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Original Article

Evaluation of Anti-Ulcer Activity of Leaf Extract of Nelumbo Nucifera in Rats

D V Kishore [1], Amatul Raheem [2,*], Tarannum Fatima [3], Shareqa Firdose [3]

[1] Department of Pharmacology- Shadaan College of Pharmacy, Hyderabad,India.
[2] Department of Pharmacology-Anwar-Ul-Ulool College of Pharmacy, Hyderabad, India.
[3] Department of Pharmaceutical Chemistry-Anwar-Ul-Ulool College of Pharmacy, Hyderabad, India.

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ABSTRACT

Nelumbo nucifera has long been used as a folk medicine in treatment of diarrhoea, fevers, bleeding and other inflammatory conditions. Therefore, the present study was designed to investigate the antiulcer effect of hydroalcoholic extract of leaves of Nelumbo nucifera. The hydroalcoholic leaf extract of Nelumbo nucifera was subjected to phytochemical screening which confirmed the presence of alkaloids, flavonoids, tannins, phytosterols and saponins. Acute gastric ulceration in rats was produced by oral administration of various noxious chemicals including indomethacin, ethanol, pylorus ligation technique and by subjecting the animals to cold restraint stress. The extract was administered in three different doses of 100, 200 and 400 mg/kg orally in all the experiments. The dose was calculated based upon acute toxicity studies. Ranitidine and Misoprostol were used as the standard drugs. The antiulcer activity was assessed by determining and comparing the ulcer index of the test group with that of the vehicle control group and standard group. The antiulcer potency was evaluated by determining and comparing the ulcer idex of the test group with that of the vehicle control group and standard group. The antiulcer activity was however less than the standard drugs. The above may be due to the presence of flavonoids, tannins and other phytoconstituents in the plant extract.

Keywords: Nelumbo nucifera, diarrhea, flavonoids, phytoconstituents.

1. INTRODUCTION

Peptic ulcer is a typical yet genuine gastrointestinal issue which is caused because of absence of harmony between the gastric aggressive and the mucosal protective factors. The etiology of peptic ulcer is influenced by different aggressive and protective factors, for example, acid-pepsin secretion, parietal cell enactment, decrease in mucus secretion, decreased mucosal blood flow, cell regeneration process and endogenous defensive components (prostaglandins and epidermal growth factors).Other factors that add to ulcers

Corresponding author *
Amatul Raheem
Department of Pharmacology
Anwar-Ul-Ulool College of Pharmacy, Hyderabad-
E Mail: amatul.raheem85@gmail.com

incorporate incorrect dietary propensities, excessive use of non-steroidal anti-inflammatory medications, stress and contamination by Helicobacter pylori.

In patients with gastric ulcer, even a low level of acid can create damage, probably because of debilitated mucosal guard and decrease bicarbonate generation. Helicobacter pylori and exogenous agents, for example, non-steroidal anti-inflammatory drugs (NSAID's) collaborate with these components unpredictably, prompting ulcer diathesis. Up to 80-90% of the ulcers are related with Helicobacter pylori contamination of the stomach[1].

Indigenous medications having fewer side effects with most extreme helpful adequacy is the region of enthusiasm of the present day investigates which goes for a superior and more secure approach for the management of peptic ulcer. A few plant species types like Allophyllus Serrata, Desmodium Gangeticum, Ocimum Sanctum, Helidesmus Indicus, Bauhinia Variegate, Canvulvulus Pluricaulis, Morinda Citrifolia, Polyathia Longifolia, Tephrosia Purpurea and numerous are known to process marked antiulcer activity. Nelumbo nucifera, otherwise called Indian lotus is one such plant with enormous therapeutic uses due to the presence of numerous bioactive principles. Consequently, this work has been carried out to assess its action against gastric ulcers[2].

Nelumbo nucifera, generally known as lotus or sacred lotus is an oceanic perpetual plant of family Nelumbonaceae.[3] N. nucifera is a vital amphibian economic plant, as a dainty and ornamental flower as well as a wellspring of herbal medicine with potent antipyretic, cooling, astringent, and demulcent properties.[4] In folk medications, seeds are used as a part of the treatment of tissue inflammation, malignancy, skin diseases, leprosy, poison antidote and large recommended to youngsters as diuretic and refrigerant. [5] The fruits and seeds of lotus are astringent and used to treat hyperdipsia, dermatopathy, halitosis, menorrhagia, leprosy and fever[6].

The whole plant is astringent and used in Chinese medicine for treating diarrhea, bleeding, ulcers and in mushroom poisoning. It is also used to treat bleeding associated with hemorrhoids, menstruation and emesis. It is used in combination with other herbs for sunstroke, fever, dizziness and diarrhea.[7] Hot water extract of the leaves is used in hyperlipidemia and in treatment obesity related disorders [8]. Quercetin isolated from the leaves is antibacterial and used against actinobacillus actinomycetemcomitans Y4, actinomyces viscosus 19246, Actinomyces Naeslundii 45, porphyromonas gingivalis 33277 and fusobacterium nucleatum.

2. MATERIAL AND METHODS

Collection of Plant Materials:

The leaf of Nelumbo nucifera where during the month of April 2011 from local area of Warangal district and where identified and authenticated by Dr.Mohammed Ismail,Research Officer, Central Research Institute Of Unani Medicine.A sample specimen has been deposited in the herbarium for further reference.

Experimental animals:

Albino Wister rats weighing 150-200 grams which were procured from the Animal house of Shadan College of Pharmacy where used throughout the experiment. The experimental animals were maintained under standard laboratory conditions with 12 hours light dark cycle under controlled temperature. All the animals were acclimatized to the laboratory conditions for at least one week before the commencement of the experiment. All the experiments were performed in accordance with the CPCSEA guidelines.

Acute toxicity assay

Acute toxicity assay was performed in mice according to OECD guidelines. Animals were divided into different groups of six each. After an overnight fast, the test drug was administered orally in graded dose (100–2000 mg/kg). In further, they were observed continuously for the first 2 h for toxic symptoms and up to 24 h for mortality. There was no lethality in any of the groups after treatment [9].

For this study 3 dose levels were chosen in such a way that, the middle dose (200 mg/kg) was approximately one tenth of the maximum dose during acute toxicity studies and a low dose (100 mg/kg) which was half of the one tenth of the maximum dose and a high dose (400 mg/kg) which was twice that of the one tenth dose of the maximum dose.

Experimental Procedures

Preparation extraction[10]:

The leaves of Nilambur nucifera were shade dried for 10 days and then powdered. The Powder was passed from 40 mesh sieve and then subjected to extraction. 800 grams of the Powder was extracted using hydro alcoholic solvent (ethanol and water in 75:25 ratio) in a Soxhlet apparatus. The residue obtained was dried on a hot water bath at a temperature of 50^0 c .The weight of the extract on dying was 98 grams. On calculation, the percentage is yield was found to be 12.25%. Further the dried extract was subjected to preliminary phytochemical screening for the identification of various phytoconstituents.

Preliminary phytochemical screening

The phytochemical examination of the hydro alcoholic extract was performed by the standard methods [11].

a) Test for Alkaloids

i. Dragendroff's test: To 2-3 ml of the extract, few drops of Dragendroff's reagent were added. Orange brown precipitation was formed which confirms the presence of alkaloids.

ii. Mayer's test: To 1ml of the extract, few drops of Mayer's reagent were added. Cream coloured precipitation was formed which confirms the presence of alkaloids.

iii. Hager's test: To 1ml of the extract, Hager's reagent was added. Yellow precipitation was formed which confirms the presence of alkaloids.

iv. ***Wagner's test:*** To 1ml of the extract, few drops of Wagner's reagent were added. Reddish brown precipitation was formed which confirms the presence of alkaloids.

b) Test for Carbohydrates:

i. ***Molisch's Test:*** To 2-3ml of the extract, add few drops of α-napthol solution in alcohol was added and shaken. To this conc. H_2SO_4 was added from the sides of the test tube. Violet ring was observed at the junction presence of carbohydrates.

ii. ***Fehling's Test:*** 1ml of Fehling's A and B reagents, add heat on a water bath for 1mins.to this equal volume of test solution on boiling water bath for 10-15 min.yellow, then brich red precipitation was observed, Presence of carbohydrates.

iii. ***Benedict's test:*** Mix equal volume of Benedict's reagent and extract were mixed in a test tube, Heat on water bath for 5mins.Green colour was observed, Presence of reducing sugars.

c) *Test for Flavanoids:*

i. ***Shinoda Test:***To 1-2 ml of extract, few magnesium turning were added and to this concentrated HCL was added drop wise. Pink scarlet, crimson red or occasionally green blue colour appeared after few minutes which indicate the presence of flavanoids.

ii. ***Alkaline Reagent Test:***To the extract solution, few drops of NaOH solution was added ,intense yellow colour was formed which turns to colorless on addition of few drops of dilute acid. This indicates the presence of Flavanoids.

d) Test for Glycosides:

i. ***Borntragers test:*** 3 ml of extract was boiled with 1ml of sulphuric acid in a test tube for five minutes and was filtered while hot. The filtrate was cooled and shaken with equal volume of chloroform. Lower chloroform layer was separated and shaken with half of its volume of dilute ammonia. A rose oink to red colour produced in ammonical layer indicates the presence of anthraquionone glycosides

ii. ***Balgets test:*** The extract was treated with picric acid. Orange colour was formed which indicates the presence of cardiac glycosides.

iii. ***Keller Kiliani :*** To 2 ml of extract, glacial acetic acid,1 drop of 5% ferric chloride and concentrated sulphuric acid were added. Reddish brown colour appears at the junction of two liquid layers and the upper layer appeared bluish green which confirms the presence of glycosides.

iv. ***Picric Acid test:*** Filter paper strip was first soaked in 10% picric acid and then in 10% of sodium carbonate and dried. In conical flask moistened powdered extract was place and corked. The above filter paper strip was placed in the slit in a cork and gently wormed at 37^0C. The filter paper turns brick red or maroon which indicates the presence of cytogenetic glycosides.

e) Test for Saponins:

i. ***Forth formation test:*** 2ml of extract was placed in a test tube containing water and shaken well. Stable forth was formed which indicates the presence of saponins

ii. ***Hemolytic test:*** The extract was added to 1 drop of blood placed on glass slide. Hemolytic zone appears which indicates the presence of saponins.

f) Test for Tannins:

i. ***Ferric chloride test:*** To 2ml of extract, ferric chloride solution was added. Blue colour appears which indicates the presence of tannins.

ii. ***Bromine water test:*** 2 ml of the extract was treated with bromine water. Discoloration of bromine water occurs which indicates of tannins.

Pylorus ligation induced ulcers[12]:

Procedure:

Albino rats 150 t8 200gms were used for the experiment maintained under the standard conditions of the temperature and humidity. Rats of either sex were randomly allocated to 5 groups containing 6 in each group, the drug treatment was carried out for 7 days. on the seventh day, the rats were fasted for 36 hours with waters ad libitium and housed singly in cages with raised bottoms of wire mesh to prevent cannibalism coprophagy.

Group 1: control rats (n=6) only given vehicle p.o (5 ml/kg)

Group 2: treated (n=6) with hydro alcoholic extract of "nelumbo nucifera" p.o (50mg/kg).

Group 3: treated (n=6) with hydro alcoholic extract of "nelumbo nucifera" p.o (100mg/kg).

Group 4: treated (n=6) with hydro alcoholic extract of "nelumbo nucifera" p.o (200mg/kg).

Group 5: treated (n=6) with hydro alcoholic extract of "nelumbo nucifera" p.o (400mg/kg)(50).

Under light ether anesthesia, one-inch abdominal incision was given below the xipoid process.

He Pylorus was carefully lifted out with minimal handling and traction and the abdominal wall was closed with interrupted sutures.

Around 6 to 7 hours after Pylorus ligation, the animals were sacrificed and the stomach dissected out. The contents of the stomach were depleted into graduated centrifuge tube and their volume and pH of the gastric acid was measured. The stomach was opened along its more noteworthy arch pinned on a wax plate and inner surface was inspected for ulceration with binocular microscope. The ulcer severity was reviewed based on mean ulcerative index.

Collection of gastric acid:

The stomach was isolated precisely by keeping the esophagus shut and the luminal contens were gathered in a graduated centifuge tube and centrifuged at 1000 rpm for 10mins. The volume of the supernatant was expressed as

ml/100gm body weight and centrifuged samples were emptied and dissected for gastric volume, pH and total acidity. The mucosa was flushed with saline and watched for gastric injuries utilizing a dismembering magnifying lens, ulcers were scored and the ulcer record was determined.

Estimation of total free acidity[13]:

1ml of the supernatant fluid was pipette out and diuted to 10ml with distilled water. pH of this solution was noted with the assistance of d7gital pH meter. The solution was titrated against 0.01N NaOH using Topfer's reagent as a indicator (it is dimethyl-amino-azo benzene with phenaphthalein and utilized for the identification and estimation of HCl and total acidity in gastric liquids). The end point was noted when the solution changes to orange shading. The volume of NaOH consumed was noted, which compares to free acidity. Further it was titrated till the solution recovers pink colour. The total volume of NaOH was noted, which compares to total acidity.

$$Acidity = \frac{Volume\ of\ NaOH \times Normality\ of\ NaOH \times 100}{0.1} = nEq/L$$

Mean ulcerative index:

Ulcer index= (No. of lesions I) + (No.of lesions II) + (No. of lesions III)

Where,

I= Presence of edema, hypreamia ane single, submucosal punctiform haemorrhages(petechiae).

II= Presence of submucosa, haemorrhagic lesions with small erosion.

III= presence of deep ulcer with erosion and invasive lesions.

The percentage inhibition was determined as follows:

Control mean lesion index-test mean lesion index/control mean lesion index x 100

Statistical analysis:

The data is represented as mean ± SEM. The data of anti ulcer activity of hydro alcoholic extract of leaves of *Nelumbo nucifera* was analysed by 1 way ANOVA followed by Dunnet's t-test using graph pad prism '5.04' trial version. 'P' value less than 0.05 was considered as statistically significant.

3. RESULTS

Preliminary phytochemical screening:

The weight of the hydro alcoholic leaf extract of *Nelumbo nucifera* on drying was found to be 98gms. On circulation, the percentage yield was found to be 12.25%. Preliminary phytochemical screening of *Nelumbo nucifera* indicated the presence of alkaloids, flavonoids, glycosides, tannins, terpinoids, carbohydrates, saponins and steroids. The results have been tabulated in table no.1

Acute toxicity study:

The hydroalcoholic extract of leaves of *Nelumbo nucifera* was found to be safe at maximum dose of 2000mg/kg body weight by oral route. There were no signs of toxicity and no mortality was observed. General behavior, neurological and autonomic profiles were found to be normal. The results have been tabulated in table no.2

Antiulcer activity:

Pylorus ligated ulcer model:

Volume of Gastric Acid:

The volume of gastric acid in Ranitidine treated group decreased significantly upto 2.24±0.21, compared to control group in which the value was 7.18±0.16. In groups treated with hydro alcoholic extract, the volume of gastric acid was significantly reduced to 6.11±0.41, 4.91±0.23 and 3.82±0.28 at doses of 100, 200, and 400 mg/kg(p.o), respectively when compared to control group. The results have been tabulated in table no. 3

pH of gastric acid:

The pH of gastric acid in Ranitidine treated group was found to increase significantly upto 5.18±0.31 compared to control group in which the value was 1.33±0.09. In groups treated with hydro alcoholic extract, the pH value increased to 2.01±0.24, 3.08±0.31 and 4.78±0.43 at doses of 100, 200 and 400 mg/kg (p.o), respectively when compared to control group.

The results have been tabulated in table no. 4 and represented through graph no.4.

Free Acidity:

In Ranitidine treated group, the value of free acidity decreased significantly upto 21.45±1.19 when compared with control group in which the value was 94.21±1.23. In test group treated with hydro alcoholic extract, the value of free acidity reduced significantly to 69.19±2.38, 45.10±1.76 and 29.32±1.41 at doses of 100, 200 400 mg/kg(p.o), respectively when compared to control group. The results have been tabulated in table no. 5 and represented through graph no.5.

Total Acidity:

Total acidity in Ranitidine treated group decreased significantly upto 43.66±4.86. In groups treated with hydro alcoholic extract, the value of total acidity was reduced significantly to 89.01±3.15, 63.31±2.36, 51.03±2.11 at doses of 100, 200, and 400 mg/kg respectively(p.o), compared to the control group. The results have been tabulated in table no. 6 and represented through graph no.6.

Ulcer Index:

Ulcer index in ranitidine treated group decreased significantly up to 6.57±0.44 compared to control group in which the value was 16.52±0.99. In groups treated with hydro alcoholic extract, the ulcer index was reduced significantly to 15.23±1.07, 13.56±0.87, 9.57±0.68 at doses of 100, 200 and 400 mg/kg respectively(p.o), compared to the control group. The results have been tabulated in table no. 7 and represented through graph no.7.

4. DISCUSSION

Pylorus ligation induced ulcer because of stress initiated elevated in hydrochloric acid which causes auto digestion of

the gastric mucosa and breakdown of the gastric mucosal membrane as a result of the acid pepsin accumulation because of pyloric deterrent. These factors are related with the improvement of upper gastrointestinal damage like sores, ulcers, aperture and haemorrage[14].

Gastric mucus is known to secure the gastric mucosa against tissue damage by abundance acid formed by the parietal cells and different noxious substances. It comprises of viscus, flexible, adherent and clear gel framed by 95% water and half glycoprotein that covers the whole gastric mucosa. Additionally, mucus is able to perform as a antioxidant and consequently can diminish mucosal damage interceded by reactive oxygen species. Subsequently the defensive property of the mucosal hindrance depends on the gel structure as well as on the thickness of the layer covering the mucosal layer. Abatement in the gastric mucus renders the mucosa susceptible to injury caused by abundance aid secretion, alcohol and NSAID's. [15]

Antisecretory action of Nelumbo nucifera in pylorus ligation strategy is clear from its noteworthy decreasing in the volume of gastric acid, free acridity, total acidity, ulcer index and an elevation in pH contrasted with the control group. Consequently it is recommended that the plant significantly reduced the gastric damage instigated by agressive factors in a dose subordinate way which is ascribed to the antisecretory activity which causes a decline in gastric acid secrtion.

Table: 1 Preliminary phytochemical screening of hydro alcoholic leaf extract of *N. nucifera*

Sl.no	Tests	Result
1.	Alkaloids	
a.	Dragendroff,s test	+ve
b.	Mayer's test	+ve
c.	Hager's test	+ve
d.	Wager's test	+ve
2.	Carbohydrates	
a.	Molisch's test	+ve
b.	Fehling's test	+ve
c.	Benedict's test	+ve
3.	Flavonoids	
a.	Shinoda's test	+ve
b.	Alkaline reagent test	+ve
4.	Glycosides	
a.	Borntrager's test	+ve
b.	Baljet's test	+ve
c.	Keller-killiani test	+ve
d.	Picric acid	+ve
5.	Saponins	
a.	Forth formation tests	+ve
b.	Heamolytic test	+ve
6.	Tannins	
a.	Ferric chloride test	+ve
b.	Bromine water test	+ve
7.	Triterpinoids	
a.	Liebermann-Burchard	+ve
b.	Salkowski test	+ve
c.	Sulfur test	+ve

Table 2: Acute oral Toxicity Studies

1	Alertness	Behavioural response	+
2	Stereotypy		–
3	Irritability		–
4	Fearfulness		+
5	Pain response		–
6	Touch response		–
7	Grooming		+
8	Restlessness		-
9	Spontaneous activity		-
1	Righting reflex	Neurological response	-
2	Corneal reflex		-
3	Pinna reflex		-
4	Abdominal tone		+
5	Limb tone		-
6	Grip strength		-
7	Twitching		-
8	Convulsions		-
9	Straub's tail		-
10	Tremors		-
1	Defecation	Autonomic response	-
2	Urination		-
3	Writhing		+
4	Piloerection		-
5	Respiration		-
6	Heart rate		-
7	Pupil size		-
8	Skin colour		-

ulcer

Table 3: Effect of hydroalcoholic leaf extract of *N.nucifera* on volume of gastric acid

Treatment	Dose	Volume of Gastric Acid (ml)
Control	**5 ml/kg p.o(d.w)**	**7.18+0.16**
Ranitidine	**50 mg/kg p.o(d.w)**	**2.24+0.21*** **
Nelumbo nucifera	**100 mg/kg p.o(d.w)**	**6.11+0.41***
Nelumbo nucifera	**200 mg/kg p.o(d.w)**	**4.91+0.33****
Nelumbo nucifera	**400 mg/kg p.o(d.w)**	**3.82+0.28*** **

All the values are expressed as Mean + SEM (n=6)
***P<0.05,**P<0.001, ***P<0.001 compared with control and analysed by one way ANOVA followed by Dunnet's t-test.**

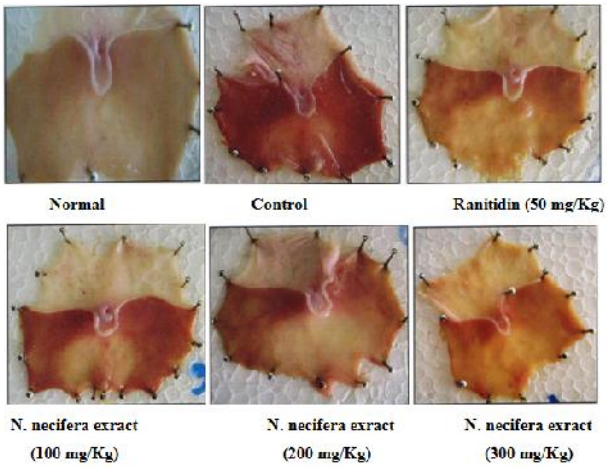

Fig 1: Effect of hydroalcoholic extract of leaves of N. necifera on pyrolus ligated ulcer

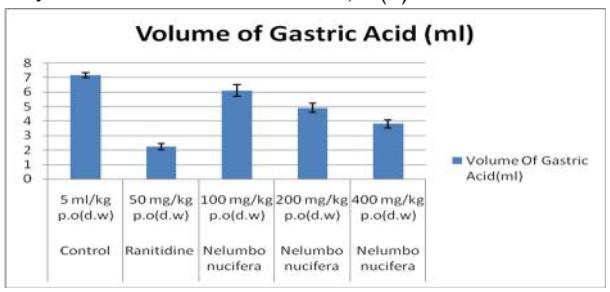

Fig 2: Effect of hydroalcoholic leaf extract of *N.nucifera* on volume of gastric acid

Table 4: Effect of hydroalcoholic leaf extract of *N.nucifera* on pH of gastric acid

Treatment	Dose	pH of gastric acid
Control	5 ml/kg p.o(d.w)	1.33+0.09
Ranitidine	50 mg/kg p.o	5.18+0.31***
Nelumbo nucifera	100 mg/kg p.o	2.01+0.24
Nelumbo nucifera	200 mg/kg p.o	3.08+0.31*
Nelumbo nucifera	400 mg/kg p.o	4.78+0.43**

All the values are expressed as Mean + SEM (n=6)
***P<0.05,**P<0.001, ***P<0.0001 compared with control and analysed by one way ANOVA followed by Dunnet's t-test.**

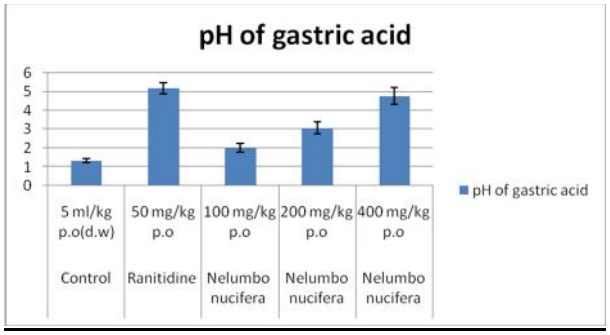

Fig 3: Effect of hydroalcoholic leaf extract of *N.nucifera* on pH of gastric acid

Table 5: Effect of hydroalcoholic leaf extract of *N.nucifera* on Free acidity

Treatment	Dose	Free acidity
Control	5 ml/kg p.o(d.w)	94.21+1.23
Ranitidine	50 mg/kg p.o	21.45+1.19***
Nelumbo nucifera	100 mg/kg p.o	69.19+2.38*
Nelumbo nucifera	200 mg/kg p.o	45.10+1.76**
Nelumbo nucifera	400 mg/kg p.o	29.32+1.41**

.All the values are expressed as Mean + SEM (n=6)
***P<0.05,**P<0.001, ***P<0.0001 compared with control and analysed by one way ANOVA followed by Dunnet's t-test.**

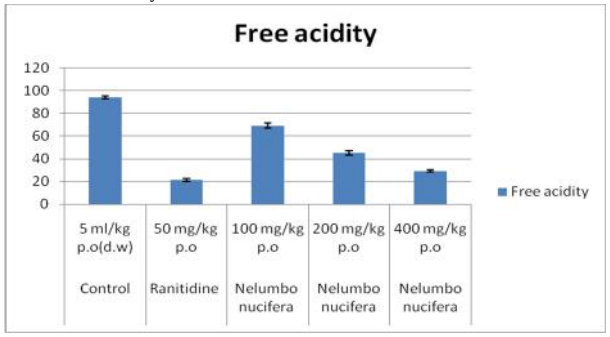

Fig 4: Effect of hydroalcoholic leaf extract of *N.nucifera* on Free acidity

Table 6: Effect of hydroalcoholic leaf extract of *N.nucifera* on Total acidity

Treatment	Dose	Total acidity
Control	5 ml/kg p.o(d.w)	125.69+4.86
Ranitidine	50 mg/kg p.o	43.66+2.09***
Nelumbo nucifera	100 mg/kg p.o	89.01+3.15*
Nelumbo nucifera	200 mg/kg p.o	63.31+2.36**
Nelumbo nucifera	400 mg/kg p.o	51.03+2.11**

All the values are expressed as Mean + SEM (n=6)
***P<0.05,**P<0.001, ***P<0.0001 compared with control and analysed by one way ANOVA followed by Dunnet's t-test.**

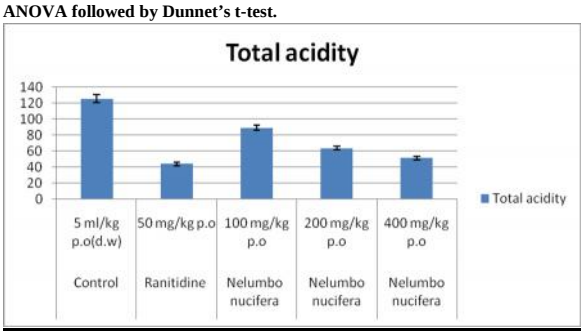

Fig 5: Effect of hydroalcoholic leaf extract of *N.nucifera* on Total acidity

Table 7: Effect of hydroalcoholic leaf extract of *N.nucifera* on Ulcer acidity

Treatment	Dose	Ulcer index	% Protection
Control	5 ml/kg p.o(d.w)	1652+0.99	
Ranitidine	50 mg/kg p.o	6.57+0.44***	76.76%
Nelumbo nucifera	100 mg/kg p.o	15.23+1.07*	24.33%
Nelumbo nucifera	200 mg/kg p.o	13.56+0.87*	55.56%
Nelumbo nucifera	400 mg/kg p.o	9.57+0.68*	61.34%

All the values are expressed as Mean + SEM (n=6)
***P<0.05,**P<0.001, ***P<0.0001 compared with control and analysed by one way ANOVA followed by Dunnet's t-test.**

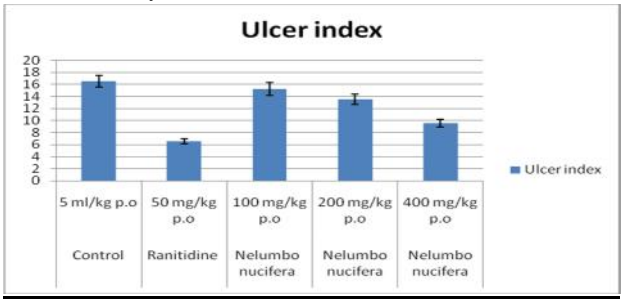

Fig 6: Effect of hydroalcoholic leaf extract of *N.nucifera* on Ulcer index

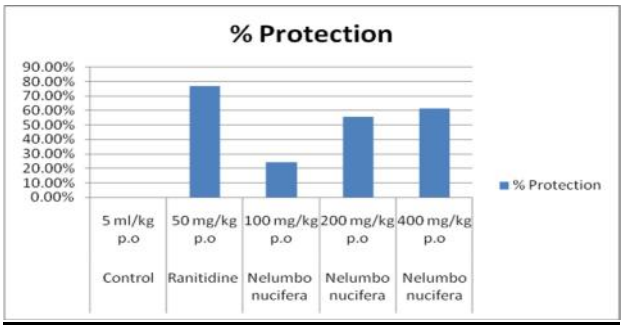

Fig 7: Effect of hydroalcoholic leaf extract of *N.nucifera* on % of Ulcer protecton

5. CONCLUSION

The preliminary phytochemical screening of the hydroalcoholic extract of *Nelumbo nucifera* revealed that the presence phytochemoicals like alkaloids, flavonoids, tannins, triterpinoids, steroids and saponins. The significant antiulcer potential could be attributed to the presence of flavonoids such as quercetin has been reported. Flavonoids prevent gastric mucosal lesions in various experimental models by elevating the level of neutral glycoproteins mucosal prostaglandins and by inhibiting the action of histidine decarboxylase in the mast cells. The anti oxidant property of flavonoids has been reported to protect the gastrointestinal tract from ulcerative lesions. They additionally act by chelation of transition metal particles, restraint of oxidant enzyme age of a tocopherol of α-tocopherol from α-tocopheroxyl radicals in this manner promoting mucus formation, reducing acid secretion and hindering the generation of pepsinogen causing a diminishing in the ulcer seriousness[16].

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