Pharmacological ev Olifera Lam

Pharmacological evaluation of antiulcer activity of *Moringa Olifera Lam root extract* for antiulcer activity

Sunil Kumar¹, Payal Rani²*, Kirteebala P Pawar³, R.Hari Babu⁴, Nilesh O. Chachda⁵, Richa Dayaramani⁶, Areeg Anwer Ali⁷, Abhijit M Kanavaje⁸

¹Faculty of Pharmacy, P. K. University, Village. Thanra, District. Karera, Shivpuri, M.P., India
 ²Seth GL Bihani SD College of Tech. Edu., Sriganganagar, Rajasthan, India
 ³D Y Patil School of Pharmacy, D Y Patil Deemed To Be University, Navi Mumbai.
 ^{4,8}M B School of Pharmaceutical Sciences, Mohan Babu University, A. Rangampeta, Tirupati, A.P, India
 ⁵Shri Chhatrapati Shahu Maharaj Shikshan Sanstha's Institute of Pharmacy, Maregaon, Yavatmal, Maharashtra, India
 ⁶Silveroak Institute of Pharmacy and research, Ahmdebad, India
 ⁷RAK College of Pharmacy, RAK Medical & Health Sciences University, Ras Al Khaimah, UAE Oriental College of Pharmacy, Sanapada, University of Mumbai, Thane, Maharashtra, India

sunilsinghpharmacy@gmail.com

Corresponding Author: Payal Rani*

palamitchawla@gmail.com

Abstract

The current study aimed to evaluate the potential of ethanolic and aqueous root extracts of *Moringa oleifera* Lam, a member of the Moringaceae family, as agents with antisecretory and antiulcer properties. Through oral administration at doses of 100, 200, and 400 mg/kg, the extracts were assessed for their effectiveness against ulcers induced by ethanol and cold stress, both considered toxicants. Notably, both extracts exhibited notable protection against ulcers, particularly at the highest dosage of 400 mg/kg, surpassing the standard reference.

To substantiate its antiulcer effects, the plant's free radical scavenging ability was probed via lipid peroxidation assays. The extracts demonstrated considerable antioxidant capacity, which could be attributed to the presence of flavonoids and polyphenols in the extracts. These compounds are recognized for their ability to neutralize free radicals and prevent oxidative damage.

In conclusion, this study reveals the significant gastric ulcer protective potential of both ethanolic (EE) and aqueous (AE) root extracts of *Moringa oleifera* Lam (MO). The findings underscore the plant's valuable antisecretory and antiulcer attributes. The observed antioxidant activity lends further support to its potential therapeutic application. However, future research to decipher the precise mechanisms involved and to conduct

clinical trials is warranted for a more comprehensive understanding and validation of its gastroprotective properties.

Keywords: Moringa Olifera, Moringceae.; Antiulcer, Ethanol, stress

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Introduction

Peptic ulcers arise from the imbalance between aggressive factors (such as acid, pepsin, and Helicobacter pylori) and protective elements (like mucin, prostaglandin, bicarbonate, and nitric oxide). Contemporary lifestyles, existing health conditions, and heightened anxiety also play a contributory role in the genesis of ulcers. Stress, in particular, significantly contributes to ulcer development, causing mucosal damage and ischemia, a pattern observed more frequently than the excessive gastric acid secretion seen in cancer patients (1,2). The control of the microcirculation within the gastric mucosa, which plays a crucial role in upholding gastric well-being, has been closely associated with endogenous nitric oxide (NO) function. Furthermore, the preservation of mucosal integrity is intrinsically linked to reduced glutathione (GSH). Depletion of GSH from the gastric mucosa by electrophilic substances has been demonstrated to lead to visible mucosal ulceration (3,4). Peptic ulcer management focuses on mitigating aggressive factors or bolstering mucosal defense in the stomach and duodenum through the use of cytoprotective agents. However, the existing allopathic treatments for peptic ulcers remain suboptimal, as they can alleviate morbidity and mortality but often lead to undesired effects such as arrhythmias, impotence, gynaecomastia, and hematopoietic alterations (5,6). Moreover, recurrence rates remain elevated, while *Moringa oleifera* has been documented to exhibit hepatoprotective, antidiabetic, and antioxidant properties across diverse experimental models (7). In traditional Ayurvedic literature Moringa oleifera is reported to posses' antiulcer activity. Since gastric and duodenal ulcers are inner wounds, we have considered the antiulcer potential of this plant on different models of gastric ulceration.

2.0 Materials and methods

2.1. Plant material

The roots of *Moringa oleifera* was collected locally. The specimens were authenticated by renowned botanist and the voucher specimen is deposited for prospect reference. The shed dried root powder was subjected to exhaustive uninterrupted hot extraction in Soxhlet apparatus using ethanol and water. The chemical constituents of the both extracts

were identified by qualitative analysis (8). The obtained masses were dried and stored in an air tight container in cold environment for further use.

2.2. Experimental animals-

Albino rats of either sex weighing between 150-200 gm were selected for the study. The experimental protocol was approved by Institutional Animal Ethics Committee and animal were maintained under standard condition. They were allowed free access to standard dry pellet diet and water *ad libitum* under strict hygienic conditions.

2.3. Toxicity study

Acute toxicity study of both extracts of the *Moringa oleifera* was carried out for determination of LD₅₀ by using OECD guidelines (9). The female albino mice of 20-30 g were used for the study. The animals were continuously observed 12 h to detect changes in behavioral responses. Mortality was observed for 24 hours. The doses of 100, 200 and 400 mg/kg, p.o. were selected based on the results.

2.4. Ethanol-induced acute gastric ulcers (10)

Thirty-six rats were subjected to an 18-hour food deprivation period during which access to water was unrestricted. Subsequently, the rats were randomly divided into six distinct treatment groups, namely: Normal control, Toxicant, and low, medium, and high dosage categories for both ethanol and aqueous extracts. All rats were administered a 70% ethanol solution orally at a volume of 0.5 ml. In the case of the toxicant group, only ethanol was administered. Following a one-hour interval, all animals were humanely euthanized using ether anesthesia. Subsequently, their stomachs were extracted and the number of ulcers present was evaluated utilizing a subjective 0-3 point scale. The cumulative score of ulcers in each stomach contributed to its corresponding ulceration index.

2.5. Cold stress-induced acute gastric ulcers (11)

In this experimental procedure, male Wistar rats weighing between 150-200 g, of either sex, were subjected to a 24-hour fasting period while being provided unrestricted access to water. All experimental groups were subjected to a 7-day regimen of herbal drug treatment. On the seventh day, the rats, which had been fasting overnight, were confined within a metallic restraint chamber. Thirty minutes after administering the test drug, they were placed in a refrigerator set at a temperature of 4-6°C for duration of 2 hours. Following the immobilization period, the rats were euthanized through cervical dislocation, and their stomachs were extracted for the purpose of ulcer assessment.

Ulceration within the stomach was quantified using a subjective 0-3 scale. The cumulative score of ulcers present in each stomach contributed to its respective ulceration index.

2.6 Method for estimation of lipid peroxidation (12)

Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS), using malondialdehyde (MDA) as standard by the method of Buege and Aust. 1.0 ml of the sample extract was added with 2.0 ml of the TCA- TBA- HCl reagent (15% w/v TCA, 0.375% w/v TBA and 0.25 N HCl). The contents were boiled for 15 minutes, cooled and centrifuged at 10000 rpm to remove the precipitate. The absorbance was read at 535 nm and malondialdehyde concentration of the sample was calculated using extinction coefficient of 1.56×10^5 M⁻¹ cm⁻¹.

3. Results

3.1 Effect of EE and AE extracts of *MO roots* on ethanol-induced gastric ulcers:

Pretreatment of rats with either EE and AE extract of *MO* roots produced a dose dependent protection from ethanol induced ulceration, as compared to control animals. However, the protection was statistically significant at higher dose 400mg/kg. Lansoprazole (8mg/kg) produced significant gastric ulcer protection as compared to control group. (Table 1).

3.2 Effect of EE and AE extracts of *MO roots* **on Cold stress induced gastric ulcers:** Pretreatment of rats with either EE and AE extract produced a dose dependent protection from the cold stress-induced ulceration, as compared to control animals. The protection was statistically significant at 400 mg/kg dose. Lansoprazole (8mg/kg) produced significant protection as compared to control group (Table 2).

4. Discussion

The rapid modernization of lifestyles has ensnared the public in an almost ceaseless state of stress. Coupled with escalating environmental pollution and a lack of basic hygiene awareness among the general population, a fertile ground has been created for the emergence of a multitude of organ-related complications. The gastrointestinal tract (GIT), particularly the stomach and proximal intestine, stands as the principle target for these complications. Regrettably, despite remarkable advancements in modern medicine, the treatment of certain gastrointestinal ailments, such as ulcers, often falls short of clinical expectations. While synthetic drugs might provide temporary symptomatic relief, they are not immune to unfavorable incidents, prompting clinicians to discontinue such therapies. Hence, there is an imperative need to explore alternative therapies, particularly those derived from herbal sources. This aligns with the growing global trend towards embracing herbal treatments, driven by the belief in their safety and efficacy (13-17)

Stress triggers an ischemic state within the gastric mucosa by stimulating both the parasympathetic and sympathetic nervous systems. This stimulation leads to vasoconstriction, which subsequently instigates the production of free radicals (18). The findings from the present study indicate that, within the stress-induced models, the administration of either ethanolic extract (EE) or aqueous extract (AE) derived from the roots of Moringa oleifera led to a noteworthy reduction in ulcer index when compared to the control group. This observed effect is attributed to the inherent antioxidant capabilities of these extracts. The antioxidant potential can be attributed to the presence of various phytoconstituents, such as flavonoids and tannins, which have been extensively documented for their antioxidant properties (19). When introduced intragastrically in rats, narcotizing agents like ethanol induce pronounced gastric erosions. The development of gastric lesions brought about by ethanol is a complex process, involving multiple factors. One of the contributing factors is the reduction in the mucus content within the gastric walls. The damage caused by ethanol is thought to trigger the release of mucosal leukotrienes, which could play a role in the pathogenesis of these lesions (20). The formation of gastric lesions induced by ethanol could be attributed to a potential stagnation in gastric blood flow. This stasis in blood circulation might play a role in the initiation of hemorrhage and the subsequent development of necrotic tissue injury (21-64). Ethanol treatment caused significant increase in the ulcer index whereas pretreatment with EE or AE extract of MO roots showed significant inhibition (p<0.01) in ethanol induced gastric damage. The antiulcerogenic activities of Moringa oleifera Lam roots also involve its antioxidant effect apart from its effects on other defensive factors.

5. Conclusion

The gastroprotective and ulcer-healing effects of ethanolic and aqueous extracts from the roots of *Moringa oleifera* Lam are attributed to their influence on both aggressive and protective factors within the gastric environment. Notably, the extracts' antioxidant properties play a pivotal role in their antiulcer activity. Furthermore, investigating additional mucosal factors, such as nitric oxide, prostaglandins, and cAMP, could offer deeper insights into their mechanisms of action. As such, it can be inferred that *Moringa*

oleifera Lam root extracts hold promise as effective antiulcer agents. Considering these findings, it is pertinent to contemplate their clinical application for patients dealing with gastric ulcer conditions, with the intention of conducting further comprehensive evaluations.

6. References

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Table 1. Effect of EE extract of the Roots of *MO* against alcohol-induced gastric ulcer in rats

Group and dose	Ulcer	Ulcer	%Ulcer
	positive	index	protecti
	animals		on
Control (2% w/v gum	6	7.427±0.	-
acacia)		04	
Lansoprazole (8	1	1.800±0.	76.67
mg/kg)		04**	
Low dose EE	4	5.417±0.	22.45
(100mg/kg)		15**	
Medium dose EE	3	4.227±0.	40.55
(200mg/kg)		02**	
High dose EE	2	3.214±0.	61.01
(400mg/kg)		10**	
Low dose AE	4	5.217±0.	23.88
(100mg/kg)		15**	
Medium dose AE	3	4.227±0.	43.82
(200mg/kg)		06**	
High dose AE	2	3.727±0.	63.02
(400mg/kg)		10**	

Table 2. Effect of EE extract of the Roots of MO against cold stress inducedgastric ulcers in rats.

Group and dose	Ulcer positive animals	Ulcer index	%Ulcer protection
Control (2% w/v gum acacia)	6	11.43±0.14	-
Lansoprazole (8 mg/kg)	1	1.447±0.03**	81.00
Low dose EE (100mg/kg)	5	8.396 ± 0.07**	21.00
Medium dose EE (200mg/kg)	4	6.10 ± 0.16**	38.00
High dose EE (400mg/kg)	3	5.223 ± 0.06**	56.17
Low dose AE (100mg/kg)	4	7.33 ± 0.15**	37.00
Medium dose AE (200mg/kg)	3	5.23± 0.07**	58.00
High dose AE (400mg/kg)	2	3.22 ± 0.07**	70.00

Pharmacological evaluation of antiulcer activity of Moringa Olifera Lam root extract for antiulcer activity Section A-

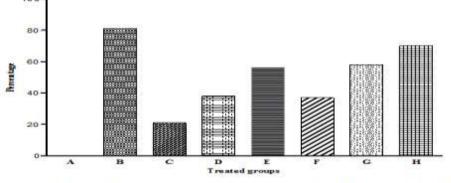
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Table 3. Lipid Peroxidation Estimation in treated groups in ethanol induced ulcer model in rats

Sl. No.	Treatment	Malondialdehyde (MDA)
		(nm/g gastric tissue)
A.	Normal control	24.03 ± 0.32
B.	Control	44.44 ± 0.50
C.	Lansoprazole	27.34 ± 0.48**
D.	Low dose EE (100mg/kg)	36.38±0.91**
E.	Medium dose EE (200mg/kg)	33.59 ± 1.43**
F.	High dose EE (400mg/kg)	29.22±1.39**
G.	Low dose AE (100mg/kg)	38.04±1.69**
H.	Medium dose AE (200mg/kg)	31.04±0.75**
I.	High dose AE (400mg/kg)	29.17±1.07**

** P < 0.01 when compared with control





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COLORE B	Lans oprazo le
00008	Low EE
-	Med EE
-	High EE
in	Low AE
1000	Med AE
ш	HighAE

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Fig 02: Percentage Ulcer protection in cold stress induced ulcer model

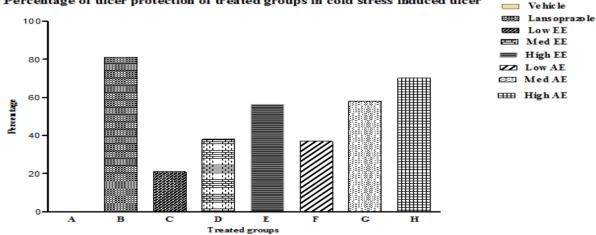




Fig 02: Percentage Ulcer protection in cold stress induced ulcer model

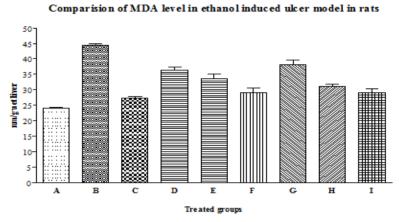


Fig 03: Comparison of MDA levels in ethanol induced ulcer model in rats.

Ethanol induced ulcer model



Normal Control







Lansoprazole

Cold stress induced ulcer model



Normal Control



Control



Lansoprazole

Pharmacological evaluation of antiulcer activity of Moringa Olifera Lam root extract for antiulcer activity Section A-Research paper



High dose of EE



High dose of AE