Antiulcer Exploration of some Bioactive Indian Plants through Ethanol-Induced Gastric Ulcer Model

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Abstract: This investigation assessed the gastroprotective effects of extracts from five traditional Indian plants—Moringa oleifera (leaves), Plumbago zeylanica (roots), Amaranthus tricolor (leaves), Elephantopus scaber (whole plant), and Cassia tora (leaves)—using an ethanol-based gastric ulcer model in rats. The experimental setup included nine groups: a vehicle-treated normal group, an untreated ulcer group, a reference group receiving omeprazole (20 mg/kg), groups treated with individual plant extracts (200 mg/kg each), and a group given a combined polyherbal extract (200 mg/kg per extract). Ulcer severity was quantified via ulcer index scores, and protective efficacy was determined by percentage reduction in ulceration. The polyherbal mixture displayed the strongest protection, with an ulcer index of 2.01 ± 0.17 and 86.15% inhibition, exceeding omeprazole's 83.89%. Analysis of oxidative stress indicators—such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), lipid peroxidation (LPO), malondialdehyde (MDA), and total protein (TP)—revealed marked normalization in the polyherbal group, highlighting reduced oxidative damage and bolstered mucosal integrity. Histological examinations supported these observations, showing decreased epithelial injury, limited inflammation, and promoted tissue recovery across treated cohorts. These outcomes underscore the robust antiulcer capabilities of both single and combined extracts, driven by their antioxidative, barrier-strengthening, and reparative attributes. The data endorse polyherbal approaches for ulcer therapy and call for advanced research, including bioactive isolation, pathway elucidation, and human trials to address peptic ulcer conditions.

Keywords: Gastroprotection, Ethanol gastric ulceration, Multi-plant formulation, Plant-derived remedies, Oxidative markers, Tissue microscopy

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INTRODUCTION

Gastric ulcers, a key component of peptic ulcer disease (PUD), involve erosive damage to the stomach lining or proximal duodenum lining, stemming from an excess of harmful agents (like acid, digestive enzymes, bacterial infections, or pain relievers) over protective elements (such as mucus layers, alkaline secretions, lipid mediators, vasodilators, and free radical neutralizers). Worldwide, PUD affects millions, leading to frequent relapses, severe outcomes, and growing tolerance to standard treatments. Although medications like acid suppressants

(e.g., omeprazole) and histamine blockers offer initial benefits, prolonged application can trigger side effects, relapse risks, and incomplete tissue restoration.

In response, natural plant remedies have gained traction for their low toxicity, cost-effectiveness, cultural acceptance, and abundance of protective phytochemicals. Numerous plants in Indian traditional systems, like Ayurveda, have long been applied for digestive issues but require rigorous scientific scrutiny. This work explores the ulcer-preventing qualities of five promising Indian species: Moringa oleifera (foliage), Plumbago zeylanica (root), Amaranthus tricolor (foliage), Elephantopus scaber (entire herb), and Cassia tora (foliage). Chosen for their folkloric use and documented radical-scavenging, swelling-reducing, or cell-shielding traits, these were tested individually and together in a rat model of ethanol-triggered ulcers. Evaluations focused on lesion scores, stress-related biomarkers, and microscopic tissue changes to uncover underlying safeguards.

MATERIALS AND METHODS

Materials

Reagents for this study met analytical standards and were sourced from trusted vendors. Absolute ethanol (99.9%) for lesion induction came from Merck Life Science Pvt. Ltd. (Mumbai, India). The suspension vehicle, carboxymethyl cellulose (1%), was from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Reference omeprazole was acquired from Sigma-Aldrich (USA). Kits for SOD, CAT, GSH, LPO, MDA, and TP assays were from Erba Diagnostics and Sisco Research Laboratories (India). Histology supplies, including H&E dyes, xylene, and formalin, were obtained from Merck and Loba Chemie (Mumbai, India).

Experimental Procedures

- **Animal Subjects**

Adult Wistar rats (150–200 g, mixed genders) were procured from a certified CPCSEA facility at [Institute Name]. Post-arrival, they underwent health checks and a 7-day adaptation phase under monitored conditions: 22 ± 2°C, 60 ± 5% humidity, 12:12 h light cycle, housed in groups of up to three in sanitized polypropylene units with fresh bedding. Standard pelleted feed and purified water were available unrestricted. Protocols adhered to IAEC guidelines at [Institute Name] (Approval: [Number]).

- **Safety Assessment**

Oral acute toxicity for each extract followed OECD 423 protocols. Rats (n=3/group) fasted overnight received escalating doses (300 and 2000 mg/kg) in 1% CMC. Monitoring spanned

4 h acutely and 14 days for delayed effects, including behavior, intake, weight, and survival. No adverse reactions or fatalities occurred up to 2000 mg/kg, classifying extracts as GHS Category 5 (LD50 > 2000 mg/kg). Subsequent tests used 200 mg/kg (1/10th MTD) and 400 mg/kg (1/5th MTD).

- **Study Layout**

Fifty-four rats formed nine groups (n=6):

- Group I: Vehicle (1% CMC)
- Group II: Ethanol alone
- Group III: Omeprazole (20 mg/kg, oral)
- Group IV: P. zeylanica (200 mg/kg, oral)
- Group V: M. oleifera (200 mg/kg, oral)
- Group VI: C. tora (200 mg/kg, oral)
- Group VII: A. tricolor (200 mg/kg, oral)
- Group VIII: E. scaber (200 mg/kg, oral)
- Group IX: All extracts combined (200 mg/kg each, oral)

- **Ulcer Induction Protocol**

This model replicates acute mucosal harm from irritants, involving barrier breakdown, permeability rise, cell sloughing, bleeding, and oxidant buildup. Rats fasted 24 h (water ad libitum) received treatments 30 min pre-induction. Ethanol (1 mL/200 g) was given orally to Groups II–IX. After 1 h, rats were anesthetized and sacrificed; stomachs were harvested, rinsed in chilled saline, and inspected.

Gross Lesion Scoring: Mucosa was viewed at 10× magnification. Scores: 0 (intact), 0.5 (erythema), 1 (petechiae), 1.5 (streaks), 2 (non-perforating ulcers), 3 (perforations). Ulcer index = (ulcer count + score sum + ulcer incidence %)/100.

Biomarker Assays: On day 22, post-fasting blood was drawn via retro-orbital route under ketamine/xylazine anesthesia (1.5–2 mL/rat), clotted, centrifuged (3000 rpm, 15 min, 4°C), and serum stored at −20°C.

- **SOD Assay**

Per Marklund and Marklund (1974), homogenates in 0.1 M phosphate buffer (pH 7.4) were centrifuged (10,000 rpm, 15 min, 4°C). Mixtures with Tris-HCl (50 mM, pH 8.2), DETAPAC (1 mM), and pyrogallol (0.2 mM) were read at 420 nm. One unit = 50% autoxidation inhibition; reported as U/mg protein.

- **CAT Assay**

Via Aebi (1984), homogenates in 0.1 M phosphate buffer (pH 7.0) reacted with 30 mM H2O2; absorbance drop at 240 nm (1 min) used ε = 43.6 M^{-1} cm^{-1}. One unit = 1 µmol H2O2/min; U/min/mg protein.

- **GSH Assay**

Ellman (1959): Homogenates precipitated in 10% TCA, supernatant + DTNB (pH 8.0) read at 412 nm vs. GSH standards; µmol/mg protein.

- **LPO/MDA Assay**

TBARS method: Homogenates + TCA/TBA heated (95°C, 30 min), read at 532 nm vs. tetramethoxypropane; nmol MDA/mg protein.

- **TP Assay**

Bradford (1976): Supernatant + Coomassie dye read at 595 nm vs. BSA; mg/mL.

Assays used spectrophotometry per kit instructions.

Microscopic Examination: Post-sacrifice, stomachs were fixed in 10% formalin (24–48 h), dehydrated in graded ethanol, cleared in xylene, embedded in paraffin, sectioned (5 µm), and stained with H&E for light microscopy.

Data Handling

Triplicate runs yielded mean ± SD. One-way ANOVA ($p < 0.05$) with Tukey's post-hoc used GraphPad Prism 9.0 or SPSS 25.

RESULTS AND DISCUSSION

Safety Profile

At 300 and 2000 mg/kg, no clinical signs (e.g., motor issues, secretions, seizures), intake changes, or losses occurred over 14 days, affirming LD50 > 2000 mg/kg and safety for 200/400 mg/kg dosing. This aligns with prior safety data for these plants, attributing tolerance to balanced bioactives despite isolates like plumbagin.

Table 1. Acute toxicity summary for plant extracts.

Plant Name	Doses (mg/kg)	Toxicity Signs	Mortality	Study Doses (mg/kg)
Plumbago zeylanica	300, 2000	None	0/3	200, 400
Moringa oleifera	300, 2000	None	0/3	200, 400
Cassia tora	300, 2000	None	0/3	200, 400
Amaranthus tricolor	300, 2000	None	0/3	200, 400
Elephantopus scaber	300, 2000	None	0/3	200, 400

Ulcer Protection

Normal rats (Group I) had zero lesions (UI = 0.00 ± 0.00). Ethanol alone (Group II) caused extensive harm (UI = 14.52 ± 0.33, 0% inhibition). Omeprazole (Group III) lowered UI to 2.34 ± 0.19 (83.89% inhibition), validating its acid-suppressing role.

Individual extracts reduced UI by 55–60%: P. zeylanica (5.87 ± 0.28, 59.57%), M. oleifera (6.12 ± 0.31, 57.86%), C. tora (6.48 ± 0.30, 55.39%), A. tricolor (6.05 ± 0.27, 58.35%), E. scaber (5.76 ± 0.25, 60.33%). The blend (Group IX) achieved UI = 2.01 ± 0.17 (86.15% inhibition), implying additive phytochemistry for enhanced barrier support and oxidant control.

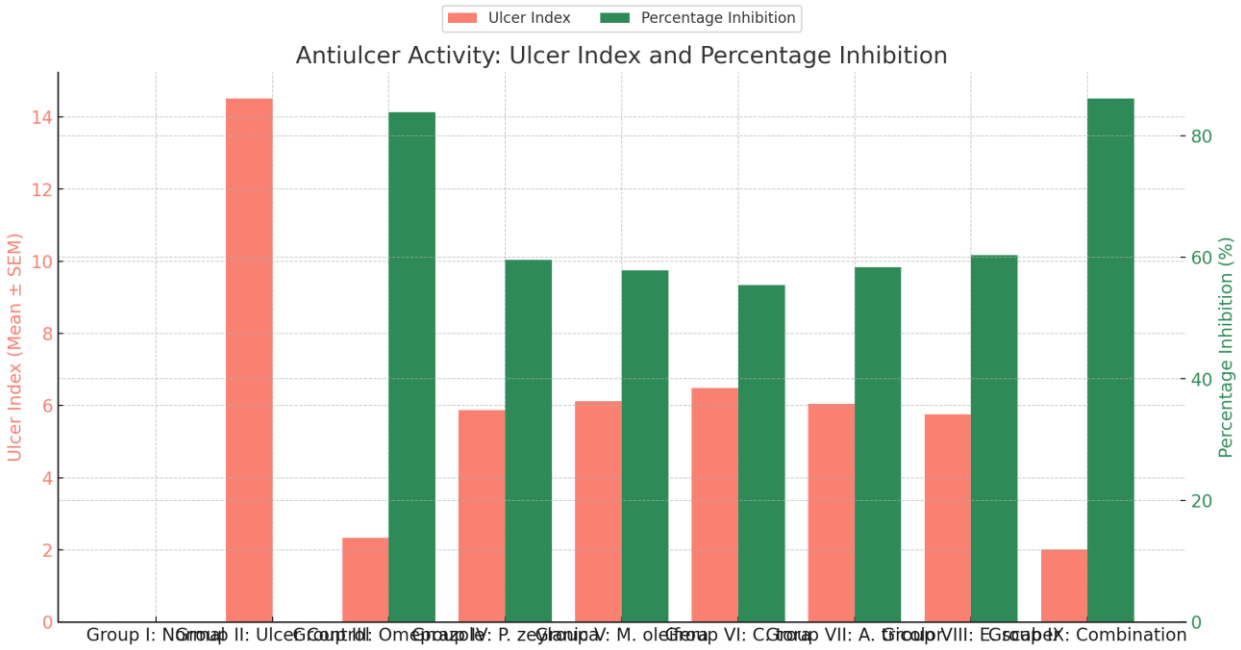

Figure 1. Comparative ulcer protection by extracts.

Table 2. Ulcer index and inhibition rates.

Group	Ulcer Index (Mean ± SEM)	Inhibition (%)
I: Normal	0.00 ± 0.00	–
II: Ulcer	14.52 ± 0.33	0
III: Omeprazole	2.34 ± 0.19	83.89
IV: P. zeylanica	5.87 ± 0.28	59.57
V: M. oleifera	6.12 ± 0.31	57.86
VI: C. tora	6.48 ± 0.30	55.39
VII: A. tricolor	6.05 ± 0.27	58.35
VIII: E. scaber	5.76 ± 0.25	60.33
IX: Combination	2.01 ± 0.17	86.15

Oxidative Markers

SOD fell in ulcers (2.34 ± 0.38 U/mg) from normal (8.50 ± 0.45); treatments restored it, peaking in blend (7.92 ± 0.43). CAT dropped to 6.92 ± 0.67 U/mg in ulcers vs. 22.51 ± 1.55

normal; blend neared baseline (21.50 ± 1.15). GSH declined to 1.92 ± 0.30 µmol/g; blend raised it to 4.50 ± 0.42. LPO/MDA surged in ulcers (1.45 ± 0.10 µmol/g, 7.05 ± 0.55 nmol/g); blend curbed to 0.49 ± 0.05 and 2.20 ± 0.22. TP fell to 4.50 ± 0.45 mg/g; blend recovered to 9.30 ± 0.80. E. scaber and A. tricolor excelled singly, with blend synergy evident.

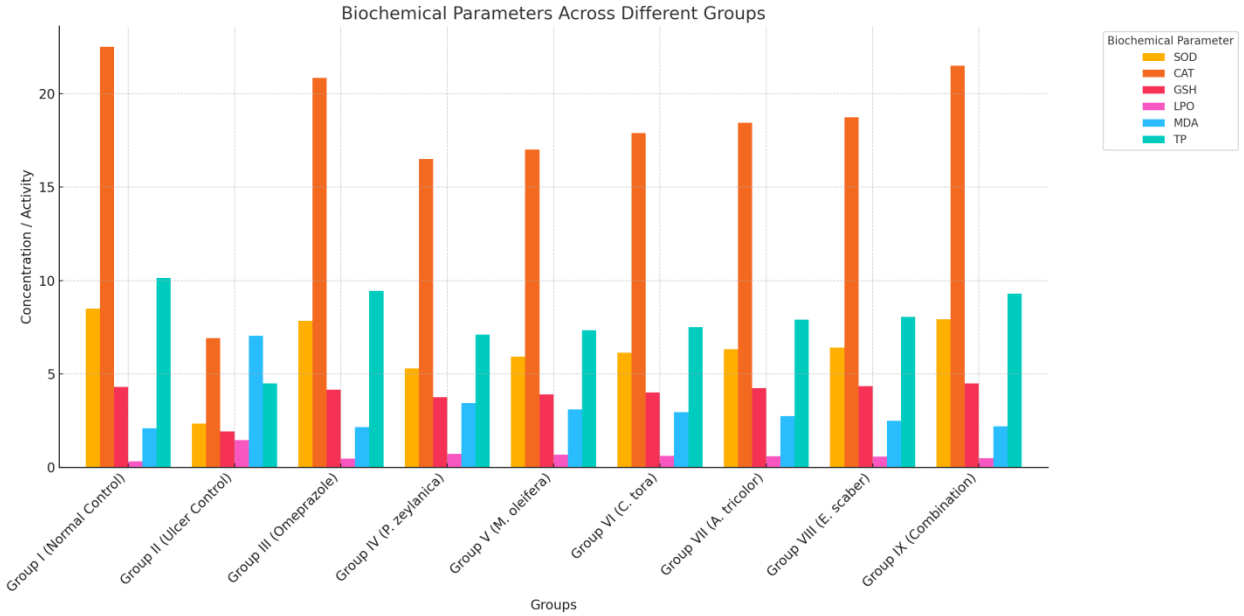

Figure 2. Oxidative biomarker profiles.

Table 3. Marker levels across groups.

Parameter	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**
SOD (U/mg)	8.50± 0.45	2.34± 0.38	7.85± 0.50	5.29± 0.42	5.92± 0.41	6.14± 0.39	6.33± 0.40	6.41± 0.38	7.92± 0.43
CAT (U/mg)	22.51 ±1.55	6.92± 0.67	20.85 ±1.12	16.50 ±1.05	17.02 ±1.03	17.90 ±1.06	18.45 ±1.08	18.75 ±1.10	21.50 ±1.15
GSH (µmol/g)	4.30± 0.38	1.92± 0.30	4.15± 0.40	3.75± 0.36	3.90± 0.38	4.00± 0.39	4.25± 0.40	4.35± 0.41	4.50± 0.42

LPO (µmol /g)	0.32± 0.05	1.45± 0.10	0.48± 0.06	0.72± 0.08	0.68± 0.07	0.62± 0.06	0.60± 0.05	0.58± 0.04	0.49± 0.05
MDA (nmol /g)	2.10± 0.20	7.05± 0.55	2.15± 0.25	3.45± 0.30	3.10± 0.27	2.95± 0.28	2.75± 0.29	2.50± 0.26	2.20± 0.22
TP (mg/g)	10.15 ±0.85	4.50± 0.45	9.45± 0.82	7.10± 0.68	7.35± 0.70	7.50± 0.72	7.90± 0.75	8.05± 0.77	9.30± 0.80

Tissue Histology

Normal mucosa (Group I) showed orderly epithelium and glands. Ulcers (Group II) featured necrosis, edema, hemorrhage, and leukocyte influx. Omeprazole (Group III) restored near-normalcy with mild residuals. Individuals varied: P. zeylanica/M. oleifera/C. tora/A. tricolor offered partial repair; E. scaber neared full recovery. Blend (Group IX) mirrored normals, with intact layers and no inflammation, indicating cooperative repair.

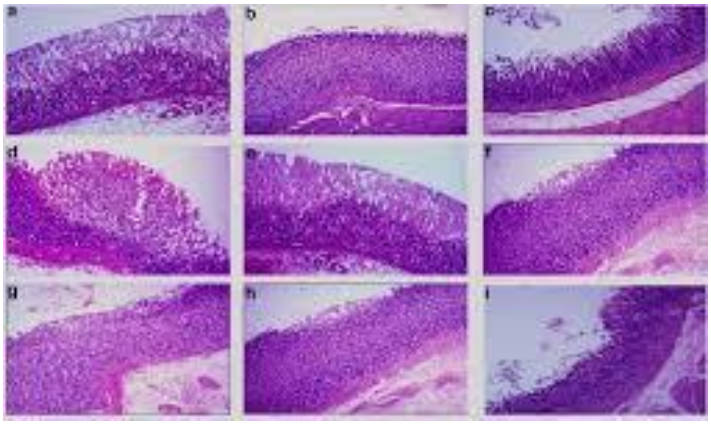

Figure 3. Gastric histology: (A) Normal; (B) Ulcer; (C) Omeprazole; (D–H) Individuals; (I) Blend.

CONCLUSION

Extracts from Moringa oleifera, Plumbago zeylanica, Amaranthus tricolor, Elephantopus scaber, and Cassia tora demonstrated strong antiulcer effects in ethanol-challenged rats, with E. scaber leading singles and the blend surpassing omeprazole (UI 2.01 ± 0.17, 86.15% inhibition). Markers normalized, peroxidation dropped, and proteins rose, signaling oxidant mitigation and repair. Microscopy confirmed integrity gains, especially synergistically.

Polyphenol/flavonoid/tannin/saponin synergies drive this, validating natural ulcer aids and urging compound purification, mechanism probes, and trials.

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