



EVALUATION OF NYCTANTHES ARBOR-TRISTIS LINN. (NAT) FLOWERS EXTRACT AGAINST EXPERIMENTALLY INDUCED PEPTIC ULCER DISEASE IN SWISS ALBINO MICE

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DOI: <https://doi.org/10.5281/zenodo.23280156>

How to cite this Article: ¹Sazzad Ansari, ²Dr. Ashutosh Mishra, ³Dr. Rekha Gour. (2026). Evaluation of *Nyctanthes arbor-tristis* Linn. (NAT) flowers extract against experimentally induced peptic ulcer disease in swiss albino mice. *European Journal of Biomedical and Pharmaceutical Sciences*, 13(10), 450–455.

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Article Received on 14/09/2026

Article Revised on 05/10/2026

Article Published on 10/10/2026

ABSTRACT

The present study was undertaken to evaluate the gastroprotective and anti-peptic ulcer activity of the methanolic flower extract of *Nyctanthes arbor-tristis* Linn. in an ethanol-induced ulcer model in Swiss albino mice. Flowers of *N. arbor-tristis* were collected, authenticated by a taxonomist, shade-dried, powdered, and extracted with 90% methanol. Preliminary phytochemical screening of the extract revealed the presence of alkaloids, glycosides, flavonoids, terpenoids, tannins, saponins, and steroids, while anthraquinones were absent. Twenty-four mice were divided into four groups and gastric ulcers were induced using absolute ethanol. The animals were treated orally with *N. arbor-tristis* flower extract at doses of 150 and 200 mg/kg, while ranitidine was used as the reference drug. Ulcer index, percentage ulcer healing, and total gastric acidity were evaluated after 6, 10, and 14 days of treatment. The ulcerated group showed an ulcer index of 2.85 ± 0.02 , whereas treatment with the extract significantly reduced the ulcer index in a time- and dose-dependent manner ($P < 0.05$). At 14 days, the high-dose extract produced 97.26% ulcer healing, compared with 95.33% for the low-dose extract and 98.59% for ranitidine. The extract also significantly reduced elevated gastric acidity. The findings suggest that the methanolic flower extract of *N. arbor-tristis* possesses significant gastroprotective and anti-ulcer activity and may have potential as a source of therapeutic bioactive compounds.

KEYWORDS: *Nyctanthes arbor-tristis*; Methanolic flower extract; Anti-ulcer activity; Gastroprotection; Ethanol-induced gastric ulcer.

INTRODUCTION

Peptic ulcer disease is a common gastrointestinal disorder characterized by damage to the gastric or duodenal mucosa resulting from an imbalance between aggressive factors, such as gastric acid, pepsin, and oxidative stress, and protective mucosal mechanisms. Although several synthetic drugs are available for ulcer management, their prolonged use may be associated with adverse effects, creating interest in safer plant-based alternatives. *Nyctanthes arbor-tristis* Linn. (Oleaceae), commonly known as night-flowering jasmine, is a medicinal plant traditionally used for various therapeutic purposes. Its flowers contain several bioactive phytoconstituents, including flavonoids, glycosides, tannins, and terpenoids. The present study was therefore designed to evaluate the gastroprotective and anti-ulcer

activity of the methanolic flower extract of *N. arbor-tristis* in an ethanol-induced gastric ulcer model in Swiss albino mice.

EXPERIMENTAL WORK

Collection of plants and identification

The plants *Nyctanthes arbor-tristis* Linn. flowers were collected from different parts of local area of chhatarpur. The identification and authentication of plant was done by taxonomist. The corresponding herbarium was after wards submitted to the same organization for future reference.

Preparation of methanolic crude extract

For preparation of the methanolic crude extract, the parts that have traditionally been employed, such as the

Nyctanthes arbor-tristis Linn. flowers were removed from the plants, cleansed with water, dried under shade and then processed in a blender to make a fine powder. The powder of all the flowers was then immersed in a solution of 90% methanol (100 g/l), in a separate beaker, for duration of 7 to 10 days. The solution was agitated every other day to ensure thorough merging of the powder with the solvent. The resulting solution was then subjected to filtration using Whatman filter paper No.1 and the solvent from the solution was subsequently separated out through a rotary evaporator. At the end, resulting methanolic crude extract were weighed and appropriately preserved at a temperature of 4°C for further utilization. Prior to treatment, the doses were prepared through the process of dissolving the extract in a solution of 0.9% Phosphate Buffer Saline (PBS) with a pH range of 7.2-7.4.

Phytochemical screening

Phytochemical analysis were conducted in accordance with the methodologies described by Trease and Evans (1996). Following is a description of the different qualitative tests that were conducted.

Alkaloids

In a beaker, 100 mg of powdered material was diluted in 5 ml of methanol and then filtered. 1 ml from each of the obtained filtrate was then placed in two different test tubes. A small quantity of Dragendorff's reagent to one test tube and Mayer's reagent was introduced to other test tube and the occurrence of orange-red and buff coloured precipitate confirms the presence of alkaloid.

Glycosides

Kellar-killiani test: Glacial acetic acid (2 ml) with one or two drops of a 2% FeCl_3 solution was added to the crude extract. To this mixture then volume of 2 ml concentrated H_2SO_4 was carefully added along the edges of the test tube. The occurrence of a brown ring at the interface implies the existence of glycosides.

Liebermann's test: The crude extract was infused with chloroform (2 ml) and acetic acid (2 ml). After that, the mixture was subjected to a cooling process utilising ice followed by addition of 2 ml of H_2SO_4 . A sequential colour transition from violet to green confirms the presence of glycoside.

Flavonoids

FeCl_3 test: A quantity of 0.5 g of the extract was subjected to heat boiling in the presence of distilled water, followed by filtration. A small volume of a 10% solution of ferric chloride was introduced into 2 ml of the filtrate. The observation of a green- blue hue serves as an indicator for the existence of a phenolic hydroxyl moiety.

Alkaline reagent test: The crude extract was dissolved with a 2 ml solution of 2% sodium hydroxide (NaOH). The presence of flavonoids can be inferred when a vibrant yellow hue is observed, which subsequently

fades to colourlessness upon the introduction of a little quantity of diluted acid.

Terpenoids

Salkowski's test: 5 ml of extract was infused with 2 ml of chloroform and then 3 ml of concentrated H_2SO_4 was added carefully. The occurrence of a reddish-brown hue near the interface is indicative of the existence of terpenoids.

Tannins

A test tube containing 0.5 g of the plant extract was subjected to boiling in 10 ml of water, followed by filtration. Further a few drops of ferric chloride (0.1%) solution were mixed to 2 ml of the filtrate. Coloration of brownish green or blue-black suggests the presence of tannin content.

Saponins

The powdered sample weighing 1 g was subjected to boiling in 10 ml of distilled water, followed by filtration. 3 ml of distilled water was added to the filter, and the mixture was violently agitated for duration of five minutes. The occurrence of foam during agitation serves as an indicator for the presence of saponins.

Anthraquinones

A volume of 1 ml of plant extract was added with a small quantity of 2% hydrochloric acid (HCl). The observation of a precipitate having a red hue serves as an indication of the existence of anthraquinones.

Steroids

The crude extract was dissolved in a 2 ml volume of chloroform. Concentrated H_2SO_4 was applied in a careful manner at the periphery. The presence of steroids can be inferred when a red coloration is observed in the bottom of chloroform layer.

6.4 Experimental animals

Healthy Swiss albino mice (males and females) with aged 8-12 weeks and weighing between 25-30 grams were used for this study. The animals were obtained from the DIPS, Chhatarpur and were maintained as per guidelines of the Committee for Purpose of Control and Supervision of Experimental Animals (CPCSEA). Animals were kept under standard housing circumstances, which included a schedule of 12 hours of light followed by 12 hours of darkness, at temperature ranging between 22-25°C. They were provided with a conventional laboratory Rodent's food diet and were given unlimited access to drinking water. Animals were acclimatized for five days prior to conducting the experiments.

Anti-pepticulcer study

Treatment protocol

Twenty four mice were divided into 4 groups (I- IV) of six animals each. Ulcer was induced in all the animals by administration of absolute ethanol following the Oates

and Hakkinen (1988) with slight modification. Before ulcer induction, animals were kept in fasting for 24 hours, but had free excess to water, and then absolute ethanol was administered orally at dose of 1 ml/100 g of mice orally. After 1 hour of ethanol treatment, animals of group I was anesthetized with ether and the abdomen was opened by a small midline incision, pyloric portion of stomach will be slightly lifted, ligated, placed it back and closed the abdominal wall by interrupted sutures. After 6 hours, the animals were sacrificed through ether anesthetization for collection of gastric juice, stomach tissue and blood. Animals of Group I considered as negative control. Group IV animals were administered reference drug Ranitidine and Group II and Group III animals were administered the crude extract of low (150mg/kg) and high dose (200mg/kg) of the *Nyctanthes arbor-tristis* Linn. for 6/10/14 days. All the doses were given orally. At the end of the 6th/10th/14th day, animals were fasted for 24hours and sacrificed after exposure to ether for collection of gastric juice, stomach tissue and blood samples for analysis.

Scoring of ulcers

Stomach of each animal was opened along the greater curvature, cleansed with water and then examined to assess the formation of ulcer. After thorough examination of each stomach, Ulcer index (U_i) was calculated by using the formula: $U_i = U_N + U_S + U_P \times 10^{-1}$ (Kulkarni, 2005) where, U_i = Ulcer Index; U_N = Average number of ulcers per animal; U_S = Average number of severity score and U_P = Percentage of animals with ulcers. Severity score was noted as follows, Normal colored stomach = 0, Red coloration = 0.5, Spot ulcer = 1, Hemorrhagic streak = 1.5, Deep ulcer = 2 and

Perforation = 3. Percentage of ulcer healing was calculated as follows-

$$\text{Percentage of ulcer healing} = \frac{[(UI_{\text{control}} - UI_{\text{treated}})/UI_{\text{control}}] \times 100}{0.1}$$

Determination of total acidity

An aliquot of 1 ml of gastric juice was diluted with 1 ml of distilled water and was taken into 50 ml conical flask and two drops of phenolphthalein indicator was added and titrated with 0.01 N NaOH until a parameter pink colour observed. The volume of 0.01 N NaOH was noted. The total acidity was expressed as mEq/L and calculated by following formula (Dashputre and Naikwade, 2011):

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times N \times 100 \text{mEq/L}}{0.1}$$

Where, N is normality

Statistical analysis

In our study, the data were analyzed using Origin software. The values were expressed as the mean $\pm$ Standard Error Mean (S.E.M). Statistical significance was determined by one-way analysis of variance (ANOVA). The result was considered statistically significant at $P < 0.05$.

RESULT AND DISCUSSION

Phytochemical screening

The phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, terpenoids, tannins, saponins, steroids in the flowers extract of *Nyctanthes arbor-tristis*. But anthraquinones were found to be absent.

Table 1: Table showing the presence (+) or absence (-) of various phytochemicals in the methanolic crude extracts of the flowers extract of *Nyctanthes arbor-tristis*.

Phytochemicals	Tests	<i>Phlogacanthus pubinervius</i>
Alkaloids	Dragendroff's test	+
	Mayer's test	+
Glycosides	Keller-Killiani's test	+
	Libersmann's test	+
Flavonoids	Alkaline test	+
	Ferric chloride test	+
Terpenoids	Salkowski's test	+
Tannins	Ferric chloride test	+
Saponins	Froth test	+
Anthraquinones	Hydrochloric test	-
Steroids	Salkowski's test	+

Measurement of ulcer index and calculation of healing percentage

In the present study, the ulcer index in ethanol induced ulcerated mice was recorded to be 2.85 ± 0.02 . Whereas, ulcerated mice treated with flowers extract of *Nyctanthes arbor-tristis* showed a significant decrease in ulcer index in time- dependent manner ($*P < 0.05$). Moreover, ulcer treatment with ranitidine and methanolic extract of

flowers extract of *Nyctanthes arbor-tristis* promoted faster healing than its negative control.

After 6 days, the ulcer healing rate in negative control was noted as 44.56%, whereas, treatment with flowers extract of *Nyctanthes arbor-tristis* and ranitidine showed better healing by 74.27%, 70.78%, and 75.30%, respectively.

After 10 days, Ulcer healing rate was found to be 65.15% in negative control, and 87.36%, 80.95%, and 89.12% in flowers extract of *Nyctanthes arbor-tristis* and ranitidine, respectively.

After 14 days, the negative control showed 77.01% healing rate. Ulcer treatment with ranitidine, flowers extract of *Nyctanthes arbor-tristis* showed significant healing rate.

Table 2: Ulcer index and healing rate (percentage) of the *Nyctanthes arbor-tristis* plants extract treated groups of mice measured after 6, 10 and 14 days of treatment.

Groups	Ulcer index and (% of healing)		
Ulcerated	2.85±0.02		
	Treatment Days		
	6 days	10 days	14 days
Negative control	1.58±0.24 (44.56)	0.99±0.09* (65.15)	0.65±0.14* (77.01)
High dose of NAT (200mg/kg)	0.73±0.08*# (74.27)	0.36±0.13*# (87.36)	0.07±0.02*# (97.26)
Low dose of NAT (150mg/kg)	0.84±0.04*# (70.78)	0.54±0.12* (80.95)	0.12±0.02*# (95.33)
Ranitidine	0.70±0.10*# (75.30)	0.31±0.14*# (89.12)	0.04±0.02*# (98.59)

Data are expressed as mean ± S.E.M; (n=3). *Significantly different from ulcerated group ($P<0.05$). #Significantly different from its respective negative control group ($P<0.05$). Healing percentage has been mentioned in brackets.

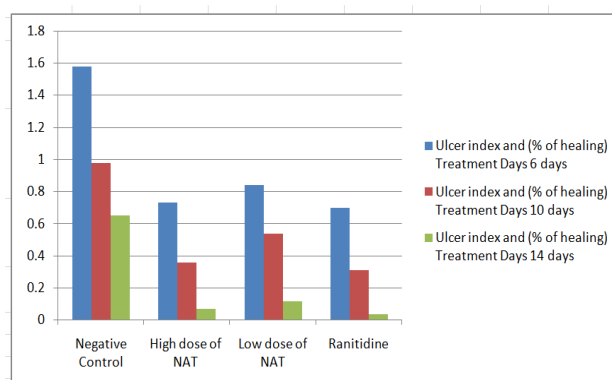


Figure 1: Ulcer index and healing rate (percentage) of the *Nyctanthes arbor-tristis* plants extract treated groups of mice measured after 6, 10 and 14 days of treatment.

Total acidity

In the healthy group total acidity was found to be 26.74±0.66 mEq/L., on the other hand ulcerated mice the value was 67.36±1.46 mEq/L. Total acidity in mice left without treatment group i.e., negative control group was found to be 59.72±3.70, 54.77±1.58 and 45.15±2.18 mEq/L after 6, 10 and 14 days, respectively. After 6 days of ulcer treatment with flowers extract of *Nyctanthes arbor-tristis* total acidity of the stomach was found to be significantly low ($P<0.05$) as compared to the ulcerated

mice i.e., 37.02±0.97, 49.79±2.32, 36.04±1.49, 40.14±2.49 mEq/L, respectively. After 10 days of ulcer treatment, in compare to negative control total acidity was found to be significantly decreased in all the treated groups. After 14 days of treatment, the total acidity was found to be lowest in the ranitidine treated group i.e., 24.31±2.03 mEq/L. However, among plant treated groups the highest reduction in total acidity was found in the mice treated with flowers extract of *Nyctanthes arbor-tristis*.

Table 3: Total acidity (mEq/L) of healthy, ulcerated and *Nyctanthes arbor-tristis* extract treated groups of mice measured after 6, 10 and 14 days of treatment.

Groups	Total acidity (mEq/L)		
Healthy	26.74±0.66		
Ulcerated	67.36±1.46		
	Treatment Days		
	6 days	10 days	14 days
Negative control	59.72±3.70*	54.77±1.58*	45.15±2.18*
High dose of NAT	37.02±0.97*#	30.74±1.79*#	27.34±1.56*#
Low dose of NAT	49.79±2.32*#	37.99±1.45*#	32.52±0.75*#
Ranitidine	34.74±2.49*#	30.97±1.77*#	24.31±2.03*#

All the values are expressed as Mean $\pm$ S.E.M; (n=3). *Significantly different from ulcerated group ($P<0.05$). #Significantly different from its respective negative control group ($P<0.05$).

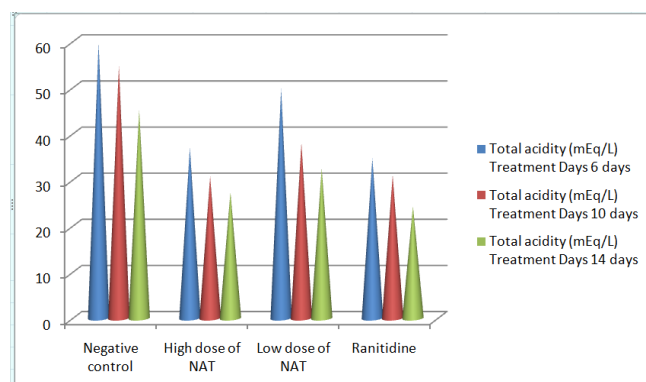


Figure 2: Total acidity (mEq/L) of healthy, ulcerated and *Nyctanthes arbor-tristis* extract treated groups of mice measured after 6, 10 and 14 days of treatment.

SUMMARY AND CONCLUSION

Herbal remedies are commonly perceived as safe due to their natural composition and lack of adverse reactions on the consumers (Ekor, 2014). Phyto-therapeutic treatments often falsely have the perception of being safe due to their natural origin (Gesler, 1992; Moreira et al., 2014). However, the toxicity and adverse effects that are associated with herbal medicines are widely recognized (Kasthuri and Ramesh, 2018). Nevertheless, the available literature suggests that these natural products include many bioactive substances that have the potential to induce harmful consequences (Bent and Ko, 2004). A chemical agent's toxicity is the degree to which it causes harmful consequences when exposed to animals in doses higher than the safe limit (Roberts et al., 2022). So, it is essential to gather preliminary data on the safety and antagonistic effects of any chemical agent or herbal extract before conducting a clinical research on it. The appropriate dosage distinguishes whether a medication is hazardous or therapeutic when evaluating its toxicity prior to its clinical administration (Gossel, 2018).

For our ulcer healing study, we have used the ethanol induced ulcer model to investigate the effectiveness of the *Nyctanthes arbor-tristis* plants and most effective in healing ulcers. Ethanol induced peptic ulcer model have been widely used experimental method for evaluation of traditional plant's anti-ulcer potency as ethanol simply infiltrates the stomach epithelium and causes damage to the stomach (Mousa et al., 2019; Fahmy et al., 2020) which closely resembles the physiological and morphological alteration observed in acute human peptic ulcer disease (Fu et al., 2018). Ethanol intake exposes the mucosal layer to HCl and pepsin, which have proteolytic and hydrolytic effects that destroy cell membranes and results in cell exfoliation, erosion and ulceration (Shams and Eissa, 2022).

In our study, the repeated oral dose with ethanolic crude extract of *Nyctanthes arbor-tristis* at a dose of 200 mg/kg bw for ethanol induced ulcer model showed a gradual reduction in ulcer index after 6, 10 and 14 days of

treatment. Besides this, the percentage of ulcer healing also increased in a time dependent manner from 6 days to 14 days treatment; which is comparable to the standard drug. However, *Nyctanthes arbor-tristis* treated mice showed highest reduction of ulcer. This indicates that the extract exhibits a gastro protective effect which is dependent on both repeated dosage and time.

Similar to our observations Ahmed et al. (2022) also recorded a significant reduction in ulcer index in repeated treatment with *Urtica simensis* extract in ethanol induced rats. Furthermore, in another *in vivo* study, a repeated pre-treatment dose with *Osyris quadripartite* in rats for 10 and 20 days showed significant reduction of ulcer index in comparison to its control groups (Abebaw et al., 2017). Similarly, the aqueous extract of *Commicarpus chinensis* significantly reduced the ulcer index and enhanced the percentage protection in a dose dependent manner (Nitin and Sayeed, 2014).

The suppression of increased gastric acidity is a critical protective factor, as ulcer formation results from the acid level surpassing the mucosal defence mechanisms (Arawawala et al., 2010). Previous research revealed that there is a strong connection between the reduction of gastric acidity and the efficacy of treatment. The ability to reduce the amount of acid that is secreted by the stomach is believed to be due to primary treatment for gastric ulcers (Schubert and Peura, 2008; Saremi et al., 2019).

In our present study, after 6, 10 and 14 days of treatment with *Nyctanthes arbor-tristis* plant extract and two doses showed significant reduction in total acidity level in comparison to its negative control and ulcerated group. Based on our comprehensive analysis of the results from our current study, we can draw the conclusion that the ethanolic crude extract of *Nyctanthes arbor-tristis* possess the ability to heal ulcers in an ethanol- induced ulcerated model.

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