavonoids and polyphenolic compounds, saponins and tannins. The gastroprotective effect exhibited by chloroform extract *Nymphaea alba Linn* is speculated to be attributed to its antioxidant property, which in turn could be linked to the presence of flavonoids and polyphenolic compounds, saponins and tannins. These compounds most likely inhibit gastric mucosal injury.

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.

ACKNOWLEDGEMENT

The authors are very grateful to all Management and Principal of RKDF College of Pharmacy for encouragement to carry out the work as well as for providing the facilities to carry out the research work.

REFERENCES

1. AlKofahi A, Atta AH., Pharmacological screening of the antiulcerogenic effects of some Jordanian Medicinal Plants in rats, J Ethnopharmacol,1999, 65, 341-5.
2. Peskar BM., Maricic N., Role of prostaglandins in gastroprotection, Dig Dis Sci, 1998, 43, S23-9.
3. Toma W., Hiruma-Lima CA, Guerrer RO., Souza AR., Preliminary studies of Mammea AmericanaL (Guttiferae) bark/latex extract point to an effective antiulcer effect on gastric ulcer models in mice,Phytomedicine, 2005, 12, 345-50.
4. Borelli F., Izzo AA., The plant kingdom as a source of anti-ulcer remedies, Phytother Res, 2000, 14, 581-91.
5. Eliana R, Ricardo T, Jose C, Galduroz F, Giuseppina N. Studies in Natural Products Chemistry. Vol.35. Brazil:Elsevier; 2008. Plants with possible anxiolytic and/or hypnotic effects indicated by three brazilian cultures - indians,afrobrazilians, and river-dwellers; pp. 549–95.
6. Adnaik RS, Pai PT, Sapakal VD, Naikwade NS, Magdum CS. Anxiolytic activity of Vitex Negundo Linn. Inexperimental models of anxiety in mice. Int J Green Pharm.2009; 3:243– 7.
7. Robin D. Nymphaea odorata: White pond lily. Medical Herbalism. Materia Medica Pharm. 2001; 11:231–3.
8. Vergeera LH, Vander VG. Phenolic content of daylight-exposed and shaded floating leaves of water lilies(Nymphaeaceae) in relation to infection by fungi. Oecologia.1997; 112:481–4.
9. James AD. Duke's Hand book of medicinal plants of the bible. USA: Taylor and Francis group; 2008. pp. 302–5.
10. Naghma K, Sarwat S. Anticarcinogenic effect of Nymphaea alba against oxidative damage and hyperproliferativeresponse and renal carcinogenis in Wistar rats.Mol Cell Biochem. 2005; 271:1–11.
11. Lipnick RL, Cotruvo JA, Hill RN, Bruce RD, Stitzel KA, Walker AP, et al. Comparison of the up-and-down, conventional LD50, and fixed-dose acute toxicity procedures. Food Chem Toxicol.1995; 33: 223–31.
12. OECD Guidelines for Testing of Chemicals [internet]. France: OECD Publishing; 2006. Section 4, Health effects: TestNo. 425: Acute oral toxicity: Up and down procedure.
13. Mukaherjee PK, Quality control of herbal drugs (an approach to evaluation of botanicals) New Delhi: BusinessHorizon's; 2002: p.380-421.
14. Ajayi IA, Ajibade O, Oderinde RA. Preliminary phytochemical analysis of some plant seeds. Res.J.Chem.Sci.2011;1(3):58-62.
15. De S, Dey YN. Phytochemical investigation and chromatographic evaluation of the different extract of tuber of Amorphaphallus paeonifolius. International journal on Pharmaceutical and Biomedical Research 2010; 1(5): 150-157.

16. Shay H, Komarov SA, Fele SS, Meranze D, Gruenstein H, Siplet H. A simple method for uniform production of gastric ulceration in rat. Gastroenterol 1945; 5:43-61.
17. Kulkarni SK. Hand book of experimental pharmacology. 3rd ed. New Delhi: Vallabh prakashan; 1999:pp148-50.
18. Brzozowski T, Konturek SJ, Kwiecien S, Pajdo R, Brzozowski I, Hahn EG et al. Involvement of endogenous cholecystokinin and somatostatin in gastro protection induced by intra duodenal fat. J Clin Gastroenterol 1998; 27:125-137.
19. Mahmod AA et al. Int J Mol Adv Sci.2005, 1:p.225.
20. Culling CFA. Handbook of Histopathological and Histochemical Techniques. 3rd ed. London: Butterworth and Company, 1974: p.126-159.
21. Piper DW, Stiel DD. Pathogenesis of chronic peptic ulcer DW, Stiel DD. Pathogenesis of chronic peptic ulcer, current thinking and clinical implications.Med Prog 1986, 2, 7-10.
22. Dhuley JN. Protective effect of Rhinax, a herbal formation against physical and chemical factors induced gastric and duodenal ulcers in rats. Indian J Pharmacol1999, 31, 128-32.
23. Soll AH. Pathogenesis of peptic ulcers and implication for therapy. New Eng J Med 1990, 322, 909- 16
24. Surendra S. Evaluation of gastric anti-ulcer activity of fixed oil of tulsi and possible mechanism. Indian J Exp Biol, 1999, 36(3), 253-57.
25. Gustaf E, Henrik H, Klaus R, Rut N, Agneta W, Mads M, Olof N. Risk of Gastric Cancer and Peptic Ulcers in Relation to ABO Blood Type: A Cohort Study, 172(11) ,451-59,2010.
26. Correa P, Haenszel W, Cuello C. A model of gastric cancer epidemiology. Lancet 2, 58-60, 1975.
27. Yamaoka Y, Ojo O, Fujimoto S. Helicobacter pylori outer membrane proteins and gastroduodenal disease. Gut. 55:775-811975.
28. Atherton JC, Peek RM, Tham KT, Cover TL, Blaser MJ. Clinical and pathological importance of heterogeneity in vacA, the vacuolating cytotoxin gene of Helicobacter pylori. Gastroenterology. 112, 92–99, 1997.
29. Atuma C, Engstrand L, Holm L. Extracts of Helicobacter pylori reduces gastric mucosal blood flow through a VacA- and CagA-independent pathway in rats. Scand. J. Gastroenterol. 33, 1256–61,1998.
30. Enno A, O'Rourke JL, Howlett CR, Jack A, Dixon MF, Lee A. MALToma-like lesions in the murine gastric mucosa after long-term infection with Helicobacter felis. A mouse model of Helicobacter pylori-induced gastric lymphoma. Am. J. Pathol. 147,217–22, 1995.
31. Warren JR, Marshall BJ. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet. 2(3), 1273–75, 1983.
32. Patchett S, Beattie S, Leen F. Helicobacter Pylori and Duodenal Ulcer Recurrence. Am J Gastroentrol.87, 24-7, 1992.
33. Konturek PC, Ernst H, Konturek SJ. Bobrzynski AJ, Faller G, Klingler C, Hahn EG.Mucosal expression and luminal release of epidermal and transforming growth factors in patients with duodenal ulcer before and after eradication of Helicobacter pylori. Gut. 40,463–69, 1997.
34. Warburton VJ, Everett S, Mapstone NP, Axon AT, Hawkey P, Dixon F. Clinical and histological associations of cagA and vacA genotypes in Helicobacter pylori gastritis. J. Clin. Pathol. 1, 51:55–61,1998
35. Graham DY. Pathogenic mechanisms leading to Helicobacter pylori-induced inflammation. 4(2), 9–16, 1975.