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EVALUATION OF THE PROTECTIVE EFFECT OF *NYMPHAEA NOUCHALI* FLOWER EXTRACT ON ULCERATIVE COLITIS IN WISTAR RATS

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ABSTRACT

Ulcerative colitis (UC) is a chronic inflammatory bowel disease characterized by recurrent inflammation and ulceration of the colonic mucosa, leading to significant morbidity and reduced quality of life. Although conventional therapies are effective, their long-term use is often associated with adverse effects, creating a need for safer and more effective alternatives. *Nymphaea nouchali* (blue water lily) is a medicinal plant rich in flavonoids and phenolic compounds with reported antioxidant and anti-inflammatory properties. The present study was undertaken to evaluate the protective effect of the ethanolic flower extract of *Nymphaea nouchali* against experimentally induced ulcerative colitis in Wistar rats. The flowers of *Nymphaea nouchali* were subjected to ethanolic extraction and the extract was administered to rats with experimentally induced ulcerative colitis. The protective efficacy of the extract was assessed through biochemical and histopathological parameters, including levels of malondialdehyde (MDA), glutathione (GSH), and microscopic examination of colonic tissues. Oxidative stress markers and tissue morphology were evaluated to determine the extent of colonic injury and recovery. The results demonstrated that the ethanolic flower extract significantly prevented the depletion of glutathione and reduced elevated malondialdehyde levels, indicating a reduction in oxidative stress. Histopathological examination revealed regeneration of the colonic mucosa, improvement in villus architecture, and restoration of collagen fibres in the submucosal layer. These findings suggest a marked protective effect of the extract against ulcerative colitis–induced tissue damage. In conclusion, the ethanolic extract of *Nymphaea nouchali* flowers exhibited significant anti-ulcerative colitis activity in Wistar rats, likely mediated through its antioxidant and anti-inflammatory properties. The study supports the potential use of *Nymphaea nouchali* as a natural therapeutic candidate for the management of inflammatory bowel diseases and warrants further investigation to identify its active constituents and clinical applicability.

KEYWORDS: *Nymphaea nouchali*, Ulcerative colitis, Ethanolic flower extract, Antioxidant activity, Histopathology, Wistar rats.

1. INTRODUCTION

1.1 Overview of Ulcerative Colitis

Ulcerative colitis (UC) is a chronic inflammatory bowel disease characterized by recurrent inflammation and ulceration of the colonic mucosa. The disease primarily affects the rectum and colon, causing symptoms such as abdominal pain, diarrhea, rectal bleeding, weight loss, and fatigue. The incidence of UC has increased worldwide, particularly in developing countries, due to changes in environmental and lifestyle factors. Despite advances in therapy, ulcerative colitis remains a significant clinical challenge because of its chronic relapsing nature and associated complications including colorectal cancer and toxic megacolon.The pathogenesis of ulcerative colitis is multifactorial and involves genetic susceptibility, dysregulated immune responses, environmental triggers, and alterations in gut microbiota.

Disruption of the intestinal epithelial barrier allows luminal antigens to activate immune cells, resulting in excessive production of inflammatory cytokines such as TNF-α, IL-1β, and IL-6. Persistent inflammation causes tissue injury, ulceration, and impairment of normal intestinal function. Oxidative stress generated by reactive oxygen species further contributes to disease progression and mucosal damage.[4–7] Animal models play an essential role in understanding the mechanisms of ulcerative colitis and evaluating potential therapeutic agents. Chemically induced models using acetic acid, dextran sulfate sodium (DSS), trinitrobenzene sulfonic acid (TNBS), and oxazolone are widely employed. Among these, acetic acid-induced colitis closely resembles human ulcerative colitis with respect to histopathological changes, oxidative stress, inflammatory cell infiltration, and mucosal ulceration.[1-3]

1.2 Role of Medicinal Plants in Inflammatory Diseases

Medicinal plants have long been utilized in traditional systems of medicine for the management of inflammatory disorders. Plant-derived secondary metabolites such as flavonoids, phenolic acids, tannins, alkaloids, and terpenoids possess significant antioxidant and anti-inflammatory activities. These phytoconstituents can suppress inflammatory mediators, neutralize free radicals, and enhance endogenous antioxidant defenses, thereby providing protection against chronic inflammatory diseases including inflammatory bowel disorders. *Nymphaea nouchali* Burm. f., commonly known as blue water lily or blue lotus, is a perennial aquatic herb belonging to the family Nymphaeaceae. It is widely distributed in India, Bangladesh, Sri Lanka, and other tropical regions of Asia. The plant has considerable medicinal importance and has been traditionally used to treat diarrhea, dysentery, fever, inflammation, liver disorders, urinary diseases, and skin ailments. Various parts of the plant including flowers, leaves, roots, and rhizomes are used in traditional medicine. *Nymphaea nouchali* is characterized by floating orbicular leaves and attractive blue-violet flowers.

The plant possesses a rhizomatous root system anchored in the muddy bottom of freshwater habitats. The flowers contain numerous petals and stamens and are known for their aesthetic and medicinal value. The plant thrives in ponds, lakes, marshes, and slow-moving water bodies under tropical climatic conditions. Phytochemical investigations have revealed the presence of various bioactive compounds in *Nymphaea nouchali*, including flavonoids, phenolic compounds, tannins, alkaloids, glycosides, terpenoids, and steroids. These constituents are responsible for the plant's antioxidant, anti-inflammatory, antimicrobial, and therapeutic activities. Flavonoids and phenolics particularly contribute to free radical scavenging and inhibition of lipid peroxidation.[4-6]

1.3 Pharmacological Activities of *Nymphaea nouchali*

Several pharmacological studies have demonstrated that *Nymphaea nouchali* possesses antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective, analgesic, and wound-healing properties. The antioxidant activity of the

plant is attributed to its high phenolic and flavonoid content, while its anti-inflammatory effects are linked to modulation of inflammatory mediators and cytokines. These biological activities support its potential application in the treatment of inflammatory bowel diseases. Oxidative stress and chronic inflammation are major contributors to the pathogenesis of ulcerative colitis. Since *Nymphaea nouchali* is rich in phytochemicals possessing antioxidant and anti-inflammatory properties, it may provide protection against colonic injury induced by ulcerative colitis. However, scientific evidence regarding its efficacy in experimental colitis remains limited. Therefore, the present study was undertaken to evaluate the protective effect of ethanolic flower extract of *Nymphaea nouchali* in acetic acid-induced ulcerative colitis in Wistar rats.[7-10]

2. MATERIAL AND METHODS

Several plants have been used by the traditional medical therapists across the globe and the actual potential of many such plants is not yet explored scientifically. The present work aimed at exploring the antiulcer potential of Nymphae nouchali using animal models. The presence of flavonoids in Nymphae nouchali was reported in literature available for the pharmacological potential of the plant. This was the main reason for the selection of the plant for the present investigation.[65] The dried flowers of Nymphae nouchali were purchased from the online platform Indian Jadi booty and was used without further authentication.[11,12]

Extraction of plant material

The dried flowers powdered using a blender at low speed. The powdered flowers were stored in air tight container until taken for further processing. The powdered flowers were used for the extraction process by hot continuous extraction method using soxhlet apparatus. 53 g of flower powder was evenly placed in the extractor of the apparatus and 250 mL of ethanol was poured over it and was allowed to collect in the attached flask. Extraction was achieved by heating the solvent at 65°C for 9 h till a colorless solution was collected in the siphon tube of the apparatus. The extract was filtered through Whatman filter and concentrated using rotary vacuum evaporator. The resinous extract was collected and stored in desiccator to remove the excessive moisture. The dried extract was stored in desiccator for further processing.[13]

Preliminary phytochemical screening

The extract was evaluated by qualitative phytochemical screening in order to identify the type of plant secondary metabolites present in it. The screening was performed for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. The color intensity or the precipitate formation was used as analytical responses to these tests.[14]

Total Phenolic Content

In order to determine the total phenolic content 0.5 g dried powder was mixed with 5 mL of ethanol and kept overnight.

The suspension was filtered through a qualitative cellulose filter paper and the filtrate was diluted to 10 mL with ethanol. The solution was stored at 4°C in amber bottles and served as the stock solution for subsequent analyses.For total phenolic content determination, 200 µL of sample was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent. After 3 min, 300 µL of 20% aqueous Na2CO3 solution was added to it and the mixture was allowed to settle for 2 h33. The absorbance was measured at 760 nm with a UV-Vis spectrophotometer. Standard solutions of

gallic acid (20-100 ppm) were treated similarly to obtain the calibration curve. The control solution contained 200 µL of water and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples.

Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.[15]

Pharmacological Study[16]

The male rat weighing between 180-230 g were used procured from approved suppliers from Bhopal. The rodents were allowed free access pellet diet (Lipton India Ltd, Mumbai, Ind.) and water *ad libitum*. All the laboratory conditions and animals were maintained as per CPCSEA guidelines throughout the experiments.

A. Acute Toxicity study

The short- and long-term toxic effects of both drugs and their extracts were performed within prescribed guideline set by OECD guideline no. 423 using single dose of 2000 mg/kg. in previous study Acute Toxicity study already performed and LD 50 is 2000.

The albino rats of 150-200 g were employed for study and kept in 12 h day-night cycle with ad libitum access to water.

The extract was suspended in 1% Tween 80, prepared in distilled water. Animals were fasted for 12 h with and extract was administered orally with 2000 mg/kg considering as maximum dose. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days. The observation made for any bizarre behavior like change in skin and fur, eye, hyperactivity, grooming, convulsions, sedation, hypothermia, salivation, tremor, coma, lethargy, body weight, and mortality. From the observations and study, 1/10th and 1/5th of the maximum dose was used as therapeutic dose & cut-off values were chosen as 200 & 400 mg/kg to evaluate dose dependent action for the assessment of activity.[17]

B. Acetic acid Ulcerative colitis

Table 1: Grouping of animals for experiment.

Group Number	Group Name	Treatment
1	Control	Administered with 0.9% NaCl
2	Inducer Group	Ulcerative colitis-induced rats that were orally treated with 0.5% CMC, daily for 6 days and intra-rectally injected with acetic acid (2 mL of 3% (v/v) in 0.9% NaCl) on day 4.
3	Standard	Ulcerative colitis-induced rats that were orally treated with sulfasalazine as a reference drug (100 mg/kg/day) for 6 days and then injected with acetic acid (2 mL of 3% (v/v) in 0.9% NaCl) intra-rectally on day 4
4	Test 1 (200 mg/kg)	Ulcerative colitis- induced rats that were orally treated with extract dose 1, daily for 6 days and intra-rectally injected with acetic acid (2 mL of 3% (v/v) in 0.9% NaCl) on day 4.
5	Test 2 (400 mg/kg)	Ulcerative colitis- induced rats that were orally treated with extract dose 2, daily for 6 days and intra-rectally injected with acetic acid (2 mL of 3% (v/v) in 0.9% NaCl) on day 4.

Induction of Colitis

Colitis was induced by injecting 2 mL of acetic acid solution (3% v/v) into the rectum through a polyurethane tube for enteral feeding (2 mm in diameter) that was inserted to a depth of 4.5 cm for a duration of 14 days. To prevent solution

leakage, the rats were held in the Trendelenburg position throughout rectal instillation and for 1 min following instillation.[18,19]

Collection of Samples

On day 6, all groups received samples one last time, and rats were sacrificed on day 7. The entire colon was removed, opened longitudinally, and the fecal content was cleared with normal saline. The evaluation of biochemical parameters such as thiol, and malonaldehyde (MDA) was conducted in the proximal part of the colon (6–7 cm). This part of the colon was stored in a physiological buffer at pH 7.4 until the homogenization of the samples. Further, a small part of the proximal colon was removed and kept in 10% formalin for histopathological analysis as well. The homogenization of tissue was performed in a cold environment at a concentration of 10% (w/v) in 11.5 g/L solutions of KCl, and the homogenized samples were centrifuged for 15 minutes at 10.000 rpm and 4 °C. The supernatant part was taken and separated for the biochemical investigations.

Measurement of thiol

Total thiol concentration was measured using reagent DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)) which reacts with the thiol to produce a yellow-coloured complex with a peak absorbance at 412 nm. One ml Trisethylene diamine tetraacetic acid (EDTA) buffer (pH 8.6) was added to 50 µl supernatant in 1 ml cuvettes and sample absorbance was read at 412 nm against Tris-EDTA buffer alone (A1). Twenty microlitre DTNB reagents (10 mmol in methanol) were then added to the mixture and keep it in laboratory temperature for 15 min and the sample absorbance was read again (A2). The absorbance of DTNB reagent was also read as a blank (B). Total thiol concentration (mmol) was calculated using the following equation [72]: [20]

Total thiol concentration (mM) = (A2–A1–B)×1.07/0.05×13.6.

Malonaldehyde (MDA) measurement

1 mL of supernatant was mixed with 2 mL of 10% (w/v) trichloroacetic acid stood in ice for approximately 15 minutes.[73-74] The precipitates were separated by centrifugation. Then 2 mL sample of the clear supernatant solution was mixed with 2 mL aqueous 0.67% thiobarbituric acid and was heated in a boiling water bath for 10 minutes. The solution was cooled in ice for 5 minutes, and absorbance was measured at 535 nm against an appropriate blank solution. The level of malondialdehyde (MDA) was calculated by using a molar extinction coefficient of 1.49 x 105 m -1cm-1.[21,22]

Assessment of Macroscopic Ulcer Score

The severity of colonic injury was assessed by evaluating the macroscopic ulcer score following the induction of ulcerative colitis. After sacrificing the animals, the colon was carefully excised, cleaned with normal saline to remove faecal contents, and an approximately 8 cm long segment of the distal colon was isolated. The colon was then longitudinally opened and mounted flat on a glass slide for gross examination. The mucosal surface was inspected visually for pathological changes such as erythema, edema, erosion, ulceration, haemorrhage, and tissue necrosis. The extent of macroscopic damage was quantified using the scoring system established by Miller , which grades the severity of colonic lesions based on observable clinical and morphological features as shown in Table 2.[23]

Table no 2 Scale bar for quantification of macroscopic ulcer score in acetic acid induced ulcerative colitis.

Macroscopic Ulcer Score	Severity of Colon Lesion
0	No macroscopic change
1	Only mucosal erythema
2	Small erosions or minor bleeding with mild edema
3	Small bleeding erosions with moderate edema
4	Tissue necrosis, severe ulceration, and edema

Histopathological Investigation

Rat colonic tissues were removed, perfused with phosphate buffer pH 7.4 and 1M EDTA (Ethylene diamine tetra acetate) and deposited in a 10% formaldehyde solution for 24h. Tissues were then dehydrated using ethanol and xylol and then embedded in paraffin blocks and cut into 3.5-mm slices using microtome. All tissue samples were examined under a light microscope after being stained with hematoxylin-eosin (HE). Morphological analyses of histopathological parameters (epithelial damage, edema, cellular infiltration and necrosis) were completed via use of a microscope.[24]

3. RESULTS

The present work focused on preparing ethanolic extract of *Nymphae nouchali* and evaluating its ulcer protective potential in rats. The results obtained from the investigation are presented in the following sections.The extraction yield of the flower of *Nymphae nouchali* flowers in ethanol by hot continuous extraction was found to be 16.41% w/w. The extract was resinous and brown in color.

Phytochemical Screening

For detecting the occurrence of alkaloids, glycosides, tannins, saponins, flavonoids and terpenoids in the extracts, a small fraction of the dried extract was subjected to the phytochemical testing procedures by re-suspending a small amount of each extract suitably into the sterile distilled water/ethanol. The extract was tested for the presence of various categories of phytochemicals and the results are presented in Table 3. The findings of the phytochemical analysis suggest the presence of phenolics and tannins, proteins and flavonoids in the ethanolic extract of the flowers.

Table 3: Phytochemical screening of *Nymphae nouchali flower* extract.

Phytochemical Class	Test Performed	Principle/Positive Observation	Result in Ethanolic Extract
Alkaloids	Mayer's Test	Cream-colored precipitate	–
	Hager's Test	Yellow precipitate	–
	Wagner's Test	Reddish-brown precipitate	–
	Dragendorff's Test	Orange to reddish-brown precipitate	–
Glycosides	Froth Test	Persistent frothing	–
	Kedde's Test	Formation of purple/violet color	–
	Borntrager's Test	Rose-pink to red color in ammoniacal layer	–
	Keller–Kiliani Test	Bluish-green color in acetic acid layer	–
Phenols and Tannins	Ferric Chloride Test	Blue-green coloration	+
	Gelatin Test	White precipitate formation	+
	Alkaline Reagent Test	Yellow to red precipitate	+
	Vanillin-HCl Test	Purplish-red coloration	+
Flavonoids	Shinoda Test	Development of red/pink color	+
	Alkaline Reagent Test	Yellow color turning red upon acidification	+
	Zinc-HCl Reduction Test	Red coloration	+
Proteins and Amino Acids	Millon's Test	White precipitate turning red on heating	+
	Ninhydrin Test	Violet or purple coloration	+

Sterols and Triterpenoids	Liebermann–Burchard Test	Brown ring at junction and green upper layer	–
	Salkowski Test	Yellow coloration in lower layer	–

Total Phenolic content

The ethanolic extract of *Nymphae nouchali* flowers was evaluated for quantifying the total phenolic content. Standard curve of gallic acid was plotted in distilled water. The result of the total phenolic content of the extract examined using Folin-Ciocalteu method. The total phenolic content of ethanolic extract of *Nymphae nouchali* was found to be 49.30±2.619 GAE mg/g.

Table 4: Absorbance data of gallic acid (at 760 nm).

Concentration ppm	Absorbance at 760 nm
15	0.089
30	0.171
45	0.233
60	0.321
75	0.437
90	0.524

Figure 1: UV spectra of gallic acid.

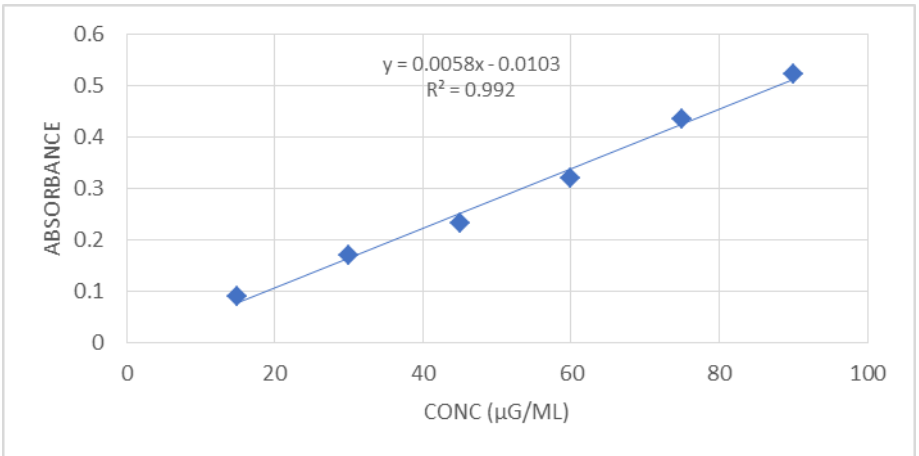

Figure 2: Calibration curve of gallic acid.

Acute toxicity Study

There was no sign and symptoms or any toxic effects in rodents for both plants even at higher dose of 2000 mg/kg body weight. Thus, 1/10th of maximum dose was selected as effectual dose. The cut off value of 200 & 1/5th dose i.e 400 mg/kg were chosen for evaluation of memory enhancing activity.

Table 5: Acute toxicity profile of Nymphae nouchali flower extract.

S. No.	Treatment	Dose (mg/kg)	No. of test animals	Mortality			Status
				24h	7d	14d	
1	Ethanolic extract	2000	3	0	0	0	Safe
2	Ethanolic extract	2000 (limit test)	3	0	0	0	Safe

Pharmacological Action

Normal colon homeostasis requires the equilibrium between pro-inflammatory and anti-inflammatory cytokines in the colon mucosa. Cytokine profile disruption causes some negative conditions, such as inflammatory bowel diseases. The colonic homogenate was examined for the levels of MDA and glutathione (Table 6).

Table 6: Effect of extract on MDA and GSH levels in colonic homogenate.

Group	GSH (µg/g)	MDA (nmol/g)
Control	446.3 ± 7.781	118.6 ± 4.338
Inducer	141.3 ± 7.451**	375.5 ± 3.452**
Standard	408.5 ± 9.928$^{\#}$	134.5 ± 4.349$^{\#}$
Extract Dose 1 (200 mg/kg)	168.5 ± 7.455$^{\$}$	337.5 ± 3.730$^{\$}$
Extract Dose 2 (400 mg/kg)	285.3 ± 10.687$^{\#}$	190.6 ± 7.431$^{\#}$
The results are expressed as mean ± SD for 6 rats in each group. **Significant difference with $p<0.05$ compared to control group, $Significant difference with $p<0.05$ compared to inducer, #Significant difference with $p<0.05$ compared to inducer		

The glutathione level was high in the control groups with tremendously decreased in the inducer administered group. The extracts were able to prevent the fall in the level of glutathione significantly at dose of 400 mg/Kg (Figure 3). The increased MDA level in the inducer group indicates the increase in oxidant levels in the colonic homogenate causing colonic ulcers. The extract was able to significantly decrease the MDA level (Figure 3).

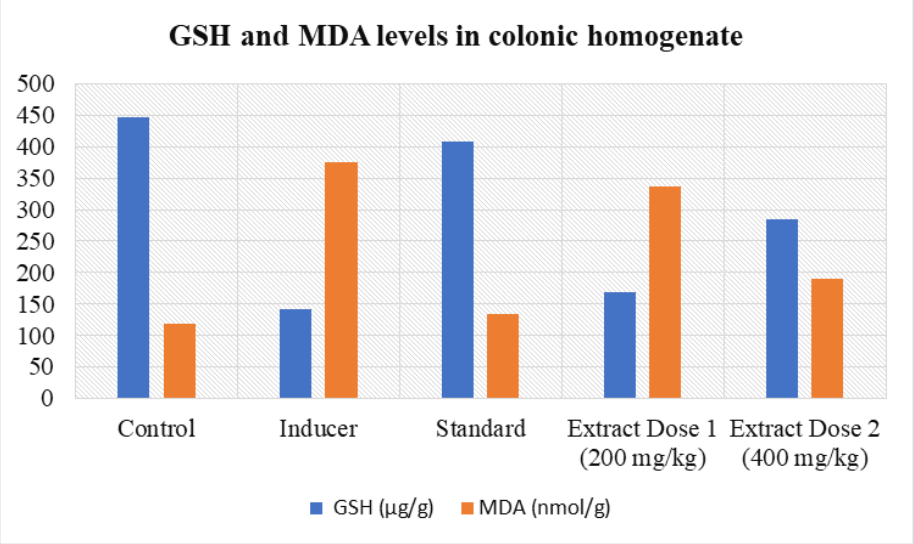

Figure 3: Effect of extract on GSH and MDA levels in colonic homogenate.

Macroscopic Assessment of Ulcer Score

Group	Lesion Score
Control	0.0 ± 0.0
Inducer	4.0 ± 0.2
Standard (sulfasalazine)	1.7 ± 0.9***
Extract 200 mg/kg	2.9 ± 1.5ns
Extract 400 mg/kg	2.01 ± 1.2**
where: ns = not significant (p > 0.05) p < 0.05 ** p < 0.01 *** p < 0.001	

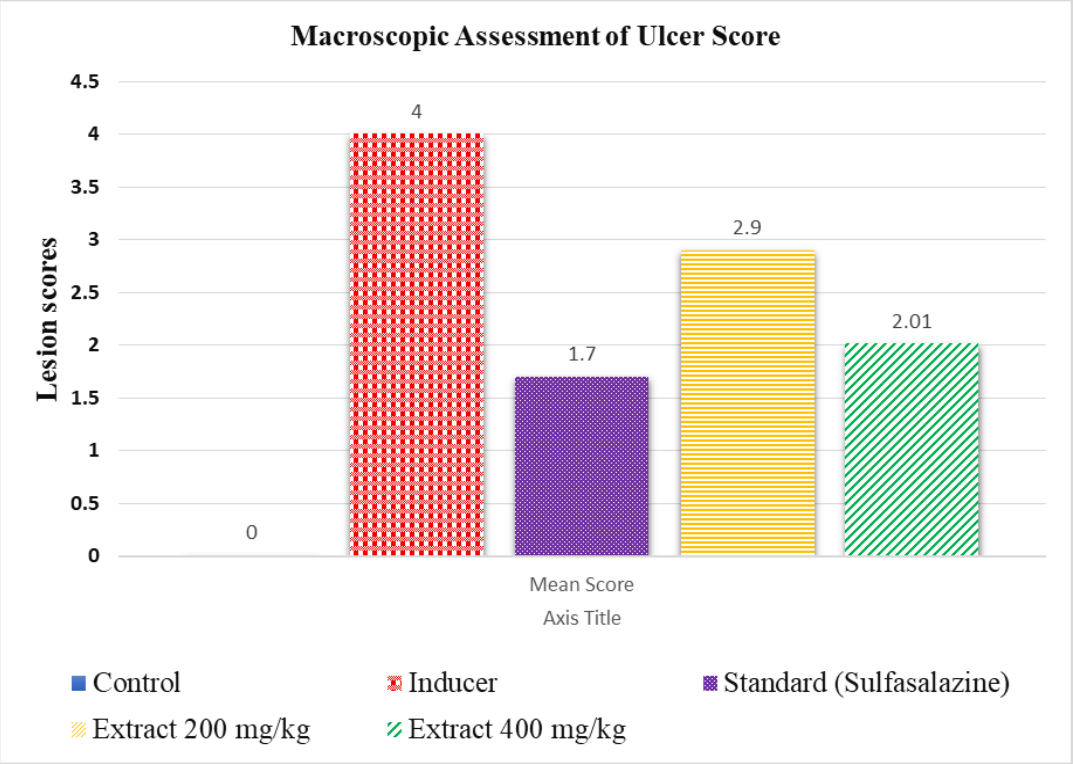

Figure 4: Macroscopic Assessment of Ulcer Score.

Microscopic Assessment of colon

In the inducer group, there were several mucosal erosions, including the invasion of certain inflammatory cells in the lamina propria, villus flattening, and some damage to the crypts and cylindric epithelium in the mucosa in the duodenum and jejunum. The ethanol extract treated groups also had some histopathological modifications, such as regeneration in the mucosa and villus degeneration as well as collagen fiber regeneration in the submucosa. In the positive control group namely the sulfasalazine-treated group, villus and collagen fiber regeneration in mucosa and submucosa were examined, respectively.

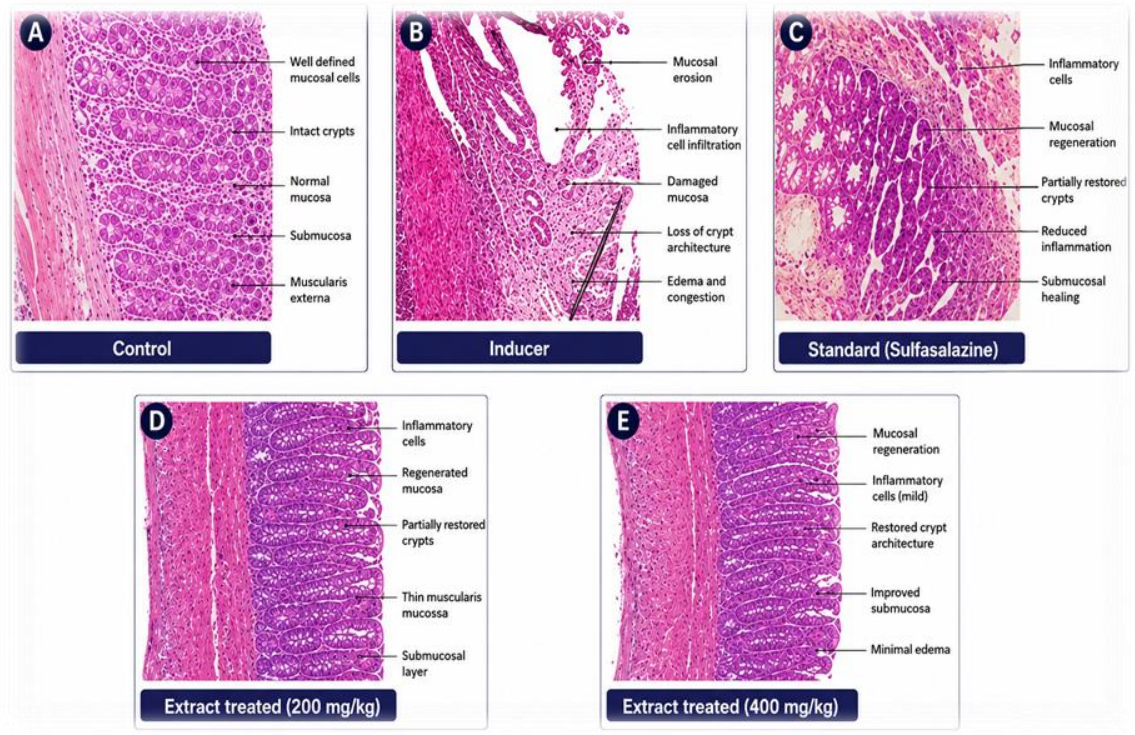

Figure 5: Section of colon (A) Control (B) Inducer (C) Standard treated (D) Extract treated (200 mg/kg) (E) Extract treated (400 mg/kg) using image J software.

4. DISCUSSION

The present study investigated the ulcer-protective potential of the ethanolic flower extract of *Nymphaea nouchali* in an experimental model of ulcerative colitis. The extraction process yielded 16.41% w/w of a brown resinous extract.

Preliminary phytochemical screening revealed the presence of phenolics, tannins, flavonoids, proteins, and amino acids, while alkaloids, glycosides, sterols, and triterpenoids were absent. The extract exhibited a considerable total phenolic content (49.30 ± 2.62 mg GAE/g), suggesting a rich source of antioxidant constituents. Acute toxicity studies demonstrated that the extract was safe up to 2000 mg/kg, indicating a wide margin of safety.

Acetic acid administration produced marked oxidative stress and colonic injury, as evidenced by a significant reduction in glutathione (GSH) levels, elevation of malondialdehyde (MDA) levels, severe macroscopic lesions, and histopathological damage. Treatment with *N. nouchali* extract, particularly at 400 mg/kg, significantly restored GSH levels, reduced MDA concentrations, and decreased ulcer severity compared with the inducer group. Histological examination further confirmed protection of colonic architecture, showing regeneration of the mucosal layer and collagen fibers with reduced inflammatory changes. These findings suggest that the ulcer-protective effect of *N. nouchali* may be attributed to its antioxidant and anti-inflammatory phytoconstituents, especially phenolic compounds and flavonoids. Overall, the study demonstrates that the ethanolic flower extract of *N. nouchali* possesses significant protective activity against experimental ulcerative colitis and may serve as a promising natural therapeutic agent for inflammatory bowel disorders. The objective of the present works was to perform ethanolic extraction of the flowers of *Nymphaea nouchali* and investigate and establish the protective effect of *Nymphaea nouchali* extract on experimentally induced ulcerative colitis in rats. The dried flowers of *Nymphae nouchali* were purchased from the online platform indianjadibooti and was used without further authentication. The powdered flowers were used for the

extraction process by hot continuous extraction method using soxhlet apparatus using ethanol as the extracting solvent. The extract was evaluated by qualitative phytochemical screening in order to identify the type of plant secondary metabolites present in it. The quantitative estimation of total phenolic content in the extract was determined by Folin-Ciocalteau method and results were expressed as gallic acid equivalent. The short and long term toxic effects of both drugs and their extracts were performed within prescribed guideline set by OECD guideline no. 423. Colitis was induced by injecting 2 mL of acetic acid solution (3% v/v) into the rectum through a polyurethane tube for enteral feeding (2 mm in diameter) that was inserted to a depth of 4.5 cm. The evaluation of biochemical parameters such as thiol, and malonaldehyde (MDA) was conducted in the proximal part of the colon. All tissue samples were examined under a light microscope after being stained with hematoxylin-eosin (HE). The extraction yield of the flower of *Nymphae nouchali* flowers in ethanol by hot continuous extraction was found to be 16.41% w/w. The extract was resinous and brown in color. The findings of the phytochemical analysis suggest the presence of phenolics and tannins, proteins and flavonoids in the ethanolic extract of the flowers. The total phenolic content of ethanolic extract of *Nymphae nouchali* was found to be 49.30±2.619 GAE mg/g. There were no sign and symptoms or any toxic effects in rodents for both plants even at higher dose of 2000 mg/kg body weight. Thus, 1/10th of maximum dose was selected as effectual dose. The cut off value of 200 & 1/5th dose i.e 400 mg/kg were chosen for evaluation of memory enhancing activity. The glutathione level was high in the control groups with tremendously decreased in the vehicle administered group. The extract was able to prevent the fall in the level of glutathione significantly at dose of 400 mg/Kg. The increased MDA level in the control group indicates the increase in oxidant levels in the colonic homogenate causing colonic ulcers. The extract was able to significantly decrease the MDA level. The ethanol extract treated groups exhibited some histopathological modifications, such as regeneration in the mucosa and villus degeneration as well as collagen fiber regeneration in the submucosa.

5. CONCLUSION

Inflammatory bowel disease is closely linked to a Westernized environment and lifestyle. Ulcerative colitis has an incidence of 9 to 20 cases per 100,000 persons per year. Roots and leaves of various species of Genus *Nymphaea* have been helpful in preventing experimentally induced ulcerative colitis in rodents. The objective of the present works was to perform ethanolic extraction of the flowers of *Nymphaea nouchali* and investigate and establish the protective effect of *Nymphaea nouchali* extract on experimentally induced ulcerative colitis in rats. The extract was able to prevent the fall in the level of glutathione significantly at dose of 400 mg/Kg. Thable increased MDA level in the control group indicates the increase in oxidant levels in the colonic homogenate causing colonic ulcers. The extract was able to significantly decrease the MDA level. The ethanol extract treated groups exhibited some histopathological modifications, such as regeneration in the mucosa and villus degeneration as well as collagen fiber regeneration in the submucosa. The results of the study led us to conclude that the ethanolic extract of the plant could significantly improve the morphology in the colon suggesting anti-ulcer potential in the extract. The improved levels of MDA and GSH suggest the involvement of anti-oxidant components in combating ulcer.

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