EVALUATION OF POLYHERBAL FORMULATION IN EXPERIMENTALLY INDUCED GASTRIC ULCERS IN RATS

Narendra R. Dighade [1], Shubhangee Tomar [2], Krupa Mansukhbhai Badava [3], Gracerity Nongrum [4], Alka Mishr [5], Farhad F Mehta [6], Sachin Dhull [7], Murali Krishna Kandukuri [8]*

1. *Professor, Nagpur College of Pharmacy, Wanadongri, Hingna road, Nagpur, Maharashtra, Pin Code: 441110*
2. *Assistant Professor, KB Institute of Pharmaceutical Education and Research, KSV, Gandhinagar, Gh 6 Circle, Gujarat, Pin Code: 382024*
3. *Assistant Professor, Parul Institute of Pharmacy and Research, Parul University, P.O. Limda, Tal. Waghodia, Vadodara, Gujarat, Pin Code: 391760*
4. *Assistant Professor, University of Science and Technology, Techno City, Kiling Road, Baridua, 9th Mile, Ri-Bhoi, Meghalaya, Pin Code:793101*
5. *Associate Professor, Goel institute of pharmaceutical sciences, Indira canal near Faizabad road, Haryana, Pin Code:226028*
6. *Assistant Professor, School of Pharmaceutical Sciences, UTD, RGPV University, Bhopal, Madhya Pradesh, Pin Code: 462033*
7. *Assistant Professor, Deen Dayal Rustagi College of Pharmacy, Khandewala, Gurgaon, Haryana, Pin Code: 122504*
8. *Assistant Professor, College of Pharmacy, Sathyabama Institute of Science and Technology, Chennai, Tamilnadu, Pin Code: 600119*

Corresponding Author: Dr. Murali Krishna Kandukuri
Designation and Affiliation: Assistant Professor, College of Pharmacy, Sathyabama Institute of Science and Technology, Chennai, Tamilnadu, Pin Code: 600119
Email Id: muralikrishna.kandukuri.pharmacy@sathyabama.ac.in

KEYWORDS

Polyherbal preparation, Peptic ulcer, Anti-ulcer activity, Pylorus ligation

ABSTRACT

A painful open break or rupture on the skin or mucous membrane that prevents the organ from performing its normal duties is called an ulcer. Due to the adverse effects of allopathic medications, ayurvedic preparations are in the lead. Previous research that shown that individual plants have antiulcer efficacy but that there was no polyherbal combination of them were used to construct this polyherbal combination. Thus, the goal of the current investigation was to assess the anti-ulcer effectiveness of a polyherbal preparation employing Wistar albino rats against stomach ulcers caused by ethanol and pylorus ligation. Rats were given polyherbal extract at 200 mg/kg and 400 mg/kg body weight, and the results were contrasted with those of omeprazole (10 mg/kg), a common medication. When compared to the disease control group animal, the extract significantly decreased the ulcer index at doses of 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.01$) in the ethanol-induced ulcer model and 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.05$) in the pylorus ligation model. Reduced gastric juice volume, ulcer protection percentage, ulcer index, and increased free acidity are indicators of antiulcer action. Due to the antioxidant, antisecretory, and cytoprotective properties of these plants, this polyherbal mixture exhibits antiulcer activity. We may therefore draw the conclusion that the polyherbal formulation exhibits strong antiulcer properties.

INTRODUCTION

Acid-related health issues are a substantial contributor to patient morbidity and mortality and primarily impact quality of life. The most prevalent gastrointestinal conditions include peptic

ulcers, gastro-esophageal reflux disease, and heartburn. 10% of people will experience an ulcer at some point in their lives, making it one of the most prevalent gastrointestinal health problems in the globe. Acid and pepsin cause the gastrointestinal mucosal lining to rupture, which is a hallmark of peptic ulcer, a disease of the gastrointestinal system. Particularly on the upper gastrointestinal tract, this rupture of the mucosal lining results in lesions. An ulcer is characterised by inflammation-induced rupture of the epithelial lining that is larger than 5 mm in diameter. Acid-pepsin secretion, prostaglandins and development factors, bloodstream, mucous secretion, and other defensive and offensive elements are all out of balance, which is the main cause of peptic ulcers. [1,2] Peptic ulcers can be caused by a wide range of additional factors, including a lifetime of stress, alcohol usage, smoking, the use of NSAIDS (steroidal and non-steroidal anti-inflammatory medicines), Helicobacter pylori infection, and more.

Triple therapy using H2 receptor blockers, proton pump inhibitors, and antimicrobial agents is now used to treat peptic ulcers. These medications frequently have unfavourable side effects. [2, 3] Many traditional medical systems around the world use plants and herbs to treat various illnesses in accordance with their cultural origins and philosophy [4, 5]. The plants Maricha (Piper nigrum, Piperaceae), Haritaki (Terminalia chebula, Combretaceae), and Tulsi (Ocimum sanctum, Lamiaceae) were previously known to exhibit antiulcer action. In order to demonstrate improved antiulcer efficacy, an attempt was made in the current study to create a polyherbal plant preparation using the leaves. [6-9]

MATERIAL AND METHODS:

Collection of plant material: The plant material like leaves of all plants were collected from the local areas of Meerut, Uttar Pradesh. Plant material was collected and washed under tap water and shade dried.

Preparation of polyherbal formulation: The formulation was created in the ratio of 1:0.5:1 of Haritaki, Maricha, and Tulsi based on the conclusions of earlier research. [10, 11]

Preparation of extract: The ethanolic extract was made using the Soxhlet extraction weight per millilitre calculations after the air-dead plant material had been coarsely pulverised. The phytochemical analysis was performed on the extract. [12, 13]

Preliminary phytochemical screening: To determine if active phytochemicals were present or absent, an initial phytochemical screening was performed on an ethanolic extract of a polyherbal preparation. [14, 15]

Experimental animals: For the investigation, Wistar strain albino rats weighing 150–180 grammes were employed. The animals were kept under conventional conditions, which included a 12-hour light/dark cycle, a temperature of 23±11°C, a relative humidity of 55±1%, and a standard rat pellet diet and unlimited water. Every experiment was carried out with approval from the Institutional Animal Ethical Committee. [16, 17]

Acute toxicity studies: In accordance with OECD guideline 423, acute toxicity investigations were conducted. Three animals of the same sex were used in this step-by-step process. Starting doses for rats ranged from 50 mg/kg to 2000 mg/kg. Determining the acute toxicity of the test material may require 2-4 steps, depending on the average mortality and moribund status of the animals. [18, 19] Animals' body weight was recorded both before and after the trial ended, along with any changes to their skin, eyes, mucous membranes, and behaviour patterns. Additionally, signs of tremors, salivation, diarrhoea, lethargy, sleep, and coma were recorded. During acute toxicity tests, the polyherbal preparation's ethanolic extract did not exhibit any death symptoms up to 2000 mg/kg p.o. On the basis of LD_{50} 1/10th dose was used for the study i.e. 200mg/kg and 400mg/kg. [20]

Aspirin plus Pylorus Ligation (PL) Induced Gastric Ulcer Model: Five groups of six Wistar rats each were created from the thirty rats. Table 1 lists the medications that were taken every day for five days. [21, 22, 23]

Table 1: Study Group for Aspirin + PL-Induced Gastric Ulcer Model

Sr. no.	Group	Dose (ml/kg body weight)
1.	Control	10 ml/kg, orally
2.	Disease Control(Aspirin + PL)	200 mg/kg, orally
3.	Standard drug	10mg/kg Omeprazole
4.	Ehanolic polyherbal extract	200 mg/kg orally
5.	Ehanolic polyherbal extract	400 mg/kg orally

On the 6th day, after 24 hrs of fasting, pylorus was ligated under anesthesia. 4 hours after ligation, the animals were sacrificed, and the stomach was removed and opened along the greater curvature. The contents were collected in graduated test tubes and centrifuged (1000 rpm. for 10 minutes). The supernatant was then subjected to biochemical analysis. The antiulcer activity was evaluated using parameters like ulcer scoring, ulcer index, gastric secretion volume, total acidity, gastric wall mucus content and pepsin estimation. [24-27]

Acetic Acid-Induced Gastric Ulcer Model: Prior to the experiment, the animals listed in Table 2 were fasted for 24 hours and divided into 5 groups of 6 animals each.

Table 2: Study Group for Acetic Acid Induced Gastric Ulcer Model

Sr. no.	Group	Dose (ml/kg body weight)
1.	Control	10 ml/kg, orally
2.	Disease Control(Aspirin + PL)	200 mg/kg, orally
3.	Standard	10mg/kg Omeprazole
4.	Ehanolic polyherbal extract	200 mg/kg orally
5.	Ehanolic polyherbal extract	400 mg/kg orally

The stomach was made visible by making a midline incision beneath the xiphoid process to open the abdomen while under anaesthesia. After adding 0.05 ml of glacial acetic acid to the 6 mm-diameter cylindrical mould, it was securely positioned over the anterior serosal surface of the stomach. This was left in place for sixty seconds. To prevent harm to the surrounding tissues, the mould was rinsed twice or three times with regular saline to remove the acid solution. After that, the abdominal wall was reopened and the stomach was carefully put back. For ten days, the medications were given every day. The stomach was removed from the animals during the anesthesia-assisted sacrifice on the eleventh day. The larger curvature of the stomach was sliced open. The antiulcer activity was evaluated using parameters like ulcer scoring and ulcer index. [28-30]

RESULTS AND DISCUSSION:

Preliminary phytochemical screening: Proteins, alkaloids, carbohydrates, saponins, and flavonoids are all present in the ethanolic extract of the polyherbal formulation according to preliminary phytochemical screening.

Acute Oral Toxicity: According to the acute oral toxicity investigation, animals did not die from the polyherbal formulation up to a dose of 2000 mg/kg. The experimental animals showed no alterations in their appearance or behaviour.

Aspirin plus Pylorus Ligation (PL) Induced Gastric Ulcer Model:

Ulcer Index and Ulcer Scoring: Both the test group and the conventional therapy group showed a significant drop in ulcer scores. Table 3 lists the scores for the data.

Table 3: Effect of polyherbal formulation on Ulcer Scoring and Ulcer Index of Aspirin + Pl-Induced Ulcer

S. No.	Group	Ulcer score	Ulcer Index
1	Control (1% CMC)	0 ± 0.0	0 ± 0.0
2	Disease control (Aspirin 200 mg/kg)	2.8 ± 0.34	1.76 ± 0.04
3	Standard (Omeprazole 10mg/kg)	0.51 ± 0.007	0.22 ± 0.003*#
4	Low dose (200 mg/kg)	0.97 ± 0.002	0.32 ± 0.09*#
5	High dose (400mg/kg)	0.62 ± 0.003	0.29 ± 0.015*#

All values are expressed as Mean ± S.E.M., n=6) analyzed by One way ANOVA followed by Tukey's test. * denotes $p<0.05$ when compared with control. # denotes $p<0.05$ when compared with disease control

Since no ulcers were discovered in the control group and the ulcer index was zero, it may be concluded that the disease control group that took (Aspirin (200 mg/kg) + PL) had a considerably higher ulcer index than the control group. Test groups and regular medication therapy show a significant ($p<0.05$) decrease in ulcer index. According to the observational results, the ulcer index significantly decreased in the high test dose group (Polyherbal formulation 400 mg/kg) compared to the lower test dose group (Polyherbal formulation 20 mg/kg).

Gastric Secretion Volume and Total Acidity: Table 4 mentions the volume of stomach secretion. The stomach secretion volume was decreased by the low dose test formulation, however this effect was not statistically significant ($P <0.05$). Gastric acid secretion is considerably decreased by the conventional and high-dose test formulation. These results imply that the polyherbal formulation's gastrointestinal protective function might result in dose-dependent anti-secretory activity. The overall acidity of the regular and high dose groups shows a discernible decline.

Table 4: Effect On Gastric Secretion Volume and Total Acidity of Aspirin + PL-Induced Ulcer

S. No.	Group	Gastric secretion volume (ml)	Total acidity (mEq/I)
1	Control (1% CMC)	2.54 ± 0.12	32.76± 1.23
2	Disease control (Aspirin 200 mg/kg)	6.53 ±0.11*	42.14 ± 3.86*
3	Standard (S Omeprazole 10mg/kg)	2.89 ± 0.64*	21.62± 1.53*#
4	Low dose (200 mg/kg)	4.52 ± 0.39*	32.76± 1.72*
5	High dose (400mg/kg)	3.01 ± 0.23*	24.11± 2.22*#

Values are expressed as Mean + S.E.M., n=6, and analysed by One way ANOVA followed by Tukey's test. * denotes $p<0.05$ when compared with control# denotes $p<0.05$ when compared with disease control

Gastric wall Mucus Content: The mucus content of the stomach wall was significantly restored in the standard group. The mucus content of the stomach wall was recovered in both the low and high dose groups (400 mg/kg of the Polyherbal formulation). With a mucosal content that is comparable to the protective effect of omeprazole, the results listed in Table 5 thus point to a strong cytoprotective efficacy of the Polyherbal formulation.

Table 5: Effect on Gastric Wall Mucus Content of Aspirin + PL Induced Ulcer

S. No.	Group	Gastric wall mucus content µg/gm
1	Control (1% CMC)	0.23± 0.034
2	Disease control (Aspirin 200 mg/kg)	0.16± 0.005*
3	Standard (Omeprazole 10mg/kg)	0.29 ±0.003*#
4	Low dose (200 mg/kg)	0.18 ± 0.004*#
5	High dose (400mg/kg)	0.24±0.0008*#

All values are expressed as Mean + S.E.M., n=6, and analyzed by One way ANOVA followed by Tukey's test.*denotes $p<0.05$ when compared with control.# denotes $p<0.05$ when compared with disease control

Pepsin Estimation: The mucus that causes an ulcer to form will be broken down by the protein pepsin and the gastric juice that results from pylorus ligation. Comparing the control group listed in Table 6 to the treatment groups, an increase in pepsin levels is evident. In comparison to the control and disease control groups, the treatment group exhibits a considerable drop in pepsin levels, as does the low dosage, high dose test formulation, and standard group.

Table 6: Effect on Pepsin Content of Aspirin + PL-Induced Ulcer

S. No.	Group	Pepsin estimation
1	Control (1% CMC)	0.17±0.001
2	Disease control (Aspirin 200 mg/kg)	0.17±0.002*
3	Standard (Omeprazole 10mg/kg)	0.10±0.002*#
4	Low dose (200 mg/kg)	0.14± 0.005*#
5	High dose (400mg/kg)	0.21±0.005*#

All values are expressed as Mean + S.E.M., n=6, and analyzed by One way of ANOVA followed by Tukey's test. * denotes $p<0.05$ when compared with control. # denotes $p<0.05$ when compared with disease control

The Aspirin + PL model's morphological examination outcomes. In comparison to the standard and test groups, the disease control group exhibits more ulcerated areas, redness, and lesions in the stomach's mucosal layer, according to a morphological analysis of the stomach.

Evaluation of Anti-Ulcer Activity by Acetic Acid Induced Gastric Ulcer Model:

Ulcer Scoring and Ulcer Index: This concept is similar to a healing sequence, relapsing traits, and pathological features. Pepsin and acid secretion are key factors in this research model that assesses ulcer healing ability. There has been a notable decline in ulcer scores in each of the other three therapy groups. Treatment groups such as standard, low dosage, and high dose test formulations show a notable reduction in their ulcer index. Therefore, it can be said that in the chronic ulcers listed in Table 7, every treatment group effectively demonstrated anti-ulcer efficacy.

Table 7: Ulcer Scoring and Ulcer Indexed in Acetic Acid-Induced Gastric Ulcer Model

S. No.	Group	Ulcer score	Ulcer Index
1	Control (1% CMC)	0 ± 0.0	0 ± 0.0
2	Disease control (Aspirin 200 mg/kg)	2.17 ± 0.040	1.456 ± 0.093
3	Standard (S Omeprazole 10mg/kg)	0.37 ± 0.041	0.161 ± 0.003*#
4	Low dose (200 mg/kg)	0.74 ± 0.091	0.286 ± 0.003*#
5	High dose (400mg/kg)	0.56 ± 0.043	0.211 ± 0.015*#

As seen below, the treatment effect shows morphological changes in the model of stomach ulcers caused by acetic acid. In comparison to the standard and test groups, the disease control group exhibits higher ulcerated diameter and morphological abnormalities in the stomach mucosa. The standard and test groups, on the other hand, experience more intense healing.

CONCLUSION: It was determined that the test formulation was safe up to 2000 mg/kg body weight. The current study's findings demonstrate that the polyherbal formulation has ulcer-healing properties, as evidenced by a dose-dependent reduction in the volume of gastric acid and an anti-secretory effect on overall acidity. Additionally, by blocking pepsin and re-establishing the mucosal content of the stomach wall, the polyherbal formulation treatment provides cytoprotection. The polyherbal formulation's cytoprotective properties are demonstrated by its anti-secretory and mucosal defensive effect. Thus, in both acute and chronic peptic ulcers, a polyherbal formulation showed promise in ulcer healing activities.

REFERENCES

1. Kumar, K., Mruthunjaya, K., Kumar, S. and Mythreyi, R. Anti ulcer activity of ethanol extract of the stem bark of Careya arborea Roxb. International Current Pharmaceutical Journal 2, 78–82 (2013).
2. Vaseem, A., Ali, M. and Afshan, K. Activity of Tulsi leaves (Ocimum sanctum linn) in protecting gastric ulcer in rats by cold restrain method. IJBCP 6, 2343–2347 (2017).
3. Vishvakrama P, Sharma S. Liposomes: an overview. Journal of Drug Delivery and Therapeutics. 2014 Jun 24:47-55.
4. Vishvakarma P. Design and development of montelukast sodium fast dissolving films for better therapeutic efficacy. Journal of the Chilean Chemical Society. 2018 Jun;63(2):3988-93.
5. Vishvakarma P, Mandal S, Verma A. A review on current aspects of nutraceuticals and dietary supplements. International Journal of Pharma Professional's Research (IJPPR). 2023;14(1):78-91.
6. Dasarapu, S. and Alluri, R. Evaluation of Antiulcer Activity of Polyherbal Formulation Against Ethanol Induced Gastric Ulcer in Albino Rats. International Journal of Innovation Sciences and Research 5, 761–765 (2016).
7. Bahmani, S., Harish, M. S., Iqbal, M. and Rao, K. S. Anti-secretory and anti-ulcer activity of Surasa, a poly herbal formulation in pylorus ligated (Shay) rat model. Biosciences Biotechnology Research Asia 5, 295–300 (2008).
8. Roopa, G., Bhat, R. S. and Dakshina, M. S. Formulation and evaluation of an antacid and anti-ulcer suspension containing herbal drugs. Biomedical and Pharmacology Journal 3, 01–06 (2010).
9. Mofleh, I. et al. Gastroprotective effect of an aqueous suspension of black cumin Nigella sativa on necrotizing agents-induced gastric injury in experimental animals. Saudi Journal of Gastroenterology 14, 128–134 (2008).

10. Mofleh, I. A., Alhalder, A. A., Mossa, J. S., Al-Soohalbani, M. O. and Rafatullah, S. Aqueous suspension of anise "Pimpinella anisum" protects rats against chemically induced gastric ulcers. World Journal of Gastroenterology 13, 1112–1118 (2007).
11. Prabhakar Vishvakarma, Jaspreet Kaur, Gunosindhu Chakraborthy, Dhruv Kishor Vishwakarma, Boi Basanta Kumar Reddy, Pampayya Thanthati, Shaik Aleesha, Yasmin Khatoon. Nephroprotective Potential of Terminalia Arjuna Against Cadmium-Induced Renal Toxicity by In-Vitro Study. J. Exp. Zool. India Vol. 28, No. 1, pp. 939-944, 2025
12. Prabhakar V, Agarwal S, Chauhan R, Sharma S. Fast dissolving tablets: an overview. International Journal of Pharmaceutical Sciences: Review and Research. 2012;16(1):17.
13. Vishvakarma P, Mandal S, Pandey J, Bhatt AK, Banerjee VB, Gupta JK. An Analysis Of The Most Recent Trends In Flavoring Herbal Medicines In Today's Market. Journal of Pharmaceutical Negative Results. 2022 Dec 31:9189-98.
14. Kunchandy E, Rao MNA: Oxygen radical scavenging activity of Curcumin. International J Pharmaceutics; 1990; 58(7): 237-240.
15. Sreejayan, Rao MN: Nitric oxide scavenging by curcuminoids. J. Pharmacy and Pharmacology; 1997; 49(1): 105-107.
16. Dursun H, Albayrak F, Bilici M, Koc F, Candar T, Kukula O: Gastroprotective and Antioxidant effects of Opipramol on Indomethacin-induced ulcers in rats. Yakugaku zasshi; 2009; 129(7): 861-869.
17. Deshpande SS, Shah GB, Parmar NS: Antiulcer activity of Tephrosia purpurea in rats. Indian J of pharmacology 2003; 35: 168-172.
18. Czinner E, Hagymasi K, Blazovics A, Kery A, Szoke E, Lemberkovics E: The in vitro effect of Helichysi flos on microsomal lipid peroxidation. J. Ethnopharmacoly; 2001; 5: 77:31.
19. Shay H, Komarov SA, Fels SS, Meranze D, Gruenstein M, Sipet H. A simple model for the uniform production of gastric ulceration in the rat. Gastroenterology, 1945; 5: 43-61
20. Nwinyl FC, Binda L, Ajoku GA. Evaluation of aqueous extract of Boswollia dalzielii stem bark for antimicrobial activities and gastrointestinal effects. African J of Biotechnol.2004; 3: 284-88.
21. P. Manimekalai, P. Maheshwari, R. Velmurugan, M. Gurumoorthy, S. Hansraj Kumar, G. Vijayakumar. Gastro Protective effect of Standardized Ethanolic leaf extract of Indigofera tinctoriao on experimental Gastric Ulcers in Rats. Research J. Pharm. and Tech 2018; 11(2):527-531.
22. P. Manimekalai, P. Maheshwari, R. Velmurugan, M. Gurumoorthy, S. Hansraj Kumar, G. Vijayakumar. Gastro Protective effect of Standardized Ethanolic leaf extract of Indigofera tinctoriao on experimental Gastric Ulcers in Rats. Research J. Pharm. and Tech 2018; 11(2):527-531.
23. Ashoka Shenoy M, Shastry C. S , Sridevi , Gopkumar P. Anti-ulcer activity of Naravelia zeylanica leaves extract . Journal of Pharmacy Research 2009, 2(7), 1218-1220
24. Ganguly AK, Bhatnagar OP, Effect of bilateral adrenalatomy on production of restraint ulcers in the stomach of albino rats, Canadian J Physiol Pharmacol 1973, 51:748-50.
25. Mariam A. Kadhem, Ahlam A. Abdul-niby, Huda K. Khassaf. Study the effect of Ethanolic extract of Anethum graveolens L. on Aspirin induced Gastric Ulcer in Male Guinea Pigs. Research J. Pharm. and Tech 2018; 11(9): 3793-3798.
26. OECD G: Guidance document on acute oral toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment 2000.

27. Jainu M and Devi CS: Gastroprotective action of Cissus quadrangularis extract against NSAID induced gastric ulcer: role of proinflammatory cytokines and oxidative damage. Chem Biol Interact 2006; 161(3): 262-270. doi:10.1016/j.cbi.2006.04.011
28. Wang Z, Hasegawa J and Wang X: Protective Effects of Ginger against Aspirin-Induced Gastric Ulcers in Rats. Yonago Acta Med 2011; 54(1): 11-19.
29. Okabe S and Amagase K: An overview of acetic acid ulcer models--the history and state of the art of peptic ulcer research. Biol Pharm Bull 2005; 28(8): 1321-41. doi: 10.1248/bpb.28.1321. PMID: 16079471.
30. Szabo S: Gastric cytoprotection is still relevant. J Gastroenterol Hepatol 2014; 29 Suppl 4: 124-32. doi: 10.1111/jgh.12735. PMID: 25521744