Protective Effects of *Marsilea minuta* Hydroalcoholic Extract Against Indomethacin-Induced Gastric Ulcers in Wistar Rats: Biochemical and Histopathological Evaluation

Ms. Shahar Bano*, Mrs. Manorama Ratre[2], Ms. Hemlata Rathore[3], Mr. Suresh Kumar Sahu[4]

[1,2,3]*School of Pharmacy, Chouksey Engineering College, Bilaspur, Chhattisgarh-495001, India*
[4]*Mark Hospital, Bilaspur, Chhattisgarh-495001, India*

*Corresponding Author
Shahar Bano
School of Pharmacy, Chouksey Engineering College, Bilaspur, Chhattisgarh-495001, India

Abstract

Peptic ulcer disease is a common gastrointestinal disorder often triggered by excessive use of non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin, which disrupt gastric mucosal integrity by inhibiting prostaglandin synthesis and increasing oxidative stress. Conventional ulcer therapies can have significant side effects and limited efficacy with prolonged use, prompting interest in natural remedies. This study investigates the protective potential of Marsilea minuta (MM), an aquatic fern traditionally used in herbal medicine, against indomethacin-induced gastric ulcers in rat models.

Hydro-ethanolic extracts of M. minuta were prepared using Soxhlet extraction, and rats were pretreated orally with two doses (50 mg/kg and 100 mg/kg) of the extract. Peptic ulcers were induced using indomethacin (50 mg/kg, intraperitoneally). The efficacy of the MM extract was evaluated through macroscopic ulcer scoring, histopathological examination, and biochemical assays measuring markers of oxidative stress and inflammation, such as, glutathione (GSH), and catalase (CAT). Additionally, prostaglandin E2 (PGE2) and Nitric oxide (NO) levels in gastric tissues were analyzed to determine the extract's effect on mucosal defense mechanisms.

The findings revealed that MM extract significantly reduced ulcer severity and preserved gastric tissue structure, particularly at the 100 mg/kg dose. Biochemical results showed improved antioxidant defense, reduced lipid peroxidation, and enhanced PGE2 synthesis in the gastric mucosa. Histological analysis confirmed reduced inflammation and maintenance of mucosal architecture in treated groups.

In conclusion, Marsilea minuta extract demonstrated notable gastroprotective activity against indomethacin-induced ulcers through antioxidant, anti-inflammatory, and prostaglandin-mediated pathways. These results support the traditional use of M. minuta in gastrointestinal ailments and suggest its potential as a safe, effective natural therapeutic agent for peptic ulcer management. Further research should focus on the isolation of active constituents and detailed mechanistic studies to support clinical applications.

Keywords: *Peptic ulcer, Indomethacine, ranitidine, M. minuta, Gastroprotective.*

1. Introduction

1.1 Peptic Ulcer

Peptic ulcers are open sores that develop on the mucosal lining of the stomach or upper part of the small intestine (duodenum), typically resulting from a breakdown in the protective mucosal barrier. These lesions occur due to an imbalance between aggressive factors such as gastric acid, pepsin, Helicobacter pylori (H. pylori), NSAIDs, and ethanol, and defensive mechanisms like mucus, bicarbonate, prostaglandins, and nitric oxide. H. pylori, a gram-negative bacterium, is one of the most common causes of peptic ulcers, implicated in nearly 75% of non-cardiac gastric cancers. NSAIDs, another leading cause, induce ulcers by inhibiting COX-1, an enzyme responsible for prostaglandin synthesis, thereby reducing mucosal protection.

Other contributing factors include chronic stress, alcohol abuse, smoking, and underlying systemic illnesses. Smoking increases oxidative stress in the gastric mucosa and lowers the availability of protective growth factors, while chronic alcohol intake damages the mucosal barrier and promotes inflammation. Although the global prevalence of H. pylori infection exceeds 50%, only 5–10% of those infected develop ulcers. The severity depends on bacterial virulence, host immune response, genetic predisposition, and environmental influences. In the United States, peptic ulcer disease results in over $6 billion in annual healthcare costs, with significant morbidity and potential progression to gastric cancer.

There are also less common causes of peptic ulcers unrelated to H. pylori or NSAIDs. These include gastric malignancies, Crohn’s disease, systemic mastocytosis, hyperparathyroidism, eosinophilic gastritis, and stress ulcers due to severe illness or trauma.

1.2 Indomethacin

Indomethacin is a potent NSAID widely used for its antipyretic, analgesic, and anti-inflammatory effects, especially in treating certain headache disorders. However, it is also known to cause significant gastrointestinal toxicity, making it a well-established agent for inducing experimental peptic ulcers in animal models. Indomethacin’s ulcerogenic potential is due to its inhibition of COX-1, which reduces protective prostaglandin levels and disrupts mucus and bicarbonate secretion, resulting in mucosal injury.

This drug also increases oxidative stress, acid secretion, and damages the mucosal barrier by altering membrane integrity. Although conventional anti-ulcer medications can mitigate these effects, they often come with significant side effects. Therefore, there is growing interest in natural therapies with fewer adverse effects.

1.3 Marsilea minuta

Marsilea minuta, commonly known as dwarf water clover, is an aquatic fern found in wetlands across Asia. Traditionally used in Ayurveda and folk medicine, it is known for its anti-inflammatory, hepatoprotective, neuroprotective, antibacterial, and antidiabetic properties. Phytochemical analysis has revealed the presence of flavonoids, alkaloids, phenolics, saponins, and other bioactive compounds. Flavonoids, in particular, are abundant and contribute to the plant’s antioxidant and anti-inflammatory activities.

These pharmacological properties support the potential of *Marsilea minuta* as a natural therapeutic agent for treating NSAID-induced peptic ulcers. Its traditional use and scientific backing make it a promising candidate for further research in ulcer management.

2. Materials and Methods

2.1 Collection and Authentication

Marsilea minuta was collected from Rajkisornagar, Bilaspur (Chhattisgarh) and authenticated by the Botany Department of Guru Ghasidas Central University. The plant was shade-dried, powdered, and stored for further use.

2.2 Extraction

About 500 g of dried plant material was subjected to Soxhlet extraction using 50:50 ethanol-water at 60–70°C for 72 hours. The extract was concentrated by air drying and stored at 4°C. For experiments, it was reconstituted in distilled water to 0.25 g/ml.

2.3 Phytochemical Screening

Hydroethanolic extract was screened for alkaloids, flavonoids, saponins, tannins, and triterpenoids using standard tests like Mayer's, Wagner's, Shinoda, foam, Liebermann–Burchard, and Salkowski tests.

2.4 Flavonoid Fractionation

The ethanolic extract was dissolved in water and partitioned with solvents like petroleum ether, chloroform, ethyl acetate, and n-butanol to isolate flavonoid-rich fractions. Ethyl acetate and n-butanol layers were collected, concentrated, and stored.

2.5 Drugs and Equipment

Indomethacin, ranitidine, and biochemical kits for CAT, NO, PGE2, and GSH were procured. All drugs and extracts were suspended in distilled water and given orally.

2.6 Animal Ethics and Housing

Male Wistar rats (150–180 g) were used. They were kept under standard conditions (25±1°C, 12-hour light/dark cycle) with ethical clearance from IAEC (Reg. No. 1275/PO/Re/S/09/CPCSEA). Cleanliness, diet, health monitoring, and record-keeping were ensured. Doses of 40, 50, and 100 mg/kg body weight were administered, and animals were monitored for 21 days.

2.7 Acute Toxicity

Acute toxicity was evaluated according to OECD guidelines. Animals were dosed orally and monitored for 48 hours (short-term) and 21 days (long-term) for signs of toxicity and mortality.

2.8 (a) In Vivo Study Design

Thirty rats were divided into five groups (n=6):

Control

Indomethacin (30 mg/kg)

Ranitidine (40 mg/kg)

Indomethacin + MM (50 mg/kg)

Indomethacin + MM (100 mg/kg)

2.8 (b) Biochemical Analysis

Catalase (CAT): Measured at 240 nm using Aebi's method.

Nitric Oxide (NO): Estimated using the Griess method at 540 nm.

Glutathione (GSH): Evaluated with DTNB at 405 nm.

PGE2 and TNF-α: Measured using ELISA kits.

2.8 (c) Histopathology

Stomach tissue was fixed in 10% formalin, embedded in paraffin, sectioned (5 μm), and stained with hematoxylin and eosin (H&E) for microscopic evaluation.

2.9 Statistical Analysis

Data were analyzed using one-way ANOVA followed by Dunnett's test. Results were expressed as mean ± SEM, and $p < 0.05$ was considered statistically significant.

3. Results and Discussions

3.1 Preparation of *Marsilea minuta* Hydro-Ethanolic Extract

The hydro-alcoholic extract of *Marsilea minuta* yielded 17.5% w/w. A schematic representation of the extraction procedure is shown in Figure 3.1.

Plant Dried Grind plant

Figure no. 3.1 Extraction process

Table no. 3.1 Physical appearance and extractive values of *Marsilea minuta*:

SR. NO.	DRUG	SOLVENT	COLOR	PLANT MATERIAL (gm)	WEIGHT OF EXTRACT (gm)	% YIELD (W/W)
1.	*MARSILEA MINUTA*	Ethanol	GREENISH COLOR	500	87.5	17.5

3.2 Preliminary Phytochemical screening

The results of our study on *Marsilea minuta* revealed the presence of tannins, terpenoids, alkaloids, saponins, and flavonoids. The extract was treated with diluted hydrochloric acid and filtered. The filtrate was used for the following test.

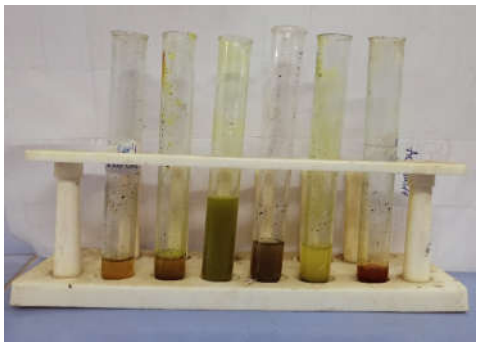


Figure no. 3.2 Phytochemical constituents Test

Table no. 3.2 Phytochemical constituents test of *Marsilea minuta* extract

Phytochemical parameters	***Marsilea minuta***
Flavonoid	+
Tannins	+
Saponin	+
Triterpenoid	+
Alkaloids	+

3.3 Acute Oral Toxicity

An acute toxicity assessment was conducted for the fractions TF-MMME, BF-MMME, and AF-MMME in accordance with OECD guideline 420 (2001) issued by the Organization for Economic Co-operation and Development. Wistar albino rats were randomly allocated into groups of six animals each. The test fractions were orally administered at doses ranging from 100 to 2000 mg/kg body weight. Following administration, the animals were closely monitored for any signs of toxicity, behavioural changes, or mortality over a period of 72 hours.

3.4 Biochemical Parameter

Effect of MM treatment on different biological parameters in indomethacin-induced peptic ulcer on rats. Indomethacine (30 mg/kg, oraly for 21 days) administration resulted in a significant elevated the biochemical parameters for peptic ulcer test like CAT, NO, GSH, PEG2, compared to the normal group. Pretreatment with ranitidine & mm significantly prevented the biochemical changes induced by indomethacin. The peptic ulcer effect offered by mm (50mg/kg,orally) was found to be significantly greater than the mm (100mg/kg, orally) and standard (ranitidine 40 mg/kg,orally) group.

Table 3.4.1 Effect of mm treatment on different biological parameters in indomethacin-induced Peptic ulcer in rats.

GROUP	TREATMENT	CAT	GSH
I	NORMAL	5.23±0.08	39.13±0.93
II	INDOMETHACIN	1.49±0.04	20.40±1.24
III	RANITIDINE	5.13±0.02	37.01±0.68
IV	INDO+MM (50MG/KG)	3.98±0.49	32.27±1.75
V	INDO+MM (100MG/KG)	4.89±0.04	35.10±1.43

The results are expressed as the mean ± S.E.M. for each group, with six animals per group (n = 6). Statistical significance was assessed using standard p-value thresholds, where * indicates $p < 0.05$, ** denotes $p < 0.01$, *** represents $p < 0.001$, and **** signifies $p < 0.0001$. Differences were considered not significant when $p > 0.05$ and are indicated as "ns." Group comparisons were made using specific notations: ‘a’ indicates a significant difference when compared to the normal control group, ‘b’ denotes a comparison with the indomethacin-treated (toxic) group, ‘c’ refers to a comparison with the MM + indomethacin (lower dose) group, and ‘d’ indicates comparison with the MM + indomethacin (100 mg/kg) group. Specifically, Group I (normal) was compared to Group II (toxic), and Group II (toxic) was compared with the treatment groups (Groups III, IV, and V).

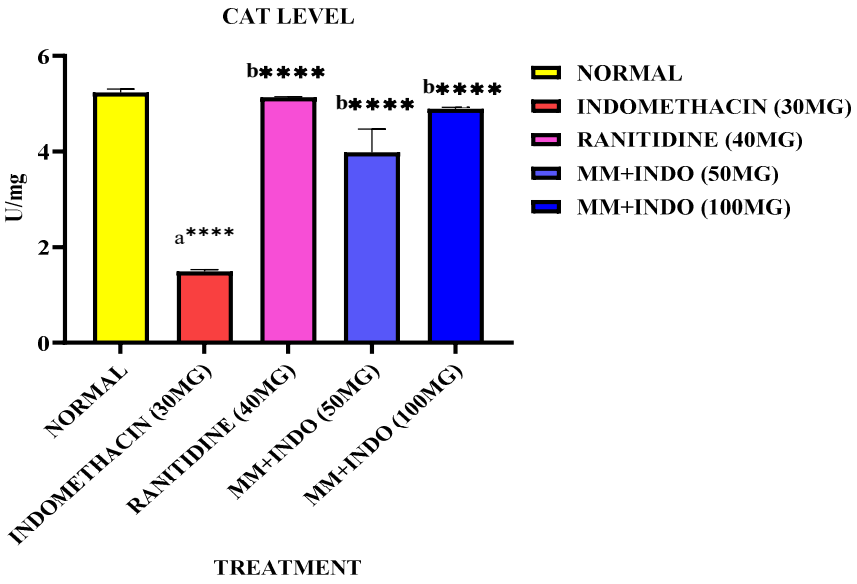


Figure no. 3.4.1 (a) Effect of MM treatment on CAT level in INDO-induced peptic ulcer in rats

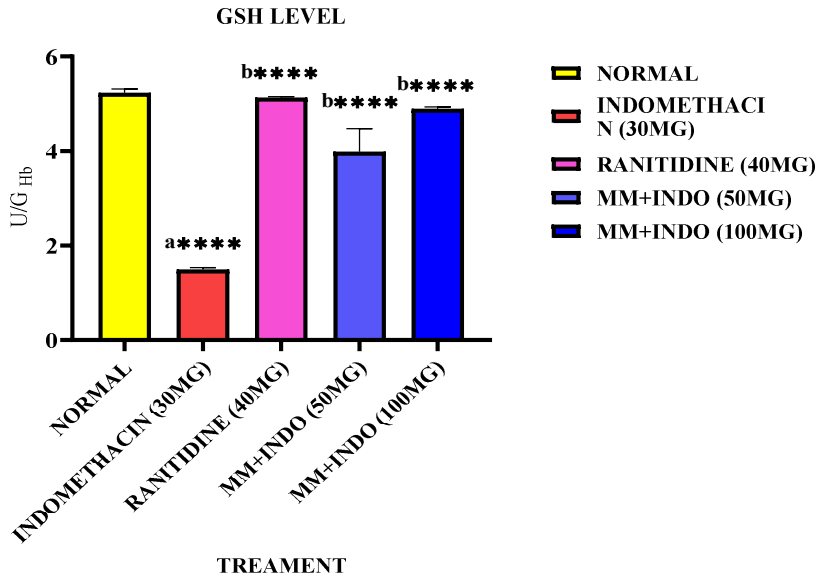


Figure no. 3.4.1 (b) Effect of MM treatment on GSH level in INDO-induced peptic ulcer in rats

Table no. 3.4.2 Effect of treatments on stomach cytoprotective mediators.

GROUP	TREATMENT	NO (pg/g tissue)	PGE2 (pg/g tissue)
I	NORMAL	819. 49±0.47	44.37±0.73
II	INDOMETHACIN	344.63±0.62	25.12±0.61
III	RANITIDINE	811.07±1.62	43.45±0.48
IV	INDO+MM (50MG/KG)	808.63±0.64	42.29±0.80
V	INDO+MM (100MG/KG)	814.93±0.38	44.10±0.71

The results are expressed as the mean ± S.E.M. for each group, with six animals per group (n = 6). Statistical significance was assessed using standard p-value thresholds, where * indicates $p < 0.05$, ** denotes $p < 0.01$, *** represents $p < 0.001$, and **** signifies $p < 0.0001$. Differences were considered not significant when $p > 0.05$ and are

indicated as "ns." Group comparisons were made using specific notations: ‘a’ indicates a significant difference when compared to the normal control group, ‘b’ denotes a comparison with the indomethacin-treated (toxic) group, ‘c’ refers to a comparison with the MM + indomethacin (lower dose) group, and ‘d’ indicates comparison with the MM + indomethacin (100 mg/kg) group. Specifically, Group I (normal) was compared to Group II (toxic), and Group II (toxic) was compared with the treatment groups (Groups III, IV, and V).

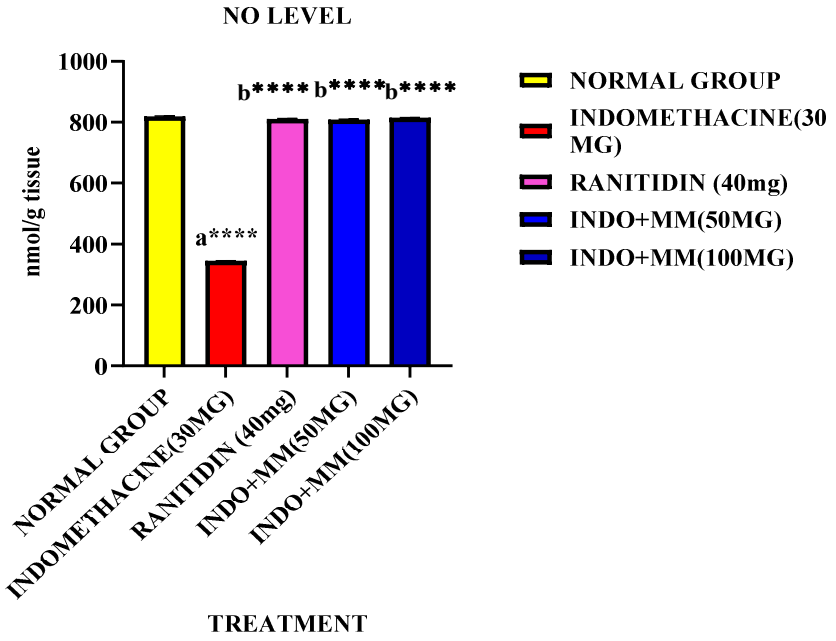


Figure no. 3.4.2 (a) Effect of MM treatment on NO level in INDO-induced peptic ulcer in rats

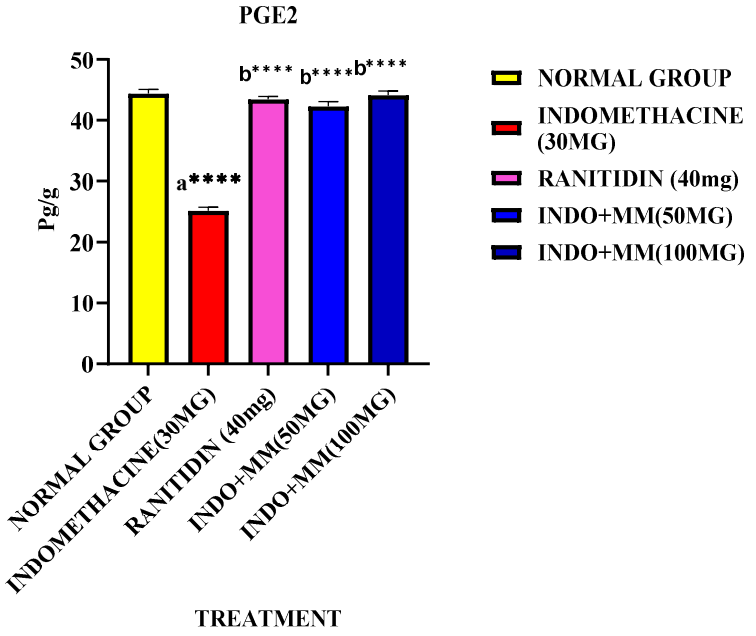


Figure no. 3.4.2 (b) Effect of MM treatment on PEG2 level in INDO-induced peptic ulcer in rats

3.5 Histopathological Analysis

Histopathological analysis revealed that rats treated with indomethacin alone exhibited severe gastric mucosal injury, characterized by exfoliation of the glandular epithelium, submucosal edema, inflammatory cell infiltration, and congestion of blood vessels—hallmarks of peptic ulceration. In contrast, rats pretreated with *Marsilea minuta* (50,100MG/KG) extract showed substantial preservation of gastric mucosal architecture. Only mild disruption of the glandular epithelium and limited submucosal edema were observed, while the muscularis mucosa remained largely intact. These findings indicate that *Marsilea minuta* exhibits protective histopathological activity against NSAID-induced peptic ulcers, likely due to its antioxidant and anti-inflammatory properties.

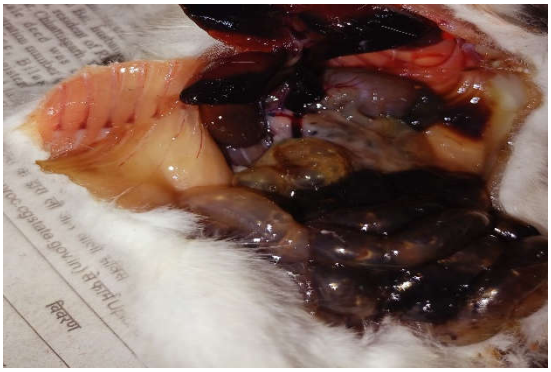
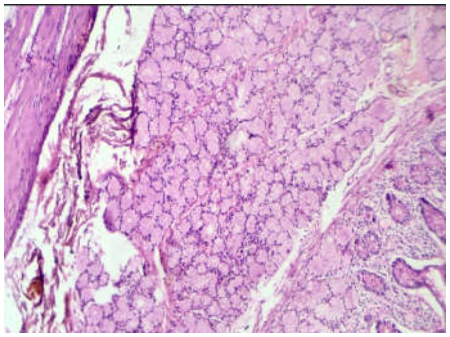
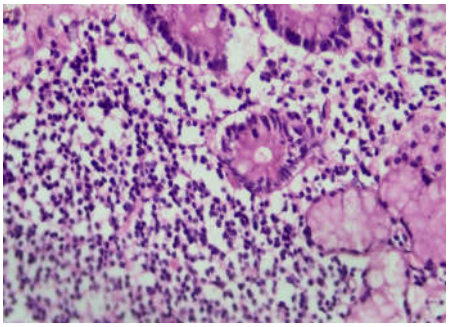


Figure no. 3.5 Collected stomach for Histopathology

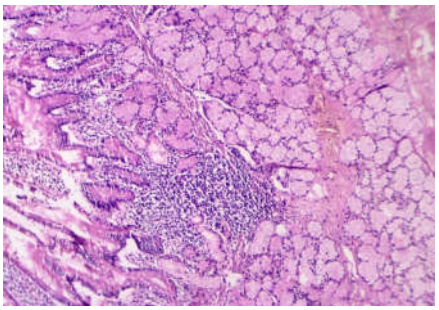
Rat Stomach Images



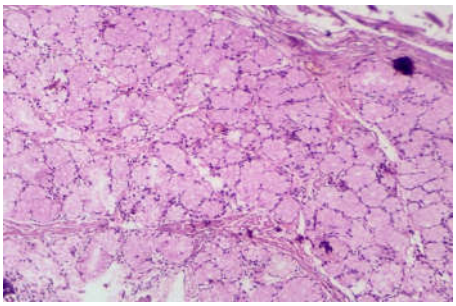
(A) Normal saline



(B) Toxin Indomethacine (30mg/kg)



(C) Indo+mm 50mg/kg (Gastro protective)



(D) Indo+mm 100mg/kg (Gastro protective)

Figure no. 3.5 Histopathological sections of rat stomach stained with hematoxylin and eosin stain (H & E) and histopathological analysis of stomach tissue was examined in control and experimental groups of rats

The normal histology of a rat stomach exhibits intact mucosal architecture without any signs of damage or inflammation. In contrast, the stomach of rats treated with indomethacin shows severe mucosal disruption, ulceration, and dense inflammatory cell infiltration, indicating significant gastric injury. However, rats pretreated with *Marsilea minuta* extract at 50 mg/kg displayed noticeable inflammatory responses in the lamina propria, though the overall tissue structure remained preserved. Notably, the group treated with 100 mg/kg of the extract demonstrated minimal inflammation and near-normal histological features, suggesting strong gastroprotective effects at this higher dose.

4. Conclusions

The present study demonstrated that *Marsilea minuta* extract possesses significant gastroprotective potential against indomethacin-induced peptic ulcers in rat models. Pretreatment with hydro-ethanolic extracts of *M. minuta* effectively reduced the severity of gastric lesions, preserved mucosal integrity, and improved antioxidant enzyme levels. The extract also restored prostaglandin E2 (PGE2) synthesis, which is essential for maintaining gastric mucosal defense. These findings suggest that *M. minuta* exerts its protective effects through multiple mechanisms, including antioxidant activity, anti-inflammatory effects, and enhancement of mucosal protective factors. The higher dose (100 mg/kg) of the extract was more effective, indicating a dose-dependent therapeutic benefit. In conclusion, *Marsilea minuta* shows promising potential as a natural and safe alternative for the prevention and treatment of NSAID-induced gastric ulcers. Further studies, including the isolation of active compounds and clinical trials, are necessary to validate its efficacy and safety for human use.

Acknowledgement

I extend my sincere gratitude to the School of Pharmacy, Chouksey Engineering College, Bilaspur, Chhattisgarh, for their invaluable support and facilities throughout this research. I am deeply thankful to my guide, Mrs. Manorama Ratre, and co-guide, Mrs. Hemlata Rathore, for their expert guidance and constant encouragement.

REFERENCES

[1] *Vimala G, Gricilda Shoba F. A review on antiulcer activity of few indian medicinal plants. Int J Microbiol. 2014;2014.*

[2] *Sajini RJ, Prema S, Chitra K. Phytoconstituents, Pharmacological Activities of Marsilea minuta L. (Marsileaceae)-an Overview. Int J Pharm Sci Res [Internet]. 2019;10(4):1582. Available from: http://dx.doi.org/10.13040/IJPSR.0975-8232.10*

[3] *Farzaei MH, Rahimi R, Abbasabadi Z, Abdollahi M. An evidence-based review on medicinal plants used for the treatment of peptic ulcer in traditional Iranian medicine. Int J Pharmacol. 2013;9(2):108–24.*

[4] *Gupta M, Kapoor B, Gupta R, Singh N. Plants and phytochemicals for treatment of peptic ulcer: An overview. South African J Bot [Internet]. 2021;138:105–14. Available from: https://doi.org/10.1016/j.sajb.2020.11.030*

[5] *Yang L, Rong GC, Wu QN. Diabetic foot ulcer: Challenges and future. World J Diabetes. 2022;13(12):1014–34.*

[6] *Kamau LK. PHYTOCONSTITUENTS , PHARMACOLOGICAL ACTIVITIES OF MARSILEA MINUTA L . (MARSILEACEAE) -AN OVERVIEW. 2019;10(January).*

[7] *Verlag Chemie; 1983. p.273-286. Risk Factors,Diagnosis, and Management of Peptic ulcer Disease. IOSR J Dent Med Sci [Internet]. 2015;14(7):2279–861. Available from: www.iosrjournals.org*

[8] *Prabhu V, Shivani A. An Overview of History , Pathogenesis and Treatment of Perforated Peptic Ulcer Disease with Evaluation of Prognostic Scoring in Adults. 2014;4(1).*

[9] *Ibrahim ME. Epidemiology, pathogenicity, risk factors, and management of Helicobacter pylori infection in Saudi Arabia. Biomol Biomed. 2024;24(3):440–53.*

[10] *Odat RM, Idrees M, Marsool MDM, Oglat SM, Tbayshat SO, Adnan ZI, et al. Stomach and duodenal ulcer as a cause of death in patients with cancer: a cohort study. Int J Emerg Med. 2024;17(1).*
[11] *Jaiswal F, Rai AK, Wal P, Wal A, Singh SP. Peptic Ulcer: a Review on Etiology, Pathogenesis and Treatment. Asian J Pharm Educ Res. 2021;10(4):1.*
[12] *Karsan N, Bose RP, Goadsby PJ. Paranoid psychosis after a single parenteral dose of indomethacin administered for headache diagnosis: A case and review of the literature. Cephalalgia Reports. 2024;7:1–6.*
[13] *In P, Minuta M, Jeya PB, Kumar R. International Journal of Pharma and Bio Sciences ISSN QUALITATIVE AND QUANTITATIVE ANALYSIS OF. 2013;4(1):800–5.*
[14] *Akpamu, Owoyele VB, Ozor M, Osifo UC. Anthonio Research Center © 2013 78 Akpamu et al INDOMETHACIN-INDUCED GASTRIC ULCER: MODEL IN FEMALE WISTAR RATS. Int J Basic, Appl Innov Res IJBAIR [Internet]. 2013;2(4):78–84. Available from: www.arpjournals.com;www.antrescentpub.com*
[15] *Weinhem: Verlag Chemie. Anti-ulcer effect of Aloe vera in non-steroidal anti-inflammatory drug induced peptic ulcers in rats. African J Pharm Pharmacol. 2011;5(16):1867–71.*